R E S E A R C H P A P E R

Winter Time Ichthyoplankton Assemblages Following Dense Mucilage Event in the Sea Of Marmara, Türkiye

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Abstract

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Introduction

Mucilage defined as a gelatinous evolving stage of amorphous aggregates released by marine organisms (Leppard, 1995). Mucilage mostly emerges Adriatic Sea and well-known around northern part (Danovaro et al., 2009). Between November 2020 and August 2021, a dense mucilage event occurred in the Marmara Sea. Although some small-scale incidences were reported previously, this is turn into a disaster with the worst consequences. The structure of mucilage consist of exopolymeric compounds that embodies carbohydrates, polysaccharides, prokaryotes and viruses. Thus, it causes anaerobe environment for other living creatures, and therefore pose a threat to the

identified. It was detected that the biodiversity of winter time ichthyoplankton was higher than previous studies. European Sprat, *Sprattus sprattus* had the highest mean biomass for both fish eggs and fish larvae, whereas Sardine, *Sardina pilchardus* had relatively low biomass. Karacabey Floodplain area, Büyükçekmece estuarine area and Erdek Bay were the most important spawning areas for winter spawners. It was found that ecosystem health of the Marmara Sea was suitable for fertilization, hatching and larval growth soon after the mucilage and hydrological regime recover itself

The community structure of ichthyoplankton after mucilage was investigated. For this purpose, three ichthyoplankton survey were conducted from 34 stations between December 2021 and March 2022. A total of 19 fish egg and 16 fish larvae species were

> environment. The worst adverse effects of mucilage affect of corals and sponges (Bianchi et al., 2019; Piazzi et al., 2018; Topçu and Öztürk, 2021). Also, mass deaths and shifting of adult fish were reported (Dalyan et al., 2021; Karadurmuş and Sarı, 2022).

> For understanding the effects of mucilage on fish biodiversity, early life stages of fish selected as a reference group due to vulnerable and fragile structure against instantaneous enviromental disestars. Ichthyoplankton was previously used as observe abnormal effects of disestars such as El Nino and La Nina events (Auth et al., 2015), global warming (Auth et al., 2018), ocean acidification (Franke and Clemmesen, 2011), and oil spill (de Souza et al., 2022). The relationship between ichthyoplankton and mucilage has

been mentioned in two studies, yet (Dulčić, 1995; Tsoukali et al., 2019).

Marmara Sea is a semi-enclosed basin, which located between Aegean Sea and the Black Sea. Due to the connection of comprised of straits, two-layered stratification originated by varied densities of water mass. The upper layer carries of low saline waters originating from the Black Sea and lower layer is comprised of high saline waters originating from the Mediterranean Sea (Beşiktepe et al., 1994). The Black Sea water serve as a nourishing characteristics due to has high nutritional elements and plankton abundance, which clearly hinge upon precipitation. A high primary production and plankton biomass reval crucial habitat especially planktivarous fish and fish larvae. Thus, the Sea of Marmara become valuable reproduction, growth and feeding area for lots of fish species (Yüksek, 2016). Beside, the inlet structure cause additional biodiversity due to create one of the most important biological migrating route in the world. In addition, the Aegean Sea welded backflow ascertain important biodiversity, especially around southwestern part. Hereit, Marmara Sea embodies nearly the half of the total fish biodiversity of Turkish seas, with 221 teleost and 36 cartilaginous fish species (Bilecenoğlu et al. 2014). Conversely, low dissolved bottom oxygen, antropogenic pressure, overfishing and marine litter pollution has been significantly effected of the biodiversity of Marmara Sea, especially in the last 20 years (Yüksek, 2016). Thus, ecological regime shifted from decrease of the top predators to dominancy of small pelagic fish species by shifted physical drivers, increased fishing mortality, and decreased water quality (Demirel et al., 2023). Nowadays, some species like Tunas, and Swordfishes are absent or represented with a few individuals in the Sea of Marmara. Also, some new fish species such as *Siganus rivulatus*(Karakulak et al., 2020), *Dentex maroccanus* (Bilecenoğlu and Yokeş, 2022), and *Ophisurus serpens* (Uzer et al., 2024) are distributed in the Sea of Marmara. The global warming effects the expansion of fish species, and the Marmara Sea and the Black Sea under the threat of the Mediterranization (Turan and Gürlek, 2016).

Ichthyoplankton studies in the Sea of Marmara has begun in the 1957s with Arım (1957)'s study. Some valuable studies were conducted in the Sea of Marmara which examine ichthyoplankton biodiversity around highly polluted areas such as Golden Horn and İzmit Bay (Yüksek et al., 2001a; 2001b), summer time ichthyoplankton (Demirel et al., 2007), seasonal variation of ichthyoplankton in the southwestern part (Kara, 2015; Daban and Yüksek, 2017). The number of ichthyoplankton studies has decreased in last years when environmental disasters and pollution load has increased. Thus, the actual status of ichthyoplankton biomass and biodiversity after these disasters arouse curiosity.

The main purposes of this study were (i) to observe the ichthyoplankton biomass and biodiversity after mucilage event (ii) to understand the habitat health (iii) to reveal large scale spatial distribution of ichthyoplankton in the Sea of Marmara.

Material and Methods

For collecting fish eggs and larvae, three ichthyoplankton survey was conducted in December 2021, February 2022 and March 2022. Samplings were realised from equally spaced (11 miles) 34 stations (Table 1) which located to cover all of the Sea of Marmara (Figure 1). Plankton samples were collected with WP-2 type plankton net with a 300 μm meshnet (57 cm frame diameter, 3 m total length) with horizontal and vertical tows from each stations. Vertical tows were used for biomass analyses, whereas the results of horizontal tows were used for biodiversity analyses. Horizontal samples were collected 0.5 munder the sea surface for a duration of 10 minutes with a vessel speed of 2 knots. Vertical tows were conducted from the upper pycnocline (~40 m) to the sea surface. All tows were conducted during daylight and samples were fixed with 4% formaldehyde-sea water solution on the vessel. Fish eggs and larvae were identified and counted under a dissecting microscope in the laboratory. The keys of Dekhnik (1973), Russell (1976), and Mater and Çoker (2004) were used for the identification of fish eggs and larvae. Dissolved oxygen, salinity and temperature were measured using a YSI 6600. Biological diversity was determined with calculating Shannon-Wiener H, the Dominance, the Margalef diversity indices. PAST Version 2.17 (Hammer et al.2001) was used for calculation of biodiversity indices. Densities of fish eggs and larvae from vertical tows standardized in a unit area ($n/10$ m²) according to using formula given "Vertical Distribution" part of FAO Fisheries Technical Papers No: 175;

C = Cv x (SR/V)

where *C* is a individual number in a unit area (n/10 m²), *Cv* is a individual number in a unit volume (n/1000 m³), *S* is a unit of area (10 m²), *R* is a depth of sampling (m) and V is a unit of volume (1000 m³).

Results

Environmental Variables (Temperature, Salinity and Dissolved Oxygen)

The mean surface temperature values for December 2021, February 2022 and March 2022 was calculated as 11.5±0.03, 7.2±0.05 and 7.4±0.1, respectively. The highest temperature was measured in P15 (Bandırma) in December, whereas the lowest value was measured from P19 (middle part). Mean surface salinity was found 26.7±0.6 in December 2021, 28.8±0.3 in February 2022 and 25.9±0.3 in March 2022. The lowest sea surface temperature was measured from stations around İstanbul Strait (P27, P28), whereas **Table 1.** Sampling stations and sea surface temperature, salinity and oxygen values

Figure 1. Study area and sampling stations

higher salinites were recorded from the western part (P1, P3). Mean dissolved oxygen (DO) values for December 2021, February 2022 and March 2022 was calculated as 8.4±0.07, 8.9±0.04 and 7.8±0.14, respectively. Highest DO values were measured at middle part (P22, P25), whereas lowest values were found in P3 where under the influence of the Gönen Stream (Table 1).

The sea surface temperature values were differ statistically important between December and February-March (*p:6.431e-12*), whereas mean surface temperature values between stations did not differ (0.999). Similarly, mean sea surface salinity values were differ statistically important between December and February (*p:1.393e-6*) and between February and March (*p:1.546e-7*), whereas mean surface salinity values between stations did not differ (0.251). The mean sea surface DO values were differ statistically important between December and February (*p:6.436e-7*), between December and March (p:0.0027) and between February and March (*p:1.045e-7*). The mean DO values statistically showed variation only between P4 and P19 (*p: 0.0047*).

Ichthyoplankton Biomass

In total, mean fish egg abundance in a unit area $(n/10 \text{ m}^2)$ was 792.6, with the mean larvae biomass of 97.6. *S. sprattus* was the most abundant fish species among egg and larvae stage, with a 713.8 $n/10$ m² (90.1%) and 60.4 $n/10$ m^2 (61.9%) density. *Pomatoschistus pictus* was the second abundant fish larvae species, with a 20.8 $n/10$ $m²$ (21.3%). The total abundance of the remaining 18 fish egg species constituted 9.9% of total egg abundance. Between of them, *Liza saliens, Sardina pilchardus,* and *Gaidropsarus*

mediterraneus were relatively abundant species. A 83.2% of total fish larvae abundance were arised from *S.sprattus* and *P.pictus*. In common with the fish eggs, *Liza saliens, Sardina pilchardus,* and *Gaidropsarus mediterraneus* were also the other dominant fish larvae species among 16 fish species (Table 2).

In terms of spatial variation of the fish egg abundance, egg patchs were seen in three varied location. 18th station where is under the influence of freshwater input (Karacabey Floodplain area) was the most abundant (23% of total biomass) among 34 station. 8th station (Hoşkoy) was the second abundant areas (8%). The stations around İstanbul Strait (26th, 28th, 30th, 32th) were the third abundant areas. By means of fish larvae biomass, similarly 18th station was the most abundant station (12% of total fish larvae biomass) among 34 areas. The lowest larvae biomass were seen the deeper middle part (25th, 27th, 29th and 31th stations). On the contrary of fish egg abundance, the

Table 2. Biomass and frequency of ichthyoplankton in the Sea of Marmara

stations around western part (2nd, 4th, 5th, 6th, 7th, and 8th) were abundant by means of fish larvae abundance.

Monthly mean fish egg biomass in December 2022, February 2023, and March 2023 was found as 83.6 n/10 m^2 (3.5%), 1815 n/10 m² (76.3%), and 480 n/10 m² (20.2%), respectively. In December 2022, abundant fish egg species in a unit area were *Sardina pilchardus* (26.4%), *Solea lascaris* (14.7%) and *Microchirus variegatus* (13.2%) between 15 fish egg species. *S.sprattus* eggs constituted 10% of the total egg biomass of December, whereas constituted 0.4% of its total biomass in the winter period. The mean biomass of *S. sprattus* eggs peaked in February 2023, with a 1719.9 $n/10$ m² (80.3%) density.

The temporal variation of fish egg biomass between months were found statistically not important, whereas temporal variation of fish larvae were important (*p:0.0086*). According to Mann-Whitney, statistically differrences were found between December 2022 and February 2023 (*p: 0.0098*) and February 2023 and March 2023 (*p: 0.011*) for fish larvae biomass. The spatial variation of fish egg and larvae biomass between stations were not found statistically important.

Ichthyoplankton Biodiversity

According to horizontal plankton tow results, 19 fish egg species belonging to 13 families and 16 fish larvae species belonging to 11 families were identified (Table 3). Sparidae and Soleidae were the most dominant families for fish eggs, with 4 and 3 species, whereas Clupeidae (2) and Soleidae (2) dominant for fish larvae. Highest fish egg biodiversity were found around İstanbul Strait (P28 and P30; 14 species) and around Karacabey Floodplain area (P21: 12 species). The highest fish larvae biodiversity was seen Erdek Bay and nearby islands, and P28, with 7 species (Figure 2).

Monthly basis, the highest fish egg biodiversity detected in December 2022 with 17 species. 12 and 14 species were identified in February 2023 and March

Figure 2. Spatial variation of fish egg and larvae biomass in the Sea of Marmara

2023. For fish larvae, 11, 10, and 11 species were found in December 2022, February 2023 and March 2023, respectively (Table 3).

Mean Shannon_H indice of fish eggs was low (0.84) during winter period. Highest Shannon indice detected in December (2.256), whereas very low values were found in February (0.115) and March (0.153). Similar values were also found for Margalef biodiversity indices for fish eggs. Conversely, relatively low dominance indice was found in December 2022, whereas very high dominancy were observed in February and March 2023. As can be seen fish eggs, highest fish larvae biodiversity was also detected in December 2022. Hovewer, a slight higher indice values were found in February and March for fish larvae biodiversity when compared with fish eggs (Figure 3). In terms of spatial varaition of biodiversity, the highest indice values detected southwestern part of the Marmara Sea (P1, P3, P5, and P7), where under the influence of Gönen stream and island ecosystem.

Discussion

The relationship between ichthyoplankton and mucilage was previously stated in two studies (Dulčić, 1995; Tsoukali et al., 2019). It was reported that the biomass of anchovy eggs and larvae and daily growth rate of anchovy larvae found lower than expected during dense mucilage around Adriatic. Similarly, Tsoukali et al. (2019) stated that biomass of anchovy eggs and larvae remain limited during mucilage around Samothraki island in 2010. Due to these studies conducted at the time of mucilage, they had a chance to reveal adverse effects directly. At the time of dense mucilage, our efforts were inconclusive due to the plankton net become clogged from sticky structure of mucilage. It may be thought that the density of mucilage around Adriatic and North Aegean Sea were relatively lower than the Marmara Sea. We could have a chance to observe the variables 4 months after the end of the mucilage. So, our ichthyoplankton based results soon after the mucilage reflects the habitat condition after the mucilage.

Table 3. Monthly variation of ichthyoplankton in the winter period in the Sea of Marmara

Figure 3. Monthly variation of the biodiversity of ichthyoplankton in the Sea of Marmara

Visible mucilage aggregations around sea surface disappeared in July 2022. During the first marine survey in December 2022, no signs of mucilage were encountered in the environment. The surface sea water parameters during surveys appeared to reflect seasonal requirements and made us think that the conditions at the time of sampling were physically healthy. When we examined the ichthyoplanktonic results, it was seen that common winter spawners in previous studies were also observed in this study. Additionally, more species richness was found against some previous studies. Alimoğlu (2002) identified 9 fish eggs and 4 larvae species in the northwestern part, Daban (2013) found 10 eggs and 3 larvae species in the Çanakkale Strait, Kara (2015) detected 11 fish eggs and/or larvae in the Erdek Bay, and Yüksek and Gül (2016) revealed 3 fish eggs and 2 larvae species in the İstanbul Strait, in the winter months. A total of 19 fish eggs and 16 fish larvae species identified in this study may be a result of sampling in a broader area and much more stations. *S. sprattus, G. mediterraneus, L. saliens, M. merlangus* and *S. pilchardus* fish eggs and larvae were the most

abundant species as in previous studies. In addition to these, the occurrence of flatfish species such as *Buglossidium luteum. M. variegatus* and *S. lascaris*, demersal fish species such as *Callionymus lyra, Chelidonichthyes lucerna, Boops boops,* and *Dicentrarchus labrax,* and important prey species such as *P. pictus, Pomatoschistus minutus* ve *Gobius niger* were important by means of ecological health of Marmara Sea ecosystem.

A 90% of total fish egg biomass in the vertical tows of this study originated from *S. sprattus*. The most abundant fish egg species in the winter period were also stated by several authors in the Marmara Sea (Yüksek, 1993; Alimoğlu, 2002; Kara, 2015). The highest fish egg biomass, fish egg survival rate and first external feeding rate of *S. sprattus* detected at sea surface temperatures (SST) between 8 and 10°C (Peck et al., 2012). The mean sea surface temperature value detected in December was 11.5°C when the lowest *S. sprattus* eggs (0.1% of total abundance) were collected. The mean biomass of *S.sprattus* found highest (71.5% of total) in February, when the sea surface temperature were lowest (7.2°C).

Similarly, Daban (2013) stated that *S. sprattus* peaked in March in the Çanakkale Strait when the SST was measured 8.8°C. These findings were also supported the hypothesis of Maynou et al. (2020) which revealed the main factor control the dispersal of small pelagic fish eggs was sea SST. The mean abundance and peaks of *Sardina pilchardus*, is the other winter spawner small pelagic fish, showed similar pattern for spawning. In 2013, it peaked in February when the SST was 11.8°C in the Çanakkale Strait, in 2015 it peaked in October at 12.3°C in Erdek Bay and in 2022 it peaked in December at 11.5°C in the Marmara Sea. Contrary to expectation mean egg and larvae biomass of *S. pilchardus* was detected relatively lower against *S. sprattus* and some previous studies (Kara, 2015, Daban and Yüksek, 2017). North Aegean coasts and Marmara Sea are the most important fishing areas of Sardine. Beside, *S. pilchardus* is an important fish species for western part of Marmara Region due to economic importance arising from traditional canned fish production. These areas are known as an important spawning grounds, as well. A relatvely low mean fish egg and larvae biomass creates concern for the sustainability of such an important species for the region. As well as fish egg biomass, *S. sprattus* was also the most abundant fish larvae species. Daban et al. (2023) detected that a 16.2% of juvenile biomass around Marmara Sea comprised from Pomatoschistus sp. species. A 21.7% of total larvae biomass were also stemmed from Pomatoschistus sp. species from ichthyoplankton samples. Also, Özen et al. (2008) were detected high abundance of the Pomatoschistus species in the Dardanelles. Pomatoschistus species are play an important role in the food chain and known as majör food item of demersal fish species in the Marmara Sea. Thus, it can be said that Marmara Sea may not be faced with food limitation in near future. Contrary to these, Yüksek (1998), Alimoğlu (2002) and Demirel (2004) were not identified any species of Pomatoschistus genus and given as rare species by Kara (2015) and Daban and Yüksek (2017) in the previous studies. One possible explaniation of this situation is Potoschistus members may have been identified on a family basis as a Gobidae larvae in order to prevent wrong identification. In addition, the biomass of Pomatoschistus genus may have begun to increase with changing environmental conditions. The possibility and reasons for the increase in the stocks of this ecologically important group should also be investigated.

This study revealed important findings related spatial variation of winter spawners such a broad geographical area which embodies whole Marmara Sea. Both the results of vertical and horizontal tows showed that the Karacabey Floodplain area (middle Western part) is the most important spawning area for winter spawners. The areas between Tekirdağ shores and İstanbul Strait and Yalova shores were also other abundant areas for fish eggs and larvae of winter spawners. The deeper middle part and western part of the Marmara Sea had relatively low mean fish egg biomass. It was clearly determined that *S. pilchardus* spawned 3 local areas which were Büyükçekmece, Karacabey Floodplain area and Gönen Stream area. The *S. pilchardus* larvae mostly centred upon the most western part (Gelibolu-Mürefte areas, Gönen Straem areas). Palomera et al. (2007) stated that the estuarine areas are favorable locations for growth of small pelagic fish larvae. Also, Ramos et al. (2009) detected relatively high biomass of *S. pilchardus* eggs and larvae around the Lima estuarine area, Atlantic Ocean. Whereas some authors stated that Sardine mostly distribute out from cloudy and oligohaline waters and prefer deeper areas (Olivar et al., 2003; Coombs et al., 2004; Santos et al., 2004). The results related spatial variation of Sardine overlapped with Palomera et al. (2007) and Ramos et al. (2009) and revealed important proof related distrubiton around oligohaline areas.

Both biomass and biodiversity of fish eggs and larvae around Istanbul Strait were relatively found higher from remaining areas. This is a data based proof that Black Sea water discharge carries Black-Sea originated eggs and larvae of winter spawners. Thus, it was understood that winds and rainfalls, control the current direction and current speed of upper layer is one of the most important factor related sustainability of the Marmara Sea. Consequently, it has been determined that the Marmara Sea ecosystem was suitable in terms of fertilization, hatching and larval growth after mucilage and the hydrological system recover itself quickly. Conversely, worsened scenarios related environmental problems such as global warming, pollution and mucilage may overthrow the fragile structure of Marmara Sea.

Ethical Statement

All process and experimental protocols have been approved by Çanakkale Onsekiz Mart University Rectorate, Animal Experiments Local Ethics Committee (No; 2021/07-04).

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Author Contribution

İsmail Burak Daban: Writing-original draft, Project Administration, Data analyses, Sampling; Ahsen Yüksek: Supervision, Data-analyses; Alkan Öztekin, Yusuf Şen, Uğur Altınağaç, Adnan Ayaz; Gençtan Erman Uğur, Oğuzhan Ayaz, Tekin Demirkıran, Buminhan Burkay Selçuk: Sampling, Laboratory works, Investigation; Ali İşmen, Uğur Özekinci, Fikret Çakır: Visualization, Investigation.

Conflict of Interest

The authors declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

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