İSTANBUL UNIVERSITY PRESS

AQUATIC SCIENCES and ENGINEERING

2024

VOLUME: 39 ISSUE: 3 E-ISSN 2602-473X

AQUATIC
SCIENCES and ENGINEERING

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Correspondence Address

Istanbul University Faculty of Aquatic Sciences,
Ordu Caddesi No: 8 34134 Laleli-Fatih / İstanbul, Türkiye
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Publisher

Istanbul University Press
İstanbul University Central Campus, 34452 Beyazıt,
Fatih / İstanbul, Türkiye
Phone: +90 (212) 440 00 00

Cover Photo

Prof. Dr. Özkan Özden
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The publication language of the journal is English.

This is a scholarly, international, peer-reviewed and open-access journal published quarterly in January, April, July, and October.

Publication Type: Periodical

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All expenses of the journal are covered by the İstanbul University Faculty of Aquatic Sciences.

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Editor in Chief: Prof. Dr. Devrim MEMİŞ
Address: İstanbul Üniversitesi Su Bilimleri Fakültesi Yetiştiricilik Anabilim Dalı Kalenderhane Mah. 16 Mart Şehitleri caddesi No: 2 Vezneciler / İstanbul, Türkiye
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Fax: +90 212 5140379
E-mail: mdevrim@istanbul.edu.tr

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EISSN 2602-473X

AQUATIC SCIENCES AND ENGINEERING

Aquat Sci Eng 2024; 39(3): 137-147• DOI: https://doi.org/10.26650/ASE20241433561

Research Article

Seasonal Zooplankton Distribution and Species Composition in the Eastern Sea of Marmara

Ezgi Türkeri[1], Melek Isinibilir[1]

Cite this article as: Türkeri, E., & Isinibilir, M. (2024). Seasonal zooplankton distribution and species composition in the Eastern Sea of Marmara. *Aquatic Sciences and Engineering, 39(3)*, 137-147. DOI: https://doi.org/10.26650/ASE20241433561

ABSTRACT

Seasonal changes in the composition and abundance of zooplankton and their responses to environmental changes were investigated at 30 stations in the upper layer and 13 stations in the lower layer (in February, May, July, and December) of the Eastern Marmara Sea in 2016. A total of 44 species/groups (including jellyfish species) were documented in the study area, with 39 identified in the upper layer water and 31 in the lower layer water. The abundances of zooplankton ranged from 29 to 2822 ind.m^{-3} in the upper layer and from 6 to 2283 ind.m^{-3} in the lower layer. *Acartia clausi, Paracalanus parvus, Penilia avirostris*, and *Oithona similis* were the dominant species in summer and autumn, whereas *Calanus euxinus*, *Pseudocalanus elongates*, *Oithona nana*, *Oithona davisae* and *Pleopis polyphemoides* were the dominant species in winter and spring. *Aetideus* spp., *Clausocalanus* spp., *Ctenocalanus vanus*, *Oncaea minuta*, *Isopoda*, and *Siphonophora* were observed only in bottom-layer waters. *M. lucens* and *O. davisae* were recorded only in the upper layer in December. In conclusion, our results suggest that zooplankton communities and some species are favorable indicators of the marine environment of the Sea of Marmara.

Keywords: Zooplankton, abundance, species diversity, Sea of Marmara

ORCID IDs of the author:
E.T 0000-0003-2017-6137;
M.I. 0000-0002-1200-6878

[1]Department of Marine Biology, Faculty of Aquatic Science, Istanbul Univerity, Istanbul, Türkiye

Submitted:
07.02.2024

Revision Requested:
07.03.2024

Last Revision Received:
14.03.2024

Accepted:
15.03.2024

Online Published:
20.05.2024

Correspondence:
Ezgi Türkeri
E-mail:
ezgi.turkeri@istanbul.edu.tr

INTRODUCTION

Marine coastal areas feature greater levels of plankton biodiversity and production, which play an important role in biogeochemical processes. However, climate change and anthropogenic pressures that degrade ecosystems threaten biodiversity and ecological functions. Increasing anthropogenic activities accelerate eutrophication, often leading to irreversible degradation (Tüfekçi et al., 2010; Kemp and Boynton, 2012; Griffith and Gobler, 2020).

Zooplanktons are highly sensitive to physico–chemical parameters and biological factors in marine systems (Isinibilir et al., 2008; Shi et al., 2015). The composition and abundance of zooplankton largely depend on environmental conditions and respond rapidly to environmental changes (Isinibilir et al., 2011). As a result, zooplankton is considered a biological indicator of environmental water quality. Zooplankton orchestrate marine ecosystems by not only transferring energy from primary producers to higher trophic levels but also regulating phytoplankton production and shaping the pelagic ecosystem (Rissik et al., 2009).

The Marmara Sea is an important transitional basin between the Black Sea and the Mediterranean, connected to the Black Sea via the Bosphorus and to the Aegean Sea via the Dardanelles. The upper layer of the Marmara Sea is influenced by low salinity Black Sea water (18%), while more saline water from the Mediterranean (up to 40%) is found at depths exceeding 20 m (Beşiktepe et al., 1994). Over the last few decades, many factors including excessive nutrients and pollutants, overfishing, the introduction of new species, and climate change have signifi-

cantly disrupted the Marmara Sea fauna temporally and spatially. These changes have resulted in red tide and mucilage aggregation, the disappearance or sharp decline in population of some species, and a rise in the number of nonindigenous species (Aktan., 2008; Isinibilir et al., 2010; Isinibilir Okyar et al., 2015; Doğan & Isinibilir, 2016; Isinibilir & Yılmaz, 2017, Turkoğlu, 2013). Zooplanktons, the most important link in energy transfer in the seas, were the first to react to these changes (Isinibilir et al., in press).

The main aim of this study is to explore the abundance and fluctuations in species composition of coastal zooplankton communities in the eastern region of the Sea of Marmara. Furthermore, the study seeks to quantify the zooplankton composition in the research area to determine the indicator species composition across environmental conditions as well as to improve existing data by measuring the relationships of indicator species in the research area with environmental parameters.

MATERIALS AND METHODS

Sampling design and analysis of samples

Upper layer samples exposed to both the Black Sea and local pollution were collected from 30 stations on the Marmara Sea in February, May, July, and December in 2016. Stations İZ9, M11, İZ4, and MD102 were located offshore where there is less anthropogenic stress, representing the open-sea ecosystem of the Sea of Marmara, although they were still impacted by the top layer flow from the İzmit Bay and the Istanbul Strait. Stations MD3, MD4, İZ8, and MD8 were considered less polluted and represented a transitional zone from the neritic to open-sea environment. G2, KÇ, M12, M13, M14, MD1, MD2, MD3, MD4, MD5, MD6, MD7, MD9, MD10, MD75, MY5 YK1, İZ7, İZ6, İZ5, İZ3, İZ2, and İZ1 were in the coastal waters of the Sea of Marmara where anthropogenic stress is greatest. Samples were collected from the Mediterranean-originated lower layer at 13 stations (MD1, MD3, MD4, MD5, MD6, MD7, MD8, MD75, MD102, M11, İZ9, İZ8, and İZ4) during periods characterized by both thermal stratification and haline stratification.

Zooplankton samples (including jellyfish species) were gathered vertically using a WP2 closing net with a 0.5 m diameter and a 157-mm mesh. Samples were collected from the bottom to the start of the mixing layer and from the interface (18–20 m) to the surface. The salinity, temperature, and dissolved oxygen of the whole water column were measured with an SBE-19 SEACAT CTD (conductivity, temperature, and pressure recorder) system. Chlorophyll-a analyses were conducted at all stations in the upper water layer using the methodology outlined in APHA (2000). The zooplankton samples were preserved on board using a 4% formalin seawater solution. Three 1-ml aliquots were obtained from the sampling bottle with a Stempel pipette; counting and diagnostics were performed in the zooplankton counting chamber and stereobinocular microscope.

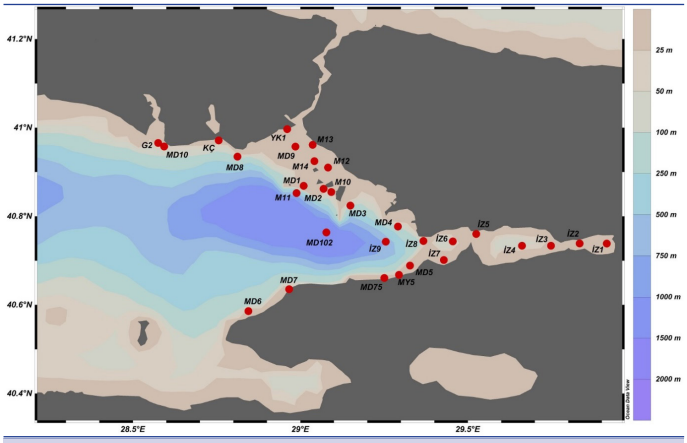

Figure 1. Locations of the sampling stations in the Sea of Marmara.

Statistical analysis

The zooplankton community was assessed according to the Shannon index of diversity (H') and the number of species (S) as described by Shannon and Weaver (1949). Furthermore, using log (x + 1)-transformed abundance data and Primer v. 6 software, multidimensional scaling (MDS) studies of similarity between sampling months were performed based on the Bray–Curtis similarity index (Clarke & Warwick, 1994). Using SPSS v22 software, Spearman's rank-correlation coefficient was calculated to find associations between biotic and abiotic factors.

RESULTS AND DISCUSSION

The two-layered structure is discernible from the profiles of salinity, temperature, and dissolved oxygen (Figure 2). The highest sea surface temperature and chlorophyll-a values were observed in July (25.3^{0}C and 7.7 µg.L^{-1}), whereas the highest salinity (30.4 ppm) and dissolved oxygen (8.8 mg.L^{-1}) values were measured in February (Figure 3). Oxygen values decreased from the surface to the bottom throughout the water column, and values below 2 mg.L^{-1} were detected deeper than 50 m.

The minimum dissolved oxygen value was recorded in May (4.57 mg.L^{-1}). Despite its considerable variability, the distribution of chlorophyll-a exhibited notable spatio-seasonal patterns and a strong correlation with temperature ($r = 0.526$, $p < 0.01$).

Seasonal variations were observed in the abundance and biomass of zooplankton (excluding the dinoflagellate *Noctiluca scintillans*) in the eastern Sea of Marmara (Figure 3). The maximum quantity of zooplankton was observed at station MD6 in December (2822 ind.m^{-3}) due to high abundance of *Paracalanus parvus* and *Penilia avirostris* (1210 ind.m^{-3} and 468 ind.m^{-3}, respectively). In contrast, the zooplankton biomass peaked at station M11 in May due to *Aurelia aurita* (Figure 3).

A total of 44 species/groups were documented in the study area, with 39 identified in the upper layer water and 31 in the lower layer water (Table 1). The most abundant zooplankton groups across all stations were copepods, cladocerans, meroplankton, appendicularians and jellyfishes. *Aetideus spp., Clausocalanus spp. Ctenocalanus vanus, Goniopsyllus rostratus, Oncaea minuta*, Isopoda, and Siphonophora were observed only in bottom-layer waters; *M. lucens* and *O. davisae* were observed only in the upper layer in December.

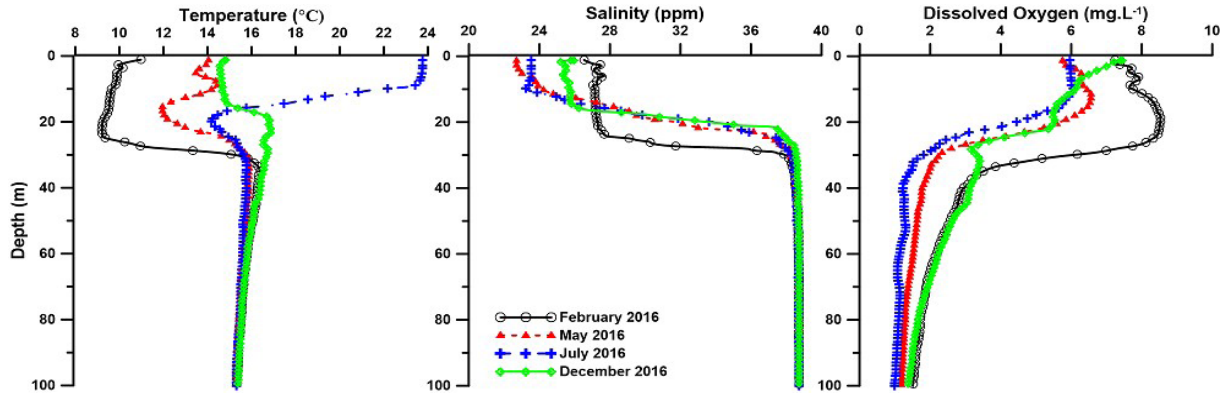

Figure 2. Temperature, salinity and dissolved oxygen profiles in station M11.

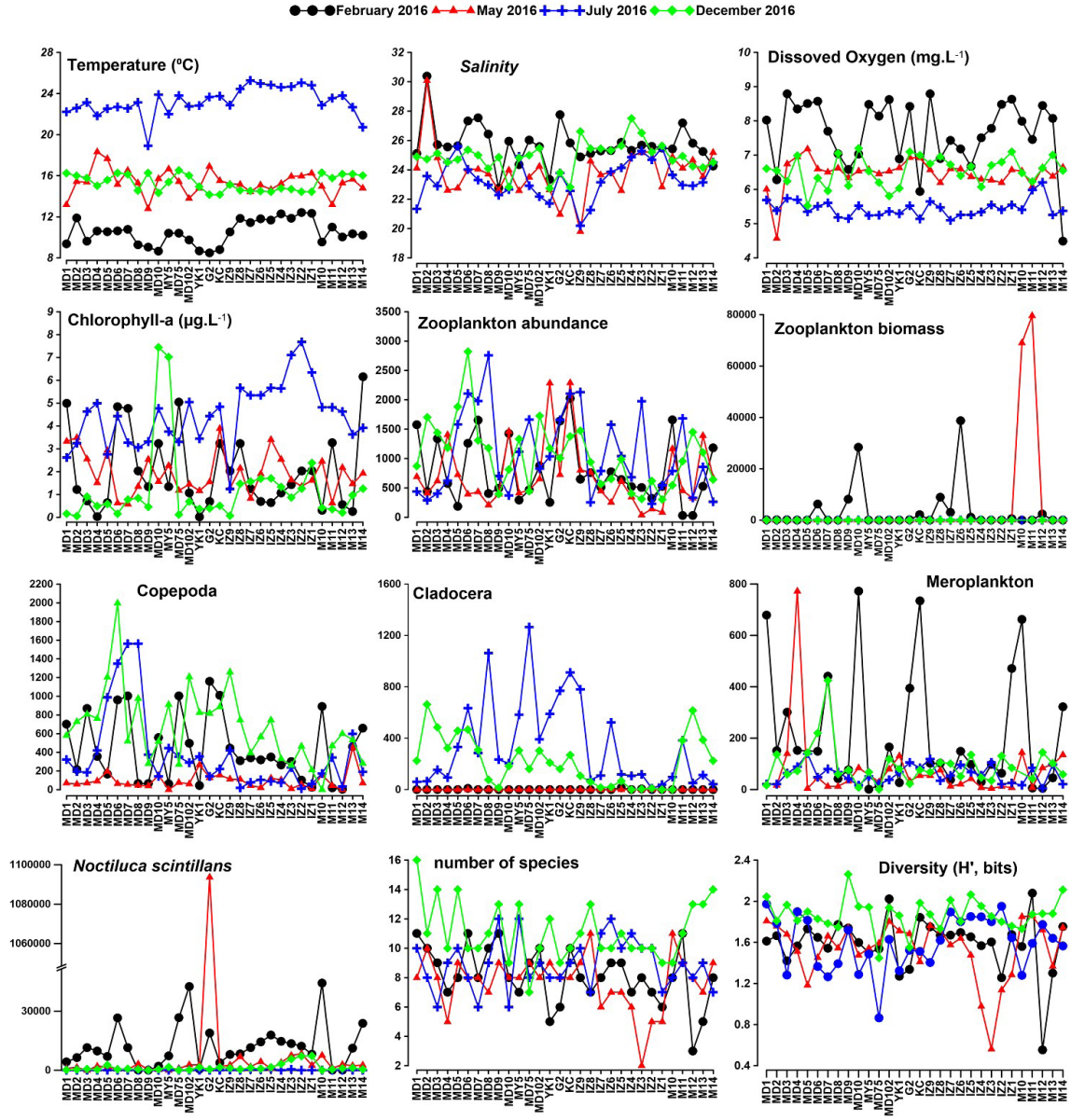

Figure 3. Seasonal fluctuation of temperature, salinity, dissolved oxygen, and chlorophyll-a at the upper layer.

Table 1. The total and seasonal mean abundances (ind.m−3) of zooplankton species/groups and Noctiluca scintillans in the eastern Sea of Marmara.

	February 2016		May 2016		July 2016		December 2016		Total mean abundance in 2016	
	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower
COPEPODA										
Acartia clausi Giesbrecht. 1889	2624.94	285.84	2133.62	166.81	7112.53	745.82	4147.87	239.37	4000.474	359,46
Aetideus spp.	0	0	0	5.27	0	5.27	0	0	0	2.635
Calanus euxinus Hulsemann. 1991	67.01	30.45	0	0	0	0	12.74	2.62	19.937	8.267
Centropages ponticus Karavaev. 1895	15.71	9.26	0	0	0	0	0	0	3.927	2.315
Centropages typicus Kröyer. 1849	51.97	0	11.89	0	8.49	4.37	33.97	7.42	26.58	2.947
Clausocalanus spp.	0	4.25	0	0.31	0	0.24	0	0.24	0	1.26
Ctenocalanus vanus Giesbrecht. 1888	0	12.74	0	5.27	0	5.27	0	12.74	0	9.005
Euterpina acutifrons (Dana. 1847)	82.31	69.78	17.83	8.49	21.23	5.27	433.12	42.48	138.622	31.505
Euchaeta marina (Prestandrea. 1833)	0	2.62	0	0	0	5.27	0	8.49	0	4.095
Metridia lucens Boeck. 1864	0	0	0	0	0	0	29.72	0	7.430	0
Microcalanus pygmaeus (Sars G.O. 1900)	2.62	8.49	0	0	0	0	2.62	4.37	1.310	3.215
Oithona davisae Ferrari & Orsi. 1984	0	0	0	0	0	0	65.19	0	16.297	0
Oithona nana Giesbrecht. 1893	1616.31	211.67	5696.53	1016.85	2005.66	617.09	2057.70	232.40	2844.050	519.502
Oithona similis Claus. 1866	146.07	37.77	325.27	39.28	430.29	95.66	2466.59	509.71	842.055	170.605
Oithona spp.	0	0	0	0	0	2.62	0	0	0.655	0
Oncaea minuta Giesbrecht. 1893	0	17.83	0	25.48	0	17.83	0	25.48	0	21.655
Oncaea spp.	0	2.62	0	0	0	0	0	5.27	0	1.972
Paracalanus parvus (Claus. 1863)	5945.53	550.47	1926.11	308.59	3841.47	652.69	6904.46	245.27	4654.39	439.255
Pseudocalanus elongatus (Boeck. 1865)	0	0	25.48	0	12.74	0	35.47	0	18.422	0
Temora stylifera (Dana. 1849)	0	0	12.74	0	2.62	0	2.62	0	4.495	0
Copepoda nauplii	89.51	27.55	50.96	15.79	56.62	14.76	83.68	13.25	70.192	17.837
CLADOCERA										
Penilia avirostris Dana. 1852	0	0	0	8.49	8980.89	1530.89	6326.71	258.35	3826.90	449.432
Pleopis polyphemoides (Leuckart. 1859)	901.40	63.09	4206.37	15.41	227.88	3.73	80.18	0	1353.957	20.557
Pseudevadne nordmanni Lovén. 1836	8.49	0.24	0	0	12.74	0	0	0	5.307	0.060
APPENDICULARIA										
Oikopleura dioica Fol. 1872	875.30	175.12	976.93	33.20	635.53	135.29	1129.51	85.28	904.317	107.222
CHAETOGNATHA										
Sagitta setosa (Müller. 1847)	74.73	21.91	0	0	0	99.86	167.27	69.52	60.5	47.822

Table 1. Continue.

	February 2016		May 2016		July 2016		December 2016		Total mean abundance in 2016	
	Upper	**Lower**	**Upper**	**Lower**	**Upper**	**Lower**	**Upper**	**Lower**	**Upper**	**Lower**
MEROPLANKTON										
Fish larvae	136.83	8.66	4.25	0	0	0	0	0	35.27	2.165
Fish egg	1524.44	17.66	619.96	13.87	97.86	45.55	31.75	6.55	568.502	20.907
Bivalvia larvae	2505.19	486.75	615.71	82.29	703.47	55.04	1694.80	217.01	1379.792	210.272
Decapoda larvae	2.14	0.20	17.24	6.09	139.79	55.04	7.79	22.21	41.74	20.885
Cirripedia larvae	21.23	0	0	0	0	0	0	0	5.307	0
Polychaeta larvae	230.29	35.41	457.44	28.77	349.61	18.04	256.53	70.22	323.467	38.11
Isopoda larvae	0	0	0	0	0	0	0	0.17	0	0.042
Gastropoda larvae	4.25	0	0	0	0	0	0	0	1.062	0
Reptantia (Brachyura) larvae	2.62	0	0	0	0	0	2.62	0	1.31	0
Pteropoda	0	0	2.62	0	2.62	0	0	0	1.31	0
OTHER GROUPS AND SPECIES										
Aglaura hemistoma Péron & Lesueur. 1810	0	0	0	0.68	0	0	4.76	0.34	1.19	0.255
Aurelia aurita (Linné. 1758)	4.62	3.52	1395.61	2.67	108.85	1.70	991.63	3.40	625.177	2.822
Beroe ovata Chamisso ve Eysenhardt. 1821	14.95	0.59	0.36	4.53	0	0	0.51	0	3.955	1.28
Mnemiopsis leidyi A. Agassiz. 1865	0.25	0	0	0	0	0	0	0	0.062	0
Pleurobrachia pileus (O.F.Müller. 1776)	26.77	7.42	24.29	5.34	0.51	0	0	0	12.892	3.19
Siphonophora (sp.)	0	0.15	0	0	0	0	0	0	0	0.037
Total zooplankton	242339.5	2092.060	51284.98	1793.48	25034.49	4061.96	42510.21	2082.16	89687.463	2978,7395
Noctiluca scintillans (Macartney. Kofoid et Swezy. 1921)	225358.08	0	32763.77	0	283.09	0	15540.40	0	68486.335	0

The abundance of most zooplankton groups displayed distinct seasonal patterns in the eastern Sea of Marmara. In general, copepods and cladocerans were the predominant groups (Table 1), whereas meroplankton became more prominent in coastal regions, eventually dominating the zooplankton community (e.g., stations MD10 and KC in February) (Figure 3). *Acartia clausi* was present year-round, reaching higher densities at station MD7 (913 ind.m^{-3}) and İZ9 (904 ind.m^{-3}) in July, whereas *P. parvus*, *E. acutifrons*, and *C. euxinus* were the primary members of the copepod community during February and December (Table 1, Figure 4). *Oithona nana, Pleopis polyphemoides*, and fine particle filter feeder, *Oikopleura dioica*, dominated the zooplankton community in all seasons, and *Oithona similis* and other fine particle filter feeder *Penilia avirostris* were abundant in July and December. High concentrations of bivalve and polychaeta larvae were measured in February and May, especially in coastal areas, whereas *Parasagitta setosa* was detected in February, July, and December. *Oithona davisae* was observed only in December and mostly at coastal stations (KC, YK1, MD1, MD3, MD5, M12, M13, M14, MY5, 1Z8, IZ7, IZ5, IZ4, IZ3, IZ1).

The highest number of species was observed at MD1 (16 species/groups) in December and the lowest number at İZ3 (3 species/groups) in May and at M12 (3 species/groups) in February. The maximum diversity index was recorded at station MD9 (2.3) in December. The minimum diversity index was 0.6 at station M12 in February and at station İZ3 in May. This decrease in diversity was due to dominance of *P. polyphemoides* and *A. clausi* (Figure 3).

The heterotrophic dinoflagellate *Noctiluca scintillans*, an important component of the net samples in the Sea of Marmara, showed sea-

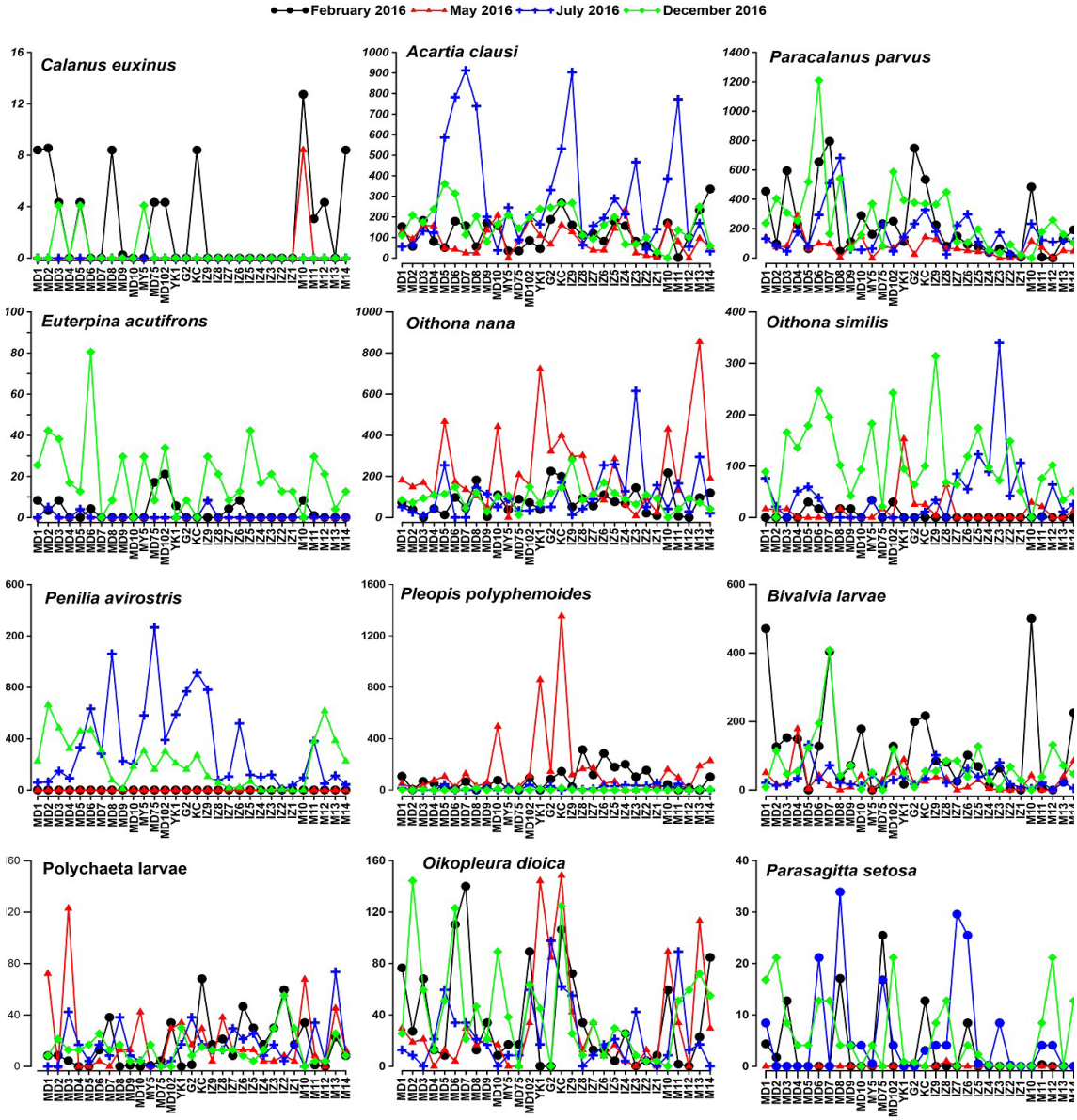

Figure 4. Variations in the abundance (ind.m−3) of primary zooplankton species and groups within the upper layer.

sonal distribution (Figure 2), peaking in February (225358 ind.m^{-3}) and reaching its minimum in July (283 ind.m^{-3}). *N. scintillans* reached its maximum abundance (1093617 ind.m^{-3}) at station G2, located at the entrance of Büyükçekmece Bay. The densities of *N. scintillans* fell to minimum levels in July but increased in December. MDS ordination of combined data across seasons showed that the zooplankton community of the eastern Sea of Marmara had high seasonality (Figure 5). Total zooplankton assemblage exhibited heterogeneity from July to December (Group I) and February to May (Group II). The species predominant from July to December included *Acartia clausi, Paracalanus parvus*, *Penilia avirostris,* and *Oithona similis* (Group I); those predominant from February to May community included *Calanus euxinus, Pseudocalanus elongates, Oithona nana, Oithona davisae, Pleopis polyphemoides*, polychaeta, and bivalve larvae. Moreover, stations M11 and M12 in February and IZ3 in May (Group III) were distinct from other samplings due to the dominance of *Pleopis polyphemoides, Oithona nana*, and *Acartia clausi* as well as low species diversity.

While *P. avirostris* was positively affected by temperature increase ($r = 0.510$, $p < 0.01$), it was negatively correlated with salinity ($r = -0.282$, $p < 0.01$) and dissolved oxygen ($r = -0.400$, $p < 0.01$). Bivalvia larvae were positively affected by salinity ($r = 0.247$, $p < 0.01$) and dissolved oxygen ($r = 0.196$, $p < 0.05$) but inversely related to temperature increase ($r = -0.305$, $p < 0.01$). *A. clausi* was positively affected by temperature ($r = 0.381$, $p < 0.05$) and negatively correlated with increased salinity ($r = -0.214$, $p < 0.05$) and dissolved oxygen ($r = -0.284$, $p < 0.01$).

Zooplankton abundance, *P. parvus,* and *P. setosa* positively correlated with temperature ($r = 0.186$, $r = 0.194$, and $r = 0.186$, $p < 0.05$, respectively), whereas polychaeta larvae ($r = -0.295$, $p < 0.05$) and *C. euxinus* ($r = -0.370$, $p < 0.01$) had a negative correlation with it. *E. acutifrons* ($r = 0.134$, $p < 0.05$) and *O. nana* ($r = 0.216$, $p < 0.05$) had a positive correlation with dissolved oxygen, whereas *N. scintillans* ($r = -0.201$, $p < 0.05$) and *P. polyphemoides* ($r = -0.244$, $p < 0.01$) were inversely related to dissolved oxygen increase. Furthermore, jellyfish abundance was weakly correlated with total zooplankton abundance, *O. similis, P. avirostris*, and *P. setosa*, ($r = -0.107$, $r = -0.112$, $r = -0.123$, $r = -0.103$ $p < 0.001$, respectively) but it was positively correlated with *P. parvus* ($r = 0.151$, $p < 0.001$) and *O. dioica* ($r = 0.181$, $p < 0.05$).

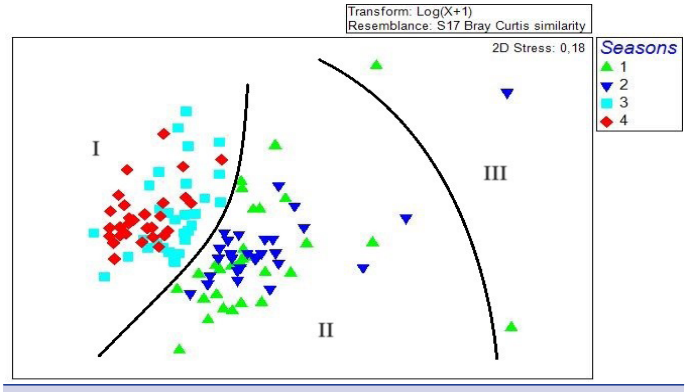

Figure 5. MDS analyses by seasons (1: February. 2: May. 3: July. 4: December)

The upper layer waters generally exhibited a greater zooplankton abundance than the lower layer waters but a lower number of species (Table 1, Figure 6, 7). The maximum average abundance in the upper layer was 2822 – 29 ind. m^{-3}, whereas the corresponding value in the lower layer was 462 – 6 ind. m^{-3}. The number of species and zooplankton abundance significantly differed between groups; zooplankton were more abundant in the upper layer than in the lower layer. The biggest difference between the lower and upper layers was measured in July and December 2016 when Cladocera dominated the upper layer of Marmara. Species such as *Aetideus* spp., *Clausocalanus* spp., *Ctenocalanus vanus, Oncaea minuta, Oithona davisae,* Pteropoda, and Siphonophora contribute to the differences in zooplankton communities between the upper and lower layers. The corresponding MDS analysis revealed significant differences between the lower and upper layers (Figure 8).

This study identified the major zooplankton species and their seasonal availability in the Marmara Sea. The most widely distributed zooplankton species were also the most prevalent and dominant species such as *Paracalanus parvus, Acartia clausi, Oithona nana, Penilia avirostris, Pleopis polyphemoides*, and bivalve larvae. These species are similar to those found in previous studies in the Marmara Sea (Yılmaz et al., 2005; Tarkan et al., 2005; Isinibilir et al., 2011). Notably, *O. davisae,* an invasive species that was first recorded in the Marmara Sea in 2014 (Doğan & Isinibilir, 2016), has expanded its distribution area to the Izmit Bay in the Sea of Marmara. The Sea of Marmara is home to several Mediterranean species, including *Oncaea minuta, Aetideus spp., Ctenocalanus vanus, Clausocalanus spp., Euchaeta marina*, and Siphanophore (Isinibilir et al., 2011).

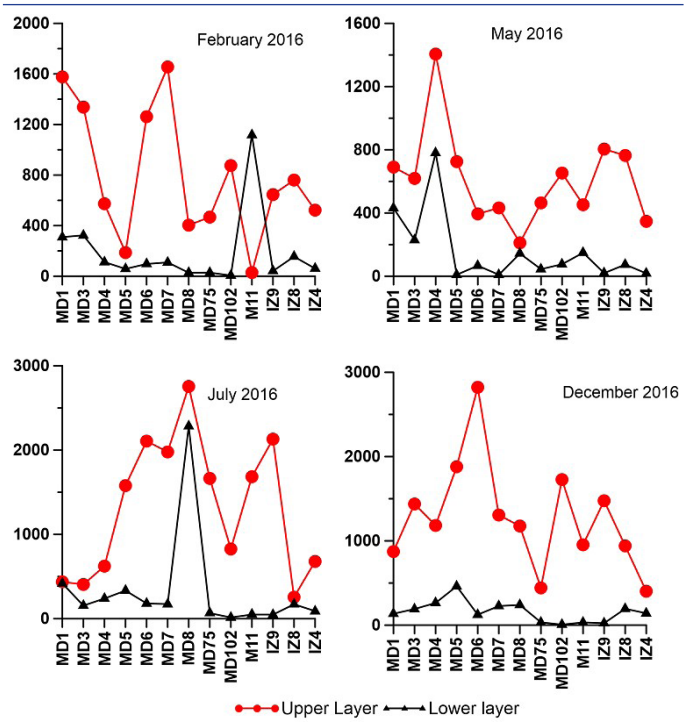

Figure 6. Seasonal distribution of zooplankton abundance (ind.m^{-3}) in the upper and lower layers at various sampling stations.

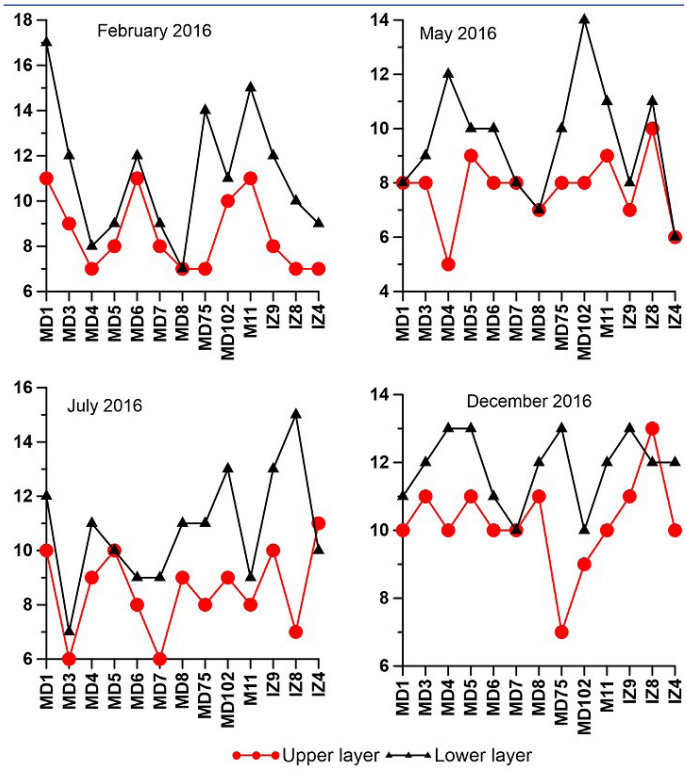

Figure 7. The variation in the number of species in the lower and upper layers at the sampling stations.

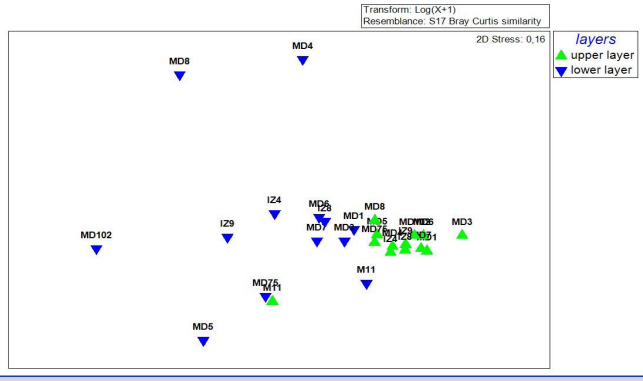

Figure 8. MDS ordination of combined data of upper- and lower layer samples.

In the Eastern Marmara Sea, zooplankton are more abundant in the upper layer than in the lower, which agrees with previous studies (Tarkan et al., 2005; Isinibilir et al., 2008, 2011). Strong stratification in the basin, depending on temperature and salinity, limits the daily vertical migration pattern of zooplankton (Isinibilir et al., 2011). Accordingly, species compositions differ between layers, and the main zooplankton biomass is concentrated in the upper layer (Mutlu, 2005; Isinibilir Okyar, et al., 2015). Thus, this study further corroborates that the top layer has larger values in terms of biomass and abundance than the lower layer. Oithona is a widespread genus in eutrophic and degraded unstable environments due to its resilience (Richard & Jamet, 2001; Castellani et al., 2005; Isinibilir et al., 2016; Svetlichny et al., 2018). In particular, *O. davisae* and *O. nana* are classified as polluted area species (Drira et al., 2017; Isinibilir et al., 2008, 2016). *O. davisae*, a cyclopoid copepod originating from the western Pacific Ocean (Hirakawa, 1988), was first recorded in the Black Sea in the Sevastopol Bay in December 2001 (Zagorodnyaya 2002). Over the last decades, this species gradually spread to the entire eastern and western coasts of the Black Sea (Altukhov et al., 2014; Gubanova & Altukhov, 2007; Mihneva & Stefanova, 2013), the Sea of Marmara (Doğan & Isinibilir, 2016; Isinibilir et al., 2016) and the Aegean Sea (Terbıyık-Kurt & Beşiktepe, 2019). Due to its thermophilic nature (Svetlichny et al., 2016), this species is most abundant during the warm seasons and least abundant during winter and early spring (Altukhov et al., 2014; Mihneva & Stefanova, 2013; Uye & Sano, 1998; Zagami et al., 2018). However, variations in the population density of *O. davisae* showed differences across regions (Ambler et al., 1985; Uye and Sano, 1995; Svetlichny et al., 2018). In this study, *O. davisae* was distributed in eutrophic coastal areas and less abundant only in December. As *O. nana* is a tolerant, opportunistic, and widely adapted species, this species can be found in sea ports and near urban wastes and/or brackish waters with varying degrees of pollution (Richard and Jamet, 2001; Beşiktepe et al., 2023). As in previous studies on the Sea of Marmara (Isinibilir et al., 2008, 2016; Isinibilir Okyar et al., 2015), this work showed that *O. nana* is the predominant zooplankton species year-round, especially in severely perturbed coastal areas.

A. clausi, widely distributed in temperate waters and dominates zooplankton in polluted areas, is also the most prevalent species in the Sea of Marmara year-round (Gubanova et al., 2001; Isinibilir et al., 2008; 2011; Svetlichny et al., 2022, Beşiktepe et al., 2023). This study supports previous findings that *A. clausi* predominates in coastal areas, reflecting the eutrophic characteristics of this sea. On the other hand, *O. similis* and *P. parvus*, the other dominant copepod species in the Marmara Sea (Isinibilir et al., 2008; Isinibilir, 2009; 2010), are nonsensitive species that can live in both polluted and unpolluted marine environments (Drira et al., 2017).

Environmental factors such as temperature, salinity, predation, food availability, and water transparency can affect the Cladocera population (Calbet et al., 2001; Marques et al., 2006; Atienza et al., 2008; Isinibilir et al., 2011). At different spatiotemporal scales, the dynamics of small-sized cladoceran abundance are influenced by predatory forces from planktivorous fish and invertebrates (Onbe & Ikeda 1995, Egloff et al., 1997; Camatti et al, 2008). In this study, the increase in *P. avirostris* abundance was triggered by water temperature, whereas salinity caused the reverse effect. In addition to being an important food source for marine pelagic fish, *P. avirostris* plays an important role in the zooplankton community due to its high abundance in tropical and temperate waters, especially during the summer (Calbet et al., 2001; Marazzo & Valentin, 2003; Rose et al., 2004). As the body length of *P. avirostris* varies between 0.70 and 1.09 mm (Zhou et al., 2022), this species may be important for fisheries by attracting many larvae and adult pelagic fish such as mackerel, sardine, horse mackerel, and anchovy (Wu et al., 2023). *Penilia avirostris* exhibits a broad dietary spectrum, encompassing small diatoms and bacterivorous microflagellates (Turner et al., 1988) as well as prymnesiophyceans (Paffenhofer & Orcutt, 1986) and

bacteria (Lipej et al., 1997). The contributions of predation to the formation of marine zooplankton ecosystems have previously been disregarded (Verity & Smetacek, 1996). However, fish larvae, chaetognaths, and ctenophores are among the predators that might wipe out marine cladoceran populations (Duró & Saiz, 2000; Barz &Hirche, 2005). Nevertheless, how predation affects *P. avirostris* population dynamics in the Sea of Marmara is unclear. Other important cladoceran species like *Pleopis polyphemoides*, the most euryhaline species (Viñas et al., 2007), transpired throughout the winter months after going dormant during the warmer ones, as in previous studies (Isinibilir et al., 2008; Isinibilir, 2009; Isinibilir Okyar et al., 2015).

In the Sea of Marmara, *N. scintillans* exhibits a year-round distribution, with the greatest abundance in the spring and a secondary increase in the autumn (November) (Isinibilir et al., 2008; Isinibilir 2009). This species competes with zooplankton for food, and its high tolerance for temperature and salinity, coupled with its feeding on zooplankton eggs, promote its ecological success (Schaumann et al., 1988; Kirchner et al., 1996; Elbrachter & Qi, 1998; Quevedo et al., 1999). Thus, it is crucial to monitor the distribution and abundance of this species in the Sea of Marmara.

CONCLUSION

In conclusion, the zooplankton communities in the Marmara Sea exhibited notable seasonal fluctuations in abundance and species diversity. Given the correlations between seasonal changes in dominant species and environmental factors such as temperature, salinity, and chlorophyll-a, zooplankton can serve as a significant indicator of changes in this marine environment. The Sea of Marmara has undergone significant changes recently due to rising temperatures and increased industrial pollution, which negatively influence zooplankton ecosystems. Extensive research conducted at the basin level is still required to elucidate the evolution and changes of this ecosystem that is vulnerable to human impacts and climate change.

Acknowledgments: We extend our sincere appreciation to the captains and crews of the R/V YUNUS-S for their invaluable support and collaboration during the research cruise conducted in the eastern Sea of Marmara in 2016. We also express our gratitude to all academic participants involved in the expedition. Special acknowledgment is owed to Onur Doğan and Esin Yüksel Durmaz for their assistance in the field and laboratory.

Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethics Committee Approval: The authors affirm that ethical approval is unnecessary for this study.

Financial Disclosure: This work was supported by the Scientific Research Projects Coordination Unit of Istanbul University with the project number 24406.

REFERENCES

Aktan, Y. (2008). Mucilage event associated with diatoms and dinoflagellates in Sea of Marmara, Turkey. *Harmful Algae News*, 36, 1-3.

Altukhov, D.A., Gubanova, A.D., & Mukhanov, V.S. (2014). New invasive copepod *Oithona davisae* Ferrari and Orsi, 1984: seasonal dynamics in Sevastopol Bay and expansion along the Black Sea coasts. *Marine Ecology*, 35, 28-34.

Ambler, J. W., Cloern, J. E., & Hutchinson, A. (1985). Seasonal cycles of zooplankton from San Francisco Bay. *Temporal Dynamics of an Estuary*, 177-197.

APHA. (2000). Standard Methods for the Examination of Water and Wastewater 20th Edition. Clesceri L.S., Greenberg A.E. and Eaton A.D. (eds). American Public Health Association. American Water Works Association and Water Environment Federation. Washington. D.C.

Atienza, D., Saiz, E., Skovgaard, A., Trepat, I., & Calbet, A. (2008). Life history and population dynamics of the marine cladoceran *Penilia avirostris* (Branchiopoda: Cladocera) in the Catalan Sea (NW Mediterranean). *Journal of Plankton Research* 30, 345-357.

Barz, K. & Hirche, H. (2005). Seasonal development of scyphozoan medusa and the predatory impact of *Aurelia aurita* on the zooplankton community in the Bornholm Basin (central Baltic Sea). *Marine Biology*, 147, 465–476.

Beşiktepe, Ş. T., Sur, H. I., Özsoy, E., Latif, M. A., Oğuz, T., & Ünlüata, Ü. (1994). The circulation and hydrography of the Marmara Sea. Progress in Oceanography, 34(4), 285-334.

Besiktepe, S., Kucuksezgin, F., Besiktepe, S.T., Eronat, C., Gonul, T., Kurt, T.T., Sayın, E., Gubanova, A. (2023). Variations in copepod composition and diversity in relation to eutrophication and hydrology in İzmir Bay, Aegean Sea. *Marine Pollution Bulletin* 197, 115745.

Calbet, A., Garrido, S., Saiz, E., Alcaraz, M., & Duarte, M. (2001). Annual zooplankton succession in coastal NW Mediterranean waters: the importance of the smaller size fractions. *Journal of Plankton Research*, 23, 319–331.

Camatti, E., Comaschi, A., De Olazabal, A., & Fonda Umani, S. (2008). Annual dynamics of the mesozooplankton communities in a highly variable ecosystem (North Adriatic Sea, Italy). *Marine Ecology*, 29, 387-398.

Castellani, C., Robinson, C., Smith, T., & Lampitt, R. S., (2005). Temperature affects respiration rate of *Oithona similis*. *Marine Ecology Progress Series*, 285, 129-135.

Clarke KR, Warwick RM (1994). Changes in Marine Communities: An Approach to Statistical Analysis and Interpretation. Plymouth, UK: PRIMER-E Ltd.

Dogan, G., & Isinibilir, M. (2016). First Report of a New Invasive Species *Oithona davisae* Ferrari and Orsi, 1984 (Copepoda: Cyclopoida) in the Sea of Marmara. *Turkish Journal of Fisheries and Aquatic Sciences*, 16, 471-475.

Drira, Z., Kmiha-Megdiche, S., Sahnoun, H., Pagano, M., Tedetti, M., & Ayadi, H. (2017). Water quality affects the structure of copepod assemblages along the Sfax southern coast (Tunisia, southern Mediterranean Sea). *Marine and Freshwater Research* ,69, 220-231.

Duró, A., & Saiz, E. (2000). Distribution and trophic ecology of chaetognaths in the western Mediterranean in relation to an inshore-offshore gradient. *Journal of Plankton Research*, 22, 339–361.

Egloff, D. A., Fofonoff, P. W., Onbé, T. (1997). Reproductive biology of marine cladocerans. *Advances in Marine Biology*, 31, 79–168.

Elbrächter, M., & Qi, Z. (1998). Aspects of Noctiluca (Dinophyceae) population dynamics. In Physiological ecology of harmful algal blooms (DM Anderson, AD Cembella, GM Hallegraeff, eds) *Springer*, Berlin, Heidelberg 315-335.

Griffith, A.W., Gobler, C.J. (2020). Harmful algal blooms: a climate change co-stressor in marine and freshwater ecosystems. *Harmful Algae* 91, 101590.

Gubanova, A.D., Prusova, I.Yu., Niermann, U., Shadrin, N.V., & Polikarpov, I.G., (2001). Dramatic change in the copepod community in Sevastopol Bay (Black Sea) during two decades (1976–1996). *Senckenbergiana Maritima*, 31, 17–27.

Gubanova, A., & Altukhov, D. (2007). Establishment of *Oithona brevicornis* Giesbrecht, 1892 (Copepoda: Cyclopoida) in the Black Sea. *Aquatic Invasions*, 4, 407–410.

Hirakawa, K. 1988. New records of the North Pacific coastal planktonic copepods, *Acartia omorii* (Acartiidae) and *Oithona davisae* (Oithonidae) from southern Chile. *Bulletin of Marine Science*, 42, 337-339.

Isinibilir, M., Kideys, A.E., Tarkan, A.N., & Yilmaz, I.N. (2008). Annual cycle of zooplankton abundance and species composition in Izmit Bay (the northeastern Marmara Sea). Estuarine *Coastal and Shelf Science*, 78, 739-747.

Isinibilir, M. (2009). Annual Crustacean Zooplankton Succession (Copepoda and Cladocera) in the Upper Layer of Ahirkapi Coastal Waters (Northeastern Sea of Marmara). *Crustaceana*, 82, 669-678

Isinibilir, M., Yilmaz, I.N., &Piraino, S. (2010). New contributions to the jellyfish fauna of the Marmara Sea. *Italian Journal of Zoology*, 77, 179-185.

Isinibilir, M., Svetlichny, L., Hubareva, E., Yilmaz, I.N., Ustun, F., Belmonte, G., & Toklu-Alicli, B. (2011). Adaptability and vulnerability of zooplankton species in the adjacent regions of the Black and Marmara Seas. *Journal of Marine Systems*, 84, 18-27.

Isinibilir Okyar, M., Üstün, F., & Orun, D.A. (2015). Changes in abundance and community structure of zooplankton population during the 2008 mucilage event in the northeastern Marmara Sea. *Turkish Journal of Zoology*, 39, 28-38.

Isinibilir, M., Svetlichny, L., & Hubareva, E. (2016). Competitive advantage of the invasive copepod *Oithona davisae* over the indigenous copepod *Oithona nana* in the Marmara Sea and Golden Horn Estuary. *Marine and Freshwater Behaviour and Physiology*, 49, 391-405.

Isinibilir, M., & Yılmaz, İ. N. (2017). Jellyfish dynamics and their socioeconomic and ecological consequences in Turkish Seas. Jellyfish: Ecology, distribution patterns and human interactions, 51, 70.

Isinibilir, M., Svetlichny, L., Türkeri, E.E., Topçu, N. E., & Kideys, A.E. (In Press). Changes in zooplankton of the eastern Sea of Marmara, In: Ecological Changes in the Sea of Marmara, ed: Isinibilir M., Kideys, A.E., Malej, A. IUPress.

Kemp, W. M., & Boynton, W. R. (2012). Synthesis in estuarine and coastal ecological research: What is it, why is it important, and how do we teach it? *Estuary and Coasts*, 35, 1–22.

Kirchner, M., Sahling, G., Uhlig, G., Gunkel, W., & Klings, K. W. (1996). Does the red tide-forming dinoflagellate Noctiluca scintillans feed on bacteria? *Sarsia*, 81(1), 45-55.

Lipej, L., Mozetič, P., Turk, V., & Malej, A. (1997). The trophic role of the marine cladoceran *Penilia avirostris* in the Gulf of Trieste. *Hydrobiologia*, 360, 197–203.

Marazzo, A., & Valentin, J. L. (2003). *Penilia avirostris* (Crustacea, ctenopoda) in a tropical bay: variations in density and aspects of reproduction. *Acta Oecologica*, 24, 251–257.

Marques, S.C., Azeiteiro, U.M., Marques, J.C., Neto, J.M., & Pardal, M.A. (2006). Zooplankton and ichthyoplankton communities in a temperate estuary: spatial and temporal patterns. *Journal of Plankton Research*, 28:297– 312.

Mihneva, V., & Stefanova, K. (2013). The non-native copepod Oithona davisae (Ferrari FD and Orsi, 1984) in the Western Black Sea: seasonal and annual abundance variability. *BioInvasions Record*, 2(2).

Mutlu, E., (2005). A comparison of the contribution of zooplankton and nekton taxa to the near-surface acoustic structure of three Turkish seas. *Marine Ecology*, 26, 17–32.

Onbe´ T., & Ikeda T. (1995). Marine cladocerans in Toyama Bay, southern Japan Sea: seasonal occurrence and day-night vertical distributions. *Journal of Plankton Research*, 17(3), 595–609.

Paffenhöfer, G.-A., & Orcutt Jr, J.D. (1986) Feeding, growth and food conversion of the marine cladoceran *Penilia avirostris*. *Journal of Plankton Research*, 8, 741-754.

Quevedo, M., Gonzalez-Quiros, R., & Anadon, R. (1999). Evidence of heavy predation by Noctiluca scintillans on Acartia clausi (Copepoda) eggs off the central Cantabrian coast (NW Spain). *Oceanologica Acta*, 22(1), 127-131.

Richard, S., & Jamet, J.-L. (2001). An unusual distribution of *Oithona nana* Giesbrecht (1892)(Crustacea: Cyclopoida) in a bay: the case of Toulon Bay (France, Mediterranean Sea). *Journal of Coastal Research*, 957-963.

Rissik, D., Shon, E. H., Newell, B., Baird, M. E., & Suthers, I. M. (2009). Plankton dynamics due to rainfall, eutrophication, dilution, grazing and assimilation in an urbanized coastal lagoon. *Estuarine Coastal and Shelf Science*, 84, 99–107.

Rose, K., Roff, J. C., & Hopcrof, R. R. (2004). Production of *Penilia avirostris* in Kingston harbour, Jamaica. *Journal of Plankton Research*, 26 (3), 1–11.

Schaumann, K., Gerdes, D., & Hesse, K. (1988). Hydrographic and biological characteristics of a Noctiluca scintillans red tide in the German Bight, 1984. *Meeresforschung*, 32, 77-91. Shannon, C.E., & Weaver, W., (1949). The Mathematical Theory of Communication. University of Illinois Press, Urbana, 125 pp.

Shi, Y. Q., Sun, S., Zhang, G. T., Wang, S. W., & Li, C. L. (2015). Distribution pattern of zooplankton functional groups in the Yellow Sea in June: A possible cause for geographical separation of giant jellyfish species. *Hydrobiologia*, 754 (1), 43–58.

Svetlichny, L., Hubareva, E., Khanaychenko, A., Gubanova, A., Altukhov, D., & Besiktepe, S. (2016). Adaptive strategy of thermophilic *Oithona davisae* in the cold Black Sea environment. *Turkish Journal of Fisheries and Aquatic Sciences*, 16, 77–90.

Svetlichny, L., Hubareva, E., & Isinibilir, M. (2018). Population dynamics of the copepod invader *Oithona davisae* in the Black Sea. *Turkish Journal of Zoology*, 42, 684-693.

Svetlichny, L., Lehtiniemi, M., Setälä, O., Lehto, A., Samchyshyna, L., Gromova, Y., Isinibilir Okyar, M., Türkeri, E.E., Eryalçın, K.M., Kayadelen, G.C. & Kıdeyş, A.E. (2022). Oxygen consumption rates and respiratory carbon losses in three species of copepods (*Acartia clausi*, *Calanus helgolandicus* and *Limnocalanus macrurus*) during starvation. *Turkish Journal of Zoology,* 46, 249-260.

Tarkan, A.N., Isinibilir, M., & Tarkan, A.S. (2005). Seasonal variations of the zooplankton composition and abundance in the Istanbul Strait. Pakistan *Journal of Biological Sciences*, 8 (9). 1327–1336.

Terbıyık Kurt, T., & Beşiktepe, Ş. (2019). First distribution record of the invasive copepod *Oithona davisae* Ferrari and Orsi, 1984, in the coastal waters of the Aegean Sea. *Marine Ecology*, 40, e12548.

Turkoglu, M. (2013). Red tides of the dinoflagellate *Noctiluca scintillans* associated with eutrophication in the Sea of Marmara (the Dardanelles, Turkey). *Oceanologia*, 55, 709-732.

Turner, J.T., Tester, P.A., & Ferguson, R.L. (1988). The marine cladoceran *Penilia avirostris* and the "microbial loop" of pelagic food webs 1. *Limnology and Oceanography,* 33, 245-255.

Tüfekçi, V., Balkis, N., Beken, Ç.P., Ediger, D., Mantikci, M. (2010) Phytoplankton composition and environmental conditions of the mucilage event in the Sea of Marmara. *Turkish Journal of Biology*, 34, 199-210.

Uye, S., & Sano, K. (1995). Seasonal reproductive biology of the small cyclopoid copepod *Oithona davisae* in a temperate eutrophic inlet. *Marine Ecology Progress Series*, 118, 121–128.

Uye, S.-I., & Sano, K. (1998). Seasonal variations in biomass, growth rate and production rate of the small cyclopoid copepod *Oithona davisae* in a temperate eutrophic inlet. *Marine Ecology Progress Series*, 163, 37-44.

Verity, P. G. & Smetacek, V. (1996). Organism life cycles, predation, and the structure of marine pelagic ecosystems. *Marine Ecology Progress Series*, 130, 277–293.

Viñas, M. D., Ramírez, F. C., Santos, B. A., & Marrari, M. (2007). Spatial and temporal distribution patterns of Cladocera in the Argentine Sea. *Hydrobiologia*, 594, 59-68.

Wu, F.-X., Gu, Y.-G., Liu, Q.-X., Zhang, S.-F., Rao, Y.-Y., Liu, H.-X., Dai, M., Wang, Y.-G. & Huang, H.-H. (2023). Research on the seasonal variation of zooplankton community in Daya Bay, South China Sea. *Frontiers in Marine Science*,10, 1110160.

Yilmaz, İ.N., Okus, E., & Yüksek, A. (2005) Evidences for influence of a heterotrophic dinoflagellate (*Noctiluca scintillans*) on zooplankton community structure in a highly stratified basin. Estuarine, *Coastal and Shelf Science*, 64, 475-485.

Zagami, G., Brugnano, C., Granata, A., Guglielmo, L., Minutoli, R., & Aloise, A. (2018). Biogeographical distribution and ecology of the planktonic copepod *Oithona davisae*: Rapid invasion in Lakes Faro and Ganzirri (Central Mediterranean Sea). Trends in Copepod Studies-Distribution, *Biology and Ecology*, 59-82.

Zagorodnyaya, Y.A. (2002). *Oithona brevicornis* in the Sevastopol Bay: is it a single event or a new invader in the Black Sea fauna? *Ecol Morya*, 61-43.

Zhou, X. N., Zhao, W., & Yin, D. P. (2022). Research progress of ecological distribution and cultivation utilization in cladoceran *Penilia avirostris*: A review. J. Dalian Ocean University. 37 (2), 345–351.

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EISSN 2602-473X

AQUATIC SCIENCES AND ENGINEERING

Aquat Sci Eng 2024; 39(3): 148-155 • DOI: https://doi.org/10.26650/ASE20241424227

Research Article

Post-Mucilage Distribution, Daily Growth, Mortality, and Hatch Date Timing of Sand steenbras *Lithognathus mormyrus* (Linnaeus, 1758) Juveniles in the Sea of Marmara

İsmail Burak Daban[1], Yusuf Şen[1], Alkan Öztekin[1], Adnan Ayaz[1], Uğur Altınağaç[1], Ali İşmen[1], Ahsen Yüksek[2], Uğur Özekinci[1], Fikret Çakır[1], Tekin Demirkıran[3], Gençtan Erman Uğur[3], Oğuzhan Ayaz[3], Buminhan Burkay Selçuk[3]

Cite this article as: Daban, İ.B., Şen, Y., Öztekin, A., Ayaz, A., Altınağaç, U., İşmen, A., Yüksek, A., Özekinci, U., Çakır, F., Demirkıran, T., Uğur, G.E., Ayaz, O. & Selçuk, B.B. (2024). Post-mucilage distribution, daily growth, mortality, and hatch date timing of sand steenbras *Lithognathus mormyrus* (Linnaeus, 1758) juveniles in the Sea of Marmara. *Aquatic Sciences and Engineering, 39(3)*, 148-155. DOI: https://doi.org/10.26650/ASE20241424227

ABSTRACT

Between 2021-2022, a dense mucilage disaster occurred in the Sea of Marmara in Türkiye. Mucilage mostly covered coastal areas, which juvenile fish use for nurseries, growth, and protection. Due to Sand steenbras having been suggested as an environmental bioindicator species for Mediterranean coastal waters, this study attempts to reveal post-mucilage distribution and potential differences on the timing of hatching, daily age, growth, and mortality as revealed by the otolith microstructure. Individuals were sampled using beach seine nets from 12 equally spaced stations along the Sea of Marmara. Its presence and significant abundance in 10 out of 12 stations showed the Sand steenbras to be a common juvenile species for the Sea of Marmara. Hatching occurs between May-January, with peak hatching occurring in October. When considering that the mucilage had completely disappeared from the environment in August, the first set of hatching months can be seen to coincide with the dense mucilage. Thus, Sand steenbras can be considered as able to reproduce under these environmental conditions. The daily age of the Sand steenbras juveniles ranged between 38-235 days, with a mean of 120.3 ± 1.8 days. The daily growth and mortality rates were also calculated respectively as 0.226 mm/day and 4.11%.

Keywords: Hatch date distribution, daily growth, juvenile fish, spatial distribution, mucilage

ORCID IDs of the author:
I.B.D. 0000-0002-2973-5698;
Y.Ş. 0000-0002-0595-4618;
A.Ö. 0000-0003-3914-9788;
A.A. 0000-0003-4839-9244;
U.A. 0000-0002-3638-9834;
A.İ. 0000-0003-2456-0232;
A.Y.: 0000-0001-7949-3001;
U.Ö. 0000-0003-2207-0168;
F.Ç. 0000-0001-5261-2365;
T.D. 0000-0003-1613-5236;
G.E.U. 0000-0002-3131-9239;
O.A. 0000-0001-8458-4872;
B.B.S. 0000-0003-4650-5592

[1]Faculty of Marine Science and Technology, University of Çanakkale Onsekiz Mart, Çanakkale, Türkiye

[2]Institute of Marine Science and Management, University of Istanbul, Istanbul, Türkiye

[3]Institute of Natural Sciences, University of Çanakkale Onsekiz Mart, Çanakkale, Türkiye

Submitted:
07.02.2024

Revision Requested:
07.03.2024

Last Revision Received:
14.03.2024

Accepted:
15.03.2024

Online Published:
21.05.2024

Correspondence:
İsmail Burak Daban
E-mail:
burakdaban@gmail.com

INTRODUCTION

The Sea of Marmara is a semi-enclosed basin located between the Black Sea and the Aegean Sea that connects them through the respective Istanbul and Dardenelles Straits. The variable physicochemical conditions of Marmara Sea such as global warming-related changes (Savun-Hekimoğlu & Gazioğlu, 2021), fluxiation (Altıok & Kayişoğlu, 2015), and pollution (Bilgili et al., 2022) cause undesirable environmental problems such as mucilage. Mucilage is defined as massive gelatinous aggregates that are produced by marine organisms under these aforementioned undesirable conditions (Mecozzi et al., 2001). A dense mucilage event occurred in the Sea of Marmara between April-July, 2021. In the first months, the mucilage covered relatively all surface areas before becoming a submersion. The coastal areas later were completely covered by mucilage in May and June.

Mucilage has direct and indirect effects on aquatic living organisms. Mucilage directly affects sessile animals such as corals, sponges, mussels, and anemones by covering them. These animals are suspension-feeders, and water flow is crucial to their survival. In addition,

marine algae and plants need light for photosynthesis. Thus, the adverse effects of mucilage on these organisms are fatal and inevitable. The indirect effects of mucilage may occur on other animals such as gastropods, decapods, cephalopods, and fish. However, relatively few studies have been conducted on this. Ertürk-Gürkan et al. (2022) found the mucilage to be consumed as a food source by benthic species *Eriphia verrucosa*. Dalyan et al. (2021) studied the effects of mucilage on cryptobenthic adult fish assemblages in the North Aegean Sea where mucilage was less impactful and stated these species to have had to change their habitats due to mucilage. Even worse, Karadurmuş and Sarı (2022) revealed mass deaths for 12 teleost, two cartilaginous, and four decapod species in the Sea of Marmara.

Due to mucilage occurring mostly along coastal areas which are known as nursery areas for new settlers and juveniles, understanding the mucilage-juvenile fish relationship may be important. Because fish in their early life stages have only been alive for a short time, retrospectively monitoring the effects of sudden environmental conditions may be more logical as these carry more noticeable signs regarding adult fish. Micro-increment studies of the sagittal otolith allows one to determine the daily age and the related time when hatched. Some pollution- and environmental change-related variations on these parameters have been previously examined (Campana, 1984; Meier et al., 2010; Isnard et al., 2015; Sardi et al., 2021) for some fish larvae and juveniles. Due to most teleost fish laying their eggs on surface areas and the fertilization and development of eggs occurring in a pelagic environment, the success of these development process is questionable in the environments where mucilage occurs. This study hypothesizes that eggs may not be fertilized as a result of the dense mucilage aggregates and the need for external fertilization. In addition, hatching larvae may get stuck in the mucilage and fail to move. Larvae may also not be able to find prey after their mouth gap opens. All these possibilities may result in high mortalities. To understand these effects, the species should be selected as a reference as their spawning season overlapped with the occurrence of dense mucilage. Thus, the study has selected Sand steenbras both for having been suggested as an environmental bioindicator species for Mediterranean coastal waters (Tom et al., 2003; Funkenstein et al., 2004) as well as for the overlap their reproduction cycle had with the dense mucilage event.

Thus, this study aims to reveal evidence for the spawning that has occurred using the spatial distribution of biomass and hatch date distributions. In addition, the study attempts to observe potential differences regarding the daily age, growth, and mortality rates compared to previous studies.

MATERIALS AND METHODS

L. mormyrus juveniles were collected using beach seine sampling from 12 equally spaced stations located along the coast of the Sea of Marmara (Figure 1). Monthly beach seine sampling hauls were carried out with two replications from each station between December 2021-March 2022. The length of wings, the height of the wings, and the length, width, and height of the bag of the beach seine were rigged as 30 m, 1.8 m and 2x2x2 m, respectively. The 4 mm nominal bar length was used in the bag net, whereas a 6.5 mm nominal bar length was used in the wing net (Figure 2). The beach seine hauls were conducted on seagrass beds and sandy habitats. Specimens were kept on ice packs and transported immediately to the laboratory. The individuals were then measured to the nearest 0.1 cm regarding total length (TL) and weighed to the nearest 0.01 g regarding total weight (W). The mean abundance at each location was given with the catch per unit effort *(CPUE)* based on the number of fish per haul. The CPUE was calculated with the following equation:

$$CPUE\ (n\ /\ \text{haul}) = N_i\ /\ H_i \quad (1)$$

where N_i is the total individual number of Sand steenbras obtained from station *i* in the study period, and H_i is the total number of hauls from station *i*. The total number of hauls was calculated by multiplying the replication number (equal to 2) with the total monthly survey number (equal to 4).

The length-weight relationship parameters are calculated using Le Cren's (1951) formula:

$$W = a * TL^b \quad (2)$$

where *W* is the total weight (g), *TL* is the total length (cm), and *a* and *b* are regression parameters. The growth type was identified using Student's t-test in accordance with the following equation (Sokal & Rohlf, 1987):

$$ts = (b - 3)\ /\ SE_b \quad (3)$$

where *ts* is the t-test value, *b* is the slope, and SE_b is the standard error of the slope. Student's t-test examines the significance of the difference of *b* - 3, which represents isometric growth (Pauly, 1984).

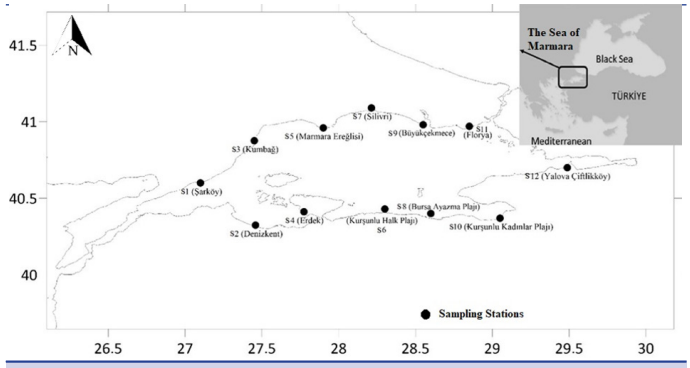

Figure 1. Study area and sampling stations.

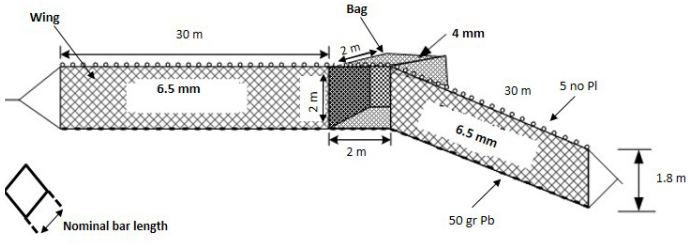

Figure 2. Technical plan of beach seine net.

A linear regression analysis ($y = bx + a$) was used to determine the relationships between *TL* (total length) and *FL* (fork length), as well as *TL*-otolith length (*OL*), total weight (*TW*)-otolith weight (*OW*), *TL*-otolith width (*OWi*), *TL-OW*, *OL-OWi*, and *OL-OW*.

For the otolith microstructure analyses, the sagittal otoliths of *L. mormyrus* juveniles were grounded and polished with abrasive papers. The sagittal otoliths of *L. mormyrus* were removed using forceps. The right otoliths were placed on a glass slide with thermoplastic glue for viewing under a microscope. After grounding and polishing, increment rings were counted from the first visible check mark succeeding the primordium to the outer edge along the maximum diameter axis, as described by Brothers (1984; Figure 3).

The daily growth rate (GR) of *L. mormyrus* was calculated based on simple linear regressions using the least-squares method between the larval lengths (SL) and juvenile lengths (TL) with respect to age in days (Leonarduzzi et al., 2010) according to the following formula:

$$L = a + b(t) \quad (4)$$

where *L* is the larval and juvenile lengths, *a* is a constant, *t* is a function of age in days, and *b* is the daily growth rate (mm). The daily mortality rates were estimated using the slope coefficient in the regression relationship of the natural log values of the abundance-per-length groups (Pauly, 1984). Hatching time was determined by subtracting the age in days of the individual from the sampling date, and the hatch peak and hatch interval were determined in order to apply the calculation over all sampling months. Dissolved oxygen, salinity, and temperature were measured using a YSI 6600 (6-series multiparameter water quality sondes).

Figure 3. A 36 mm total length juvenile Sand steenbras individual and sagittal otolith at 90 days old.

RESULTS AND DISCUSSION

In terms of the spatial variation of abundance, *L. mormyrus* juveniles were detected in 10 of 12 stations in the Marmara Sea. *L. mormyrus* juveniles were not found in Stations S9 (Büyükçekmece) or S6 (Bandırma Kurşunlu Shores). Stations S3 (Kumbağ) and S12 (Yalova Tigem) had the highest abundance with a mean of 11.6 ind./haul. Station S7 (Silivri) also had a high abundance (9.1 ind./haul). As can be seen in Figure 4, *L. mormyrus* juveniles were well distributed throughout all of the Marmara Sea.

Table 1 summarizes the minimum (min.), maximum (max.) and mean (*M*) lengths for *TL* and *FL*, as well as the weight values of the 479 individuals. The highest individual number, mean *TL*, and mean *FL* were detected in January, whereas the max. weight was found in February. The length-frequency distribution showed 27% of the individuals to be found in the 53-57 mm *TL* group. The lowest size (28-32 mm) and highest size (73-77 mm) *TL* groups were represented with low individual numbers due to selectivity and recruitment patterns.

The length frequency distribution shows two different peaks, accordingly two cohorts in one spawning season (Figure 5). The length–weight relationship curve can be seen in Figure 6. The *b* value was found to significant vary from 3 ($t = 2.41$; *t-table* = 1.96; $t > t\text{-table}$; $p < 0.05$) and to exhibit negative allometric growth (Table 2).

The measurements related to sagittal otolith length (*OL*), otolith width (*OWi*), and otolith weight (*OW*) of the *L. mormyrus* juveniles are shown in Table 3. A linear relationship has been detected between *TL-FL*, *TL-OL*, *TW-OW*, *TL-OWi*, *TL-OW*, *OL-OWi*, and *OL-OW*. The equations and related parameters are summarized in Table 4.

The pattern for age in days as estimated by the sagittal otoliths of *L. mormyrus* juveniles ranged between 38-235 days. The mean age in days of *L. mormyrus* was determined as 120.3 ± 1.8. The youngest individual was sampled on December 16, 2021, whereas the oldest one was sampled on February 18, 2022. The 120-139 (22.8%) age-in-days grouping was determined to be the dominant age group, followed by the 140-159 (17%) age-in-days grouping. The age in days-length key for juvenile *L. mormyrus* is shown in Table 5.

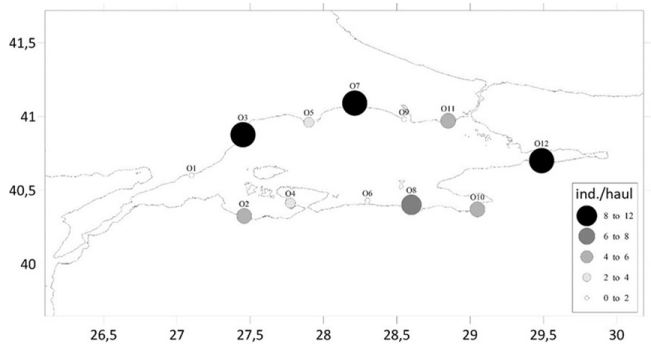

Figure 4. Spatial variaiton of mean CPUE of the *Lithognathus mormyrus* in the Sea of Marmara.

Table 1. The length and weight values and temporal variations of juvenile Sand steenbras *(Lithognathus mormyrus).*

		December	January	February	March	Total
Total Length (TL; mm)	Individual	146	149	127	57	479
	Min.	28.42	30.73	30.13	34.39	28.42
	Max.	72.61	74.88	74.84	65.04	74.88
	M	47.38	53.24	52.36	50.99	50.95
	SE	0.96	0.69	0.79	0.94	0.45
	SD	11.65	8.36	8.85	7.07	9.79
	CI	47.38 ± 1.58	53.24±1.13	52.36±1.30	50.99±1.58	50.95±0.74
Fork Length (FL; mm)	Individual	146	149	127	57	479
	Min.	26.45	28.52	28.1	32.25	26.45
	Max.	69.09	71.01	68.88	59.95	71.01
	M	43.81	50.02	48.7	46.98	47.46
	SE	0.95	0.64	0.73	0.89	0.43
	SD	11.49	7.77	8.22	6.76	9.42
	CI	43.81±1.56	50.02±1.05	48.7±1.20	46.98±1.50	47.46±0.71
Weight (g)	Individual	146	149	127	57	479
	Min.	0.2439	0.2445	0.2967	0.3479	0.2439
	Max.	3.8538	4.2984	3.9433	2.4182	4.2984
	M	1.23	1.46	1.47	1.37	1.37
	SE	0.075	0.049	0.063	0.064	0.033
	SD	0.91	0.6	0.71	0.48	0.73
	CI	1.23±0.12	1.46±0.08	1.47±0.10	1.37±0.11	1.37±0.05

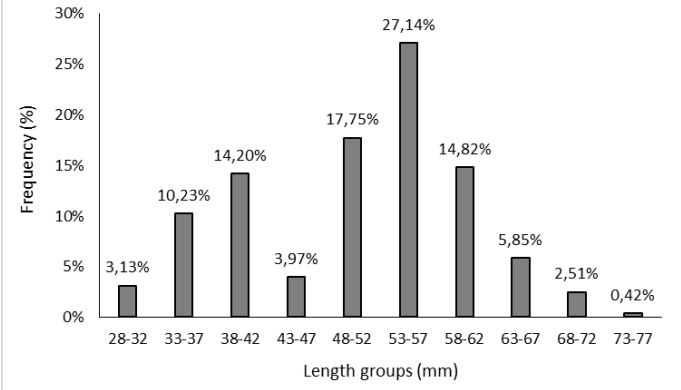

Figure 5. Length-frequency distribution of juvenile Sand steenbras (*Lithognathus mormyrus*).

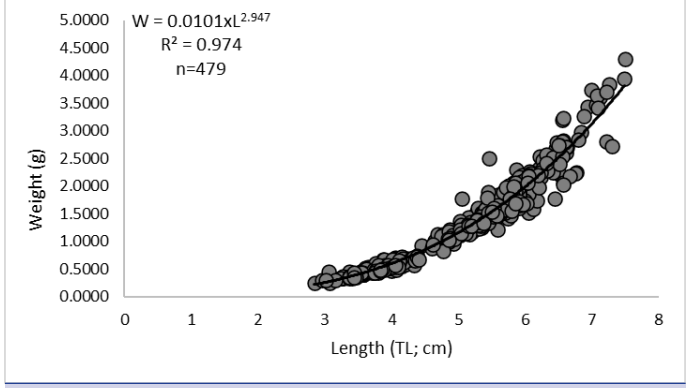

Figure 6. Length-weight relation of juvenile Sand steenbras (*Lithognathus mormyrus*).

Table 2. The length-weight relationship parameters of Sand steenbras *Lithognathus mormyrus* juveniles.

a*	b*	SE(b)*	R^2*	Growth Type	SD_a	SD_b
0.0101	2.947	0.02194	0.98	Negative allometric	0.00945 - 0.01087	2.9043 – 2.9906

**a* and *b* are regression parameters; SE = standard error of the slope (95% CI); R^2 = coefficient of determination; SD_a = standard deviation of *a* (95% CI); SD_b = standard deviation of *b* (95% CI).*

The daily growth rate of the species was found as 0.226 mm/day according to the linear regression between the age in days and juvenile lengths (Figure 7). The instantaneous and mean daily mortality rates of juvenile *L. mormyrus* were calculated as 0.0403 and 4.11%, respectively (Figure 8).

By subtracting the age in days from the time of sampling, the hatching of *L. mormyrus* was determined to have occurred between May 2021-January 2022, with an increase occurring between August-October and peaking in October (Figure 9).

Table 3. Otolith length (OL), otolith width (OWi), and otolith weight (OW) of Sand steenbras (*Lithognathus mormyrus*) juveniles.

	Individual	Min.	Max.	Mean	*SE*	*SD*
Otolith Length (OL, mm)	200	1.282	3.387	2.331	0.032	2.331 ± 0.053
Otolith Width (Owi, mm)	200	0.885	1.982	1.475	0.018	1.475 ± 0.030
Otolith Weight (OW, g)	200	0.0001	0.0042	0.0024	0.0001	0.0024 ± 0.0001

*OL = otolith length; OW = otolith weight; OWi = otolith width; *SE* = standard error; *SD* = standard deviation of *b* (95% CI).

Table 4. Linear Regression parameters of body measurements and sagittal otoliths of Sand steenbras (*Lithognathus mormyrus*) juveniles.

Relationship	Equation	SE_b*	R^2*	95% SD_b*	
TL*-FL*	FL = 0.9468TL - 0.9367	0.0073	0.99	0.9322	0.9610
TL-OL*	OL = 0.0398TL + 0.3117	0.154	0.88	0.0377	0.0419
TW*-OW*	OW = 0.0011TW + 0.0008	0.0006	0.66	0.00101	0.00123
TL-OWi*	OWi = 0.0219TL + 0.3687	0.091	0.87	0.0207	0.023
TL-OW	OW = 9E-05TL - 0.0023	0.0004	0.83	8.63E-05	9.82E-05
OL-OWi	OWi = 0.5376OL + 0.2366	0.073	0.92	0.5133	0.558
OL-OW	OW = 0.0022OL - 0.0029	0.0004	0.86	0.002112	0.002365

*TL = total length; TW = total weight; FL= fork length; OL = otolith length; OW = otolith weight; OWi = otolith width; SE_b = standard error of the slope (95% CI); R^2 = coefficient of determination; SD_b = standard deviation of *b* (95% CI).

Table 5. The age-length key of juvenile *Lithognathus mormyrus* in the Marmara Sea, Türkiye.

Length (mm)	Age in days										
	20-39	40-59	60-79	80-99	100-119	120-139	140-159	160-179	180-199	200-219	220-239
25-29	1	1									
30-34	1	14	5	4							
35-39		21	33	19	1						
40-44		4	9	23	4						
45-49			2	3	19	8					
50-54				9	31	73	12				
55-59					4	24	61	21	1		
60-64						4	7	21	6	2	
65-69							2	6	10	3	
70-74								2	4	2	2

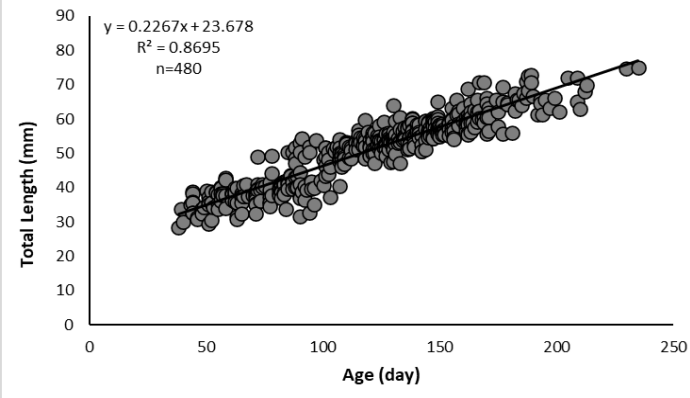

Figure 7. Age-length relationship of juvenile Sand steenbras (*Lithognathus mormyrus*).

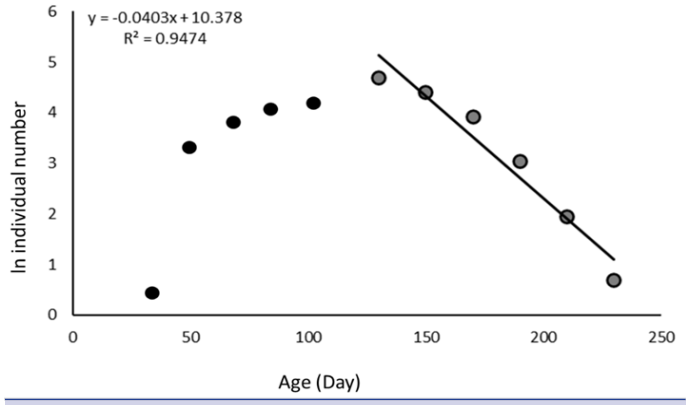

Figure 8. Linearized catch curve of juvenile Sand steenbras (*Lithognathus mormyrus*).

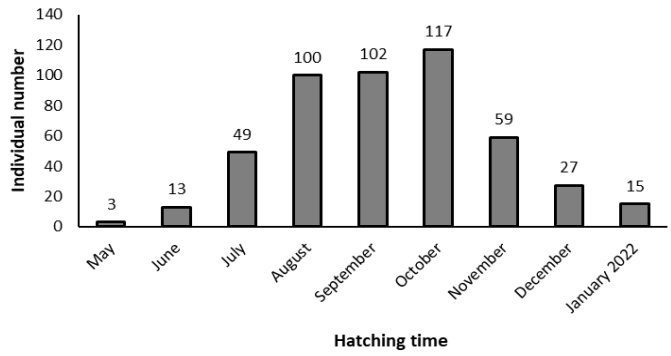

Figure 9. Hatching time of juvenile Sand steenbras (*Lithognathus mormyrus*) in the Marmara Sea.

The detection of juvenile individuals for each age-in-days class with a significance abundance from each location in the present study may be an indication that spawning might have occurred in the area and the individuals had not been sampled accidentally. According to the microstructure of the sagittal otoliths, the time of hatching had occurred between May-January, with peak hatching having occurred in October. When considered that the mucilage had completely disappeared from the environment in August, the first hatching months can be seen to have coincided with the dense mucilage. Thus, Sand steenbras can be considered as able to reproduce under these environmental conditions. Thus, the hypothesis related to "spawning being prevented due to unsuccessful external fertilization and being stuck in mucilage" is rejected.

Recruitment lengths of Sand steenbras have been stated as 60-70 mm TL (Matic-Skoko et al., 2007) and 72 mm TL (Lasiak, 1986). Ayyıldız et al. (2014) also only found 14 of 416 individual specimens to be longer than 75 mm TL. Ayyıldız and Altın (2021) additionally measured the largest juvenile individual they found as 74 mm TL. The largest individual found in the present study measured was 75 mm TL, which coincides with the findings of previous studies and confirms the relative recruitment length of *L. mormyrus*. Also, the well-distributed age and size class under 75 mm TL in the present study may be an indication that recruitment is able to occur. The spawning frequency of adult *L. mormyrus* identified has been identified as one clear seasonal peak per year (Bauchot & Hureau, 1986). Hereby, age 0 cohorts with different ages in days within a certain period of this single reproductive period need to be observed as juvenile individuals. The hatch date distribution and age-length data set presented in this study have revealed a single cohort. This may be proof of the spawning success of adult *L. mormyrus* under mucilage conditions.

Some population parameters related to stock status, such as growth type, condition factors, and daily growth and mortality rates were estimated and compared with previous studies. Matic-Skoko et al. (2007) examined the growth of juvenile *L. mormyrus* ranging from 8 mm-103 mm in length around the Adriatic Sea. They found the *b* exponent estimated from the length-weight relationship to be 3.141 and calculated the condition factor (*CF*) as 1.245. Ayyıldız and Altın (2021) calculated the *b* value as 3.106 in the Gökçeada Island of the North Aegean Sea. Reis and Ateş (2020) also found the *b* value for immature individuals to be 3.276 in the Köyceğiz Lagoon. Meanwhile, the current study detected *b* and *CF* as 2.947 and 0.94 (*SD* ± 0.04), respectively. The higher *b* and CF values in the Adriatic Sea, North Aegean Sea, and South Aegean Sea indicate those stocks to perhaps have better conditions compared to the Marmara Sea. Ayyıldız et al. (2014) evaluated the growth parameters of juvenile *L. mormyrus* around the Dardenelles Strait in Türkiye. The distribution for age in days was seen to closely correspond to length. Conversely, they found a higher mean daily growth rate (0.325 mm/d) compared to the current study's finding (0.226 mm/d). In addition, they found relatively lower daily mortality rates (2.16%) compared to the current study's result (4.11%). Ayyıldız and Altın (2021) also detected a higher mean daily growth rate (0.317 mm^{-1}) and relatively comparable mortality rate (4.61%) around Gökçeada Island of the North Aegean Sea. Some negative variations related to stock health such as conditions, growth and mortality were also detected. These differences may be a result of the species' feeding style, which feed by scratching the seabed. Reaching food may have been difficult due to the bottom being covered by mucilage. Also, the differences may be related to many other variables such as variations regarding the physicochemical parameters stemming from temporal and geographical differences, food availability, and pollutants along coastal areas. Thus, making a definitive judgement is difficult due to the studies not have been conducted simultaneously.

The timing of hatchings first occurred in May and peaked in October in the present study. The peak hatching period had been determined as June-July in the Adriatic Sea (Matic-Skoko et al., 2007), as August in the Dardenelles Strait (Ayyıldız et al., 2014), and between July-December in the North Aegean Sea (Ayyıldız & Altın, 2021). The main parameter controlling the spawning activity of Sand steenbras has been stated as sea surface temperature (SST; Vitale et al., 2011). Mandic et al. (2014) studied an ichthyoplankton community in Boka Kotorska Bay (South Adriatic Sea) and stated Sand steenbras to dominate the ichthyoplankton biomass in April, when their measured SST values ranged between 14.6°C-16.4°C (*M* = 15.5°C). According to Karadurmuş and Sarı (2022), the SST ranged between 14.5°C and 15.6°C in the Marmara Sea during the dense mucilage on April 25, 2021. The estimated onset of hatching occurred in May 2021. When additionally examining the fertilization period, the onset of hatching in May overlaps exactly with the species SST preferences for spawning. The onset of hatching, which seems to have been delayed, may actually have been caused not by the mucilage but by the factors that had caused the mucilage. In addition, hypoxia was reported (dissolved oxygen [*DO*] ranging between 2.3 mg/l-3.6 mg/l) in the Marmara Sea in April 2021 (Karadurmuş & Sarı, 2022), just before the hatching. Meanwhile, this study measured *DO* as ranging between 6.5 mg/l-9.4 mg/l (*M* = 8.4 mg/l) between December 2021-March 2022. As the mucilage density decreased, the oxygen values were observed to have increased. Thus, another possibility for the slightly delayed hatching and/or spawning times may have been caused by low oxygen levels.

CONCLUSION

Whether the differences mentioned in this study are directly related to the mucilage effect or the environmental conditions that

caused the mucilage to appear remains unclear. To better understand the direct and indirect effects of environmental factors such as mucilage on fish and their early life stages, factors such as distribution, feeding, reproduction, behavior, and pollution should be monitored more closely during and immediately after a mucilage event. Using back-calculated estimations based on the micro-increments of juvenile otoliths becomes more accurate when considering the obtained data alongside observations that were made during an incident. Simultaneously taking samples from areas affected by mucilage and unaffected areas should also increase the accuracy of back-calculation estimations. The world may encounter similar environmental disasters more frequently due to pollution and global warming, which are expected to increase in the near future. More detailed studies should be performed for understanding the direct and indirect effects environmental disasters such as mucilage events have on the early life stages of teleost fishes.

Acknowledgement: The authors would like to thank Captain Osman Odabaşı and Able Seaman Umut Tunçer for their help with the field work.

Conflicts of Interest: The authors declare that there are no conflicts of interest or competing interests.

Ethics Committee Approval: No specific ethical approval was necessary for this study.

Financial Disclosure: This work has been supported by the Scientific and Technological Research Council of Türkiye (Project No: 121G097).

REFERENCES

Altıok, H. & Kayişoğlu, M. (2015). Seasonal and interannual variability of water exchange in the Strait of Istanbul. *Mediterranean Marine Sciene*, 16(3), 644-655. https://doi.org/10.12681/mms.1225

Ayyıldız, H., Özen, O. & Altın, A. (2014). Growth, mortality and hatch-date distributions of striped sea bream *Lithognathus mormyrus* inhabiting the Çanakkale Strait, Turkey. *Journal of the Marine Biological Association of the United Kingdom*, 94(3), 607-613. https://doi.org/10.1017/S0025315413001847

Ayyıldız, H. & Altın, A. (2021). Pelagic larval duration and early growth of striped seabream, *Lithognathus mormyrus* inhabiting the Gökçeada shallow waters, Turkey. *Research in Marine Science*, 6(1), 883-895.

Bauchot, M. L. & Hureau, J. C. (1986). Sparidae. p. 883-907. In P. J. P. Whitehead, M. L. Bauchot, J. C. Hureau, J. Nielsen, & E. Tortonese (Eds.), *Fishes of the north-eastern Atlantic and the Mediterranean*. Vol. 2. UNESCO, Paris.

Bilgili, L., Çetinkaya A. Y. & Sarı, M. (2022). Analysis of the effects of domestic waste disposal methods on mucilage with life cycle assessment. *Marine Pollution Bulletin*, 180, 113813. https://doi.org/10.1016/j.marpolbul.2022.113813

Brothers, E. B. (1984). Otolith studies. In H. G. Moser (Eds.), *Ontogeny and Systematics of Fishes Based on an International Symposium Dedicated to the Memory of Elbert Halvor Ahlstrom* (pp. 50-57). American Society of Ichthyologists and Herpetologists: Lawrence.

Campana, S. E. (1984). Interactive effects of age and environmental modifiers on the production of daily growth increments in otoliths of plainfin midshipman, Porichthys notatus. *Fishery Bulletin*, 82(1), 165-177.

Dalyan, C., Kesici, N. B. & Yalgın, F. (2021). Preliminary study on cryptobenthic fish assemblages affected by the mucilage event in the northeastern Aegean Sea. *Journal of the Black Sea/Mediterranean Environment*, 27(2), 202-213.

Ertürk-Gürkan, S., Acar, S., Gürkan, M. & Özdilek, Ş. Y. (2022). Evaluation of isotopic signature of mucilage in the benthic food web in the Çanakkale Strait. *Biharean Biologist*, 16(1), 11-15.

Funkenstein, B., Dyman, A., Levavi-Sivan, B. & Tom, M. (2004). Application of real-time PCR for quantitative determination of hepatic vitellogenin transcript levels in the striped sea bream, *Lithognathus mormyrus*. *Marine Environmental Research*, 58(2-5), 659-663. https://doi.org/10.1016/j.marenvres.2004.03.058

Isnard, E., Tournois, J., McKenzie, D. J., Ferraton, F., Bodin, N., Aliaume, C., & Darnaude, A. M. (2015). Getting a good start in life? A comparative analysis of the quality of lagoons as juvenile habitats for the gilthead seabream Sparus aurata in the Gulf of Lions. *Estuaries and Coasts*, 38, 1937-1950.

Karadurmuş, U. & Sarı, M. (2022). Marine mucilage in the Sea of Marmara and its effects on the marine ecosystem: mass deaths. *Turkish Journal of Zoology*, 46(1), 93-102. https://doi.org/10.3906/zoo-2108-14

Lasiak, T. A. (1986). Juveniles, food and the surf zone habitat: implications for teleost nursery areas. *South African Journal of Zoology*, 21(1), 51-56. https://doi.org/10.1080/02541858.1986.11447956

Le Cren, E. D. (1951). The length-weight relationship and seasonal cycle in gonad weight and condition in the perch (*Perca fluviatilis*). *The Journal of Animal Ecology*, 201-219.

Leonarduzzi, E., Brown, D. R. & Sánchez, R. P. (2010). Seasonal variations in the growth of anchovy larvae (*Engraulis anchoita*) on the Argentine coastal shelf. *Scientia Marina*, 74(2), 267-274. https://doi.org/10.3989/scimar.2010.74n1267

Mandic, M., Regner, S., Gačić, Z., Đurović, M., Marković, O. & Ikica, Z. (2014). Composition and diversity of ichthyoplankton in the Boka Kotorska Bay (South Adriatic Sea). *Acta Adriatica*, 55(2), 229-244.

Matic-Skoko, S., Ferri, J., Kraljevic, M. & Dulcic, J. (2007). Growth of juvenile striped seabream, *Lithognathus mormyrus* (Teleostei: Sparidae), in the Adriatic Sea. *Journal of Applied Ichthyology*, 23(3), 286.

Mecozzi, M., Acquistucci, R., Di Noto, V., Pietrantonio, E., Amici, M. & Cardarilli, D. (2001). Characterization of mucilage aggregates in Adriatic and Tyrrhenian Sea: Structure similarities between mucilage samples and the insoluble fractions of marine humic substance. *Chemosphere*, 44(4), 709-720. https://doi.org/10.1016/S0045-6535(00)00375-1.

Meier, S., Morton, H. C., Nyhammer, G., Grøsvik, B. E., Makhotin, V., Geffen, A., ... & Svardal, A. (2010). Development of Atlantic cod (*Gadus morhua*) exposed to produced water during early life stages: Effects on embryos, larvae, and juvenile fish. *Marine Environmental Research*, 70(5), 383-394.

Pauly, D. (1984). *Fish population dynamics in tropical waters: A manual for use with programmable calculators*. Philippines: Manila. ICLARM Stud. Rev. 8. ICLARM, 325 pp.

Reis, I., & Ateş, C. (2020). Age, growth, length–weight relation, and reproduction of sand steenbras, Lithognathus mormyrus (Actinopterygii: Perciformes: Sparidae), in the Köyceğiz Lagoon, Mediterranean. *Acta Ichthyologica et Piscatoria*, 50(4), 445-451.

Sardi, A. E., Bégout, M. L., Cousin, X., Labadie, P., Loizeau, V., & Budzinski, H. (2021). A review of the effects of contamination and temperature in Solea solea larvae. Modeling perspectives in the context of climate change. *Journal of Sea Research*, 176, 102101.

Savun-Hekimoğlu, B. & Gazioğlu, C. (2021). Mucilage problem in the semi-enclosed seas: recent outbreak in the Sea of Marmara. *International Journal of Environment and Geoinformatics*, 8(4), 402-413 https://doi.org/10.30897/ijegeo.955739.

Sokal, R. R. & Rohlf, F. J. (1987). *Biostatistics*. Francise & Co, New York, 10.

Tom, M., Shmul, M., Shefer, E., Chen, N., Slor, H., Rinkevich, B. & Herut, B. (2003). Quantitative evaluation of hepatic cytochrome P4501A transcript, protein, and catalytic activity in the striped sea bream

(*Lithognathus mormyrus*). *Environmental Toxicology and Chemistry*, 22(9), 2088-2092. https://doi.org/10.1897/02-275

Vitale, S., Arkhipkin, A., Cannizzaro, L. & Scalisi, M. (2011). Life history traits of the striped seabream *Lithognathus mormyrus* (Pisces, Sparidae) from two coastal fishing grounds in the Strait of Sicily. *Journal of Applied Ichthyology*, 27(4), 1086-1094. https://doi.org/10.1111/j.1439-0426.2011.01775.x

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EISSN 2602-473X

AQUATIC SCIENCES AND ENGINEERING

Aquat Sci Eng 2024; 39(3): 156-164 • DOI: https://doi.org/10.26650/ASE20241450758

Research Article

Reproductive Biology of the Catfish *Pachypterus atherinoides* (Bloch, 1794) with Special Reference to Its Lentic and Lotic Ecosystems

Arun Jana[1], Godhuli Sit[1], Angsuman Chanda[1]

Cite this article as: Jana, A., Sit, G., & Chanda, A. (2024). Reproductive biology of the catfish *Pachypterus atherinoides* (Bloch, 1794) with special reference to its lentic and lotic ecosystems. *Aquatic Sciences and Engineering, 39(3),* 156-164. DOI: https://doi.org/10.26650/ASE20241450758

ABSTRACT

The study of reproductive biology is extremely crucial in fishery. *Pachypterus atherinoides* (Bloch, 1794) is a good food as well as an ornamental catfish in the World. The present study aims to examine the seasonal reproductive behavior of *P. atherinoides* regarding both its lentic and lotic ecosystems. The study was conducted over two years in selected lentic and lotic aquatic ecosystems of the Jhargram and Paschim Medinipur districts of West Bengal, India using the seasonally observed gonadosomatic index (GSI), fecundity, and gonad maturation stages for both habitats in the study area. The study reveals *P. atherinoides* to show single annual spawning between June-September. No significant difference was found regarding breeding period in terms of lentic and lotic aquatic ecosystems. This study can help aquaculturists regarding the culture, captive breeding, and conservation of *P. atherinoides*, as well as researchers regarding the biometric study of another fish.

Keywords: Fecundity, gonadosomatic index, gonad maturation, lentic, lotic, *Pachypterus atherinoides*

ORCID IDs of the author:
A.J. 0000-0003-3547-6247;
G.S. 0000-0002-5821-7937;
A.C. 0000-0003-1781-5057

[1]Natural and Applied Science Research Centre, Post Graduate Department of Zoology, Raja Narendralal Khan Women's College (Autonomous), Midnapore, Paschim Medinipur, West Bengal, India

Submitted:
11.03.2024

Revision Requested:
23.04.2024

Last Revision Received:
24.04.2024

Accepted:
28.04.2024

Online Published:
23.05.2024

Correspondence:
Angsuman Chanda
E-mail:
chandaangsuman182@gmail.com

INTRODUCTION

Fish reproductive biology entails gonad maturation, spawning period, spawning frequency, fecundity, and sexual development. Breeding biology, maturation, and fecundity are investigated in order to provide data that can be useful regarding fish conservation. The frequency of spawning in a year for a specific fish species can be determined by knowing its spawning periodicity. The significance of fecundity is self-evident, as it indicates the number of eggs that are laid. Understanding the fecundity of fish facilitates setting the arrangements for effectively hatching eggs on a fish farm. This type of research is also critical in fishery management. Fundamental themes in fish biology also include the description of reproductive techniques and the study of fecundity. For one to understand a fish's life history, spawning, culture, and administration, one also needs to understand fish reproduction. Reproductive strategies are one of the most important aspects of stabilizing a population in an environment. Examining the stages of gonad development for each fish, such as the period of the spawning season and the size at first maturity, is necessary for studying fish reproduction. The study of reproductive behavior has focused primarily on fish fecundity and the gonadosomatic index (GSI). The GSI is a key component of fish biology and aids in identifying the fish mating season by offering a full grasp of fish reproduction and the reproductive condition of each species (Shankar & Kulkarni, 2005). Fecundity is the quantity of maturing eggs a female has before spawning (Bagenal, 1978). Having a comprehensive understanding of fecundity is important for estimating the commercial potential of fish stocks and the spawning stock's abundance and reproductive potential (Lagler et al., 1956). *Pachypterus atherinoides* (Bloch, 1794), often known as Indian potasi, is a common cat-

fish belonging to the family Schilbeidae and is found in rivers and ponds throughout India (except Kerala), Pakistan, Bangladesh, Nepal, Myanmar, and Bunna (Menon, 1999). It has high nutritional as well as ornamental value. The fish meal of the species contains 343 Kcal in energy, 16 g of water, 58 g of protein, 9 g of fat, and 17 g of minerals (primarily 1,597 mg of calcium, 595 mg of phosphorus, and 41 mg of iron) per 100 g (Gopalan et al., 2004). Aquarists are attracted to it because of its bright color and small size. This species has been gradually decreasing due to pollution, habitat destruction, selective captive breeding, and removal from aquatic bodies due to their carnivorous nature, as well as the high demand for exotic catfish (African catfish *Clarius gariepinus* [Burchell, 1822]; Thai catfish *Pangasius sutchi* [Fowler, 1937]). The world has seen very little research on the reproductive biology of *Pachypterus atherinoides* (Bloch, 1794). India has yet to have a study on the reproductive biology of *Pachypterus atherinoides*. Gogai et al. (2020) and Jana et al. (2024) only studied this species' feeding biology. Gosavi et al. (2020) studied the reproductive biology of *Pachypterus khavalchor* in the Panchaganga River of Western Ghat, India. Various researchers (Dasgupta, 2004; Chattopadhyay et al., 2014; Paul and Chanda, 2017; Gupta, 2015; Jana et al., 2021A; Jana et al., 2021B; Jana et al., 2022A; Jana et al. 2022B; Sit et al. 2020; Chanda and Jana., 2021; Sahil et al., 2023; Sit et al. 2022A; Sit et al. 2022B; Sit et al., 2023A; Sit et al., 2023B; Jana et al., 2024) have also studied some aspects of various indigenous fish species in West Bengal, but none of these observed the reproductive behavior of *Pachypterus atherinoides*. Therefore, the present study will depict the seasonal reproductive biology of two different habitats that will be important for the conservation and propagation of this species.

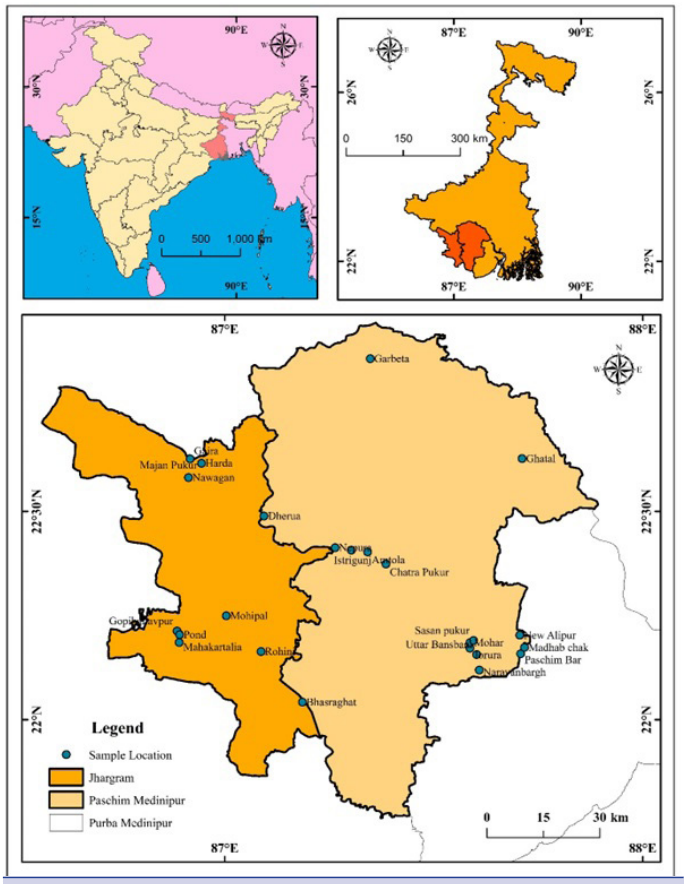

Figure 1. Specimen collection sites.

MATERIALS AND METHODOLOGY

Collection of fish specimens: Specimens were collected every 15 days from the selected lentic (ponds) and lotic (river sites) habitats of the Paschim Medinipur and Jhargram districts of West Bengal, India, during the pre-monsoon (March-June), monsoon (July-Oct), and post-monsoon (Nov-Feb) seasons between March 2020-February 2022 (Figure 1).

Sex determination: Male and female specimens were identified based on genital papilla, with males having a distinct extended papilla (Figure 2).

Length and Weight Measurements: Seasonally and ecosystem/habitat-wise, each specimen's total weight and length were measured using a digital scale with 0.01 g accuracy and a digital slide caliper with 0.01 mm accuracy, respectively.

Dissection and Internal Organ Measurement: Gonads were collected by dissection with the help of scissors, forceps, needles, and a brush. Digital slide calipers were used to measure the length, and an electronic balance was used to weigh each gonad.

Gonadosomatic Index (GSI): Howaida et al.'s (1998) formula was used to determine the GSI as follows:

$$GSI = \frac{Weight\ of\ gonad}{Weight\ of\ fish} \times 100 \quad (1)$$

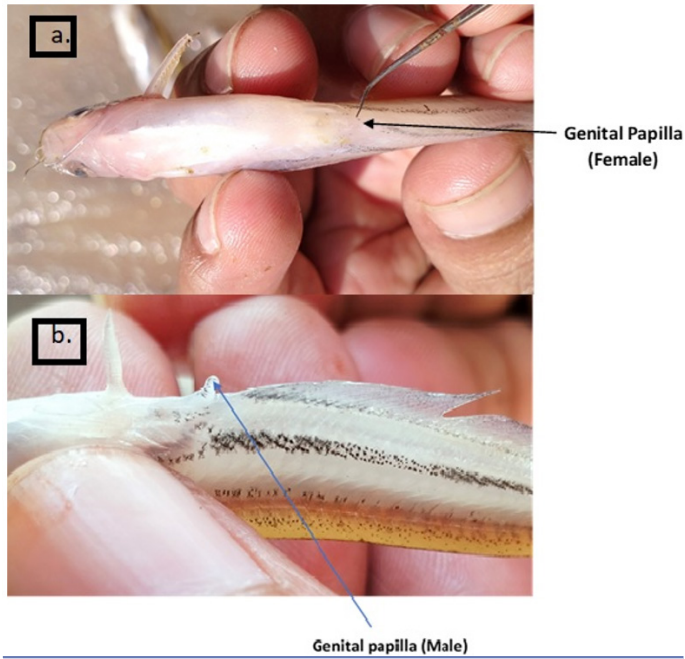

Figure 2. Genital apparatus of *P. atherinoides*. a.) Female; b.) Male.

Fecundity: The egg sac's front, middle, and posterior regions were divided into equal numbers of mature and immature eggs, which were then separated and counted using a needle and magnifying glass for each portion. The total number of eggs (i.e., the fish's fecundity) is measured using Dewan and Doha's (1979) gravimetric method as follows:

$$F = n\,G\,/\,g \quad (2)$$

where F is fecundity, n is the number of eggs in the subsample, G is the total weight of the ovary, and g is the weight of the subsample in grams.

Observed gonad maturation: Examinations were carried out at the macro and microscopic (Model-XSP L101) levels to examine both species' cycles of gonad maturation. Female and male gonad maturation stages have been divided into the various stages of development (Heins & Baker, 1988; Gomes & Araaujo, 2004; Gupta & Banerjee, 2013).

Data Analysis: Lastly, the study analyzed the data using the Microsoft Excel (2019), SPSS (2021), and Origin Pro (2023) software systems.

RESULTS AND DISCUSSION

GSI values were highest during the monsoon season ($GSI_{J\text{-}Lentic}$ = 20.29 ± 6.926 and $GSI_{PM\text{-}Lentic}$ = 19.94 ± 8.075; $GSI_{J\text{-}Lotic}$ = 20.72 ± 6.29 and $GSI_{PM\text{-}Lotic}$ = 20.50 ± 7.56) followed by pre-monsoon ($GSI_{J\text{-}Lentic}$ = 16.02 ± 4.77 and $GSI_{PM\text{-}Lentic}$ = 15.166 ± 4.52; $GSI_{J\text{-}Lotic}$ = 16.06 ± 4.65 and $GSI_{PM\text{-}Lotic}$ = 15.99 ± 4.91), post-monsoon ($GSI_{J\text{-}Lentic}$ = 8.85 ± 1.24 and $GSI_{PM\text{-}Lentic}$ = 8.52 ± 2.00; $GSI_{J\text{-}Lotic}$ = 8.65 ± 1.46 and $GSI_{PM\text{-}Lotic}$ = 9.05 ± 1.56) in both habitats of the two districts (Table 1 & Figures 3-4). The maximum GSI in both habitats occurred from June to September, with the peak value occurring in July for the lentic habitats and August for the lotic habitats, while the lowest value was in December for both habitats (Figures 5-6). The GSI values indicate the breeding season to occur between June-September for *P. atherinoides*. Hossian et al. (2019) reported the GSI to be high between April-June and very low between January-March, with the spawning season from April to June peaking in April and being at its lowest in May for the *P. atherinoides* from Kangsha River in Bangladesh. Paul et al. (2021) observed the same result as Hossian et al. (2019): After GSI peaked in April, it gradually decreased up to July. However, the present findings are not related to these two works due to the different physicochemical parameters. The current study measure fecundity as 482 ± 203.02 (4.44g -6.57g TW) and 492 ± 236.91 (4.31g -6.85g TW) for the respective lentic and lotic areas of Jhargram district; while this was measured as 526 ± 243.32 (4.23g -6.77g TW) and 538 ± 245.12 (4.36g -6.83g TW) for Paschim Medinipur's respective lentic and lotic areas (Table 2). Hossain et al. (2019) reported fecundity values of 1541-10,043 (2.33-5.59 g), while Paul et al. (2021) observed values of 1,805.17 ± 965.71 to 4,553.07 ± 1,755.36 for the *P. atherinoides* from Bangladesh. Gosavi et al. (2020) measured absolute fecundity to varied between 932-24,642 eggs/fish and relative fecundity to range between 226-

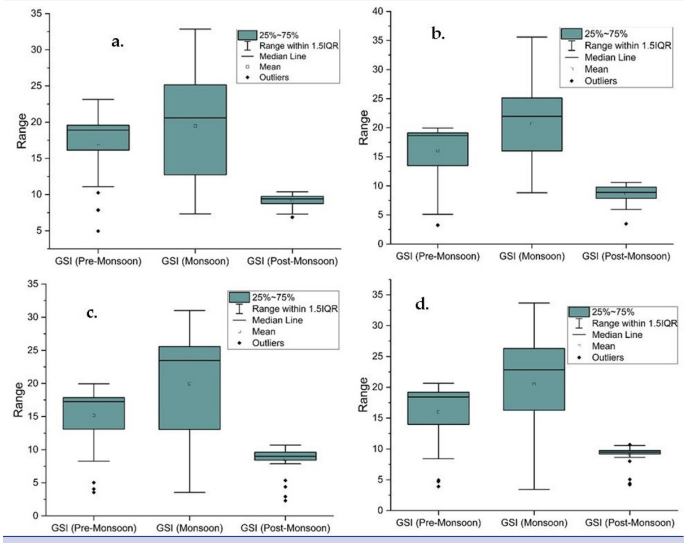

Figure 3. Seasonal GSI values of *P. atherinoides* in Jhargram (a & b) and Paschim Medinipur (c & d) districts: a & c are ponds; b & d are rivers.

Table 1. Seasonal GSI of *P. athertenoides* for both lentic and lotic habitats in Jhargram and Paschim Medinipur districts (N = 128)

District	Habitat	Season	GSI			
			Min.	Max.	Mean	SD
JHARGRAM	Pond	*Pre-monsoon*	4.60	23.14	16.0244	4.77928
		Monsoon	7.33	32.87	20.2942	6.92698
		Post-monsoon	4.73	10.38	8.8555	1.24614
	River	*Pre-monsoon*	3.25	19.93	16.0655	4.65761
		Monsoon	8.82	35.60	20.7204	6.29151
		Post-monsoon	3.48	10.57	8.6504	1.46845
PASCHIM MEDINIPUR	Pond	*Pre-monsoon*	3.55	19.97	15.1664	4.52530
		Monsoon	3.55	31.02	19.9467	8.07572
		Post-monsoon	2.30	10.70	8.5228	2.00369
	River	*Pre-monsoon*	3.90	20.64	15.9921	4.91183
		Monsoon	3.44	33.67	20.5057	7.56303
		Post-monsoon	4.21	10.70	9.0554	1.56233

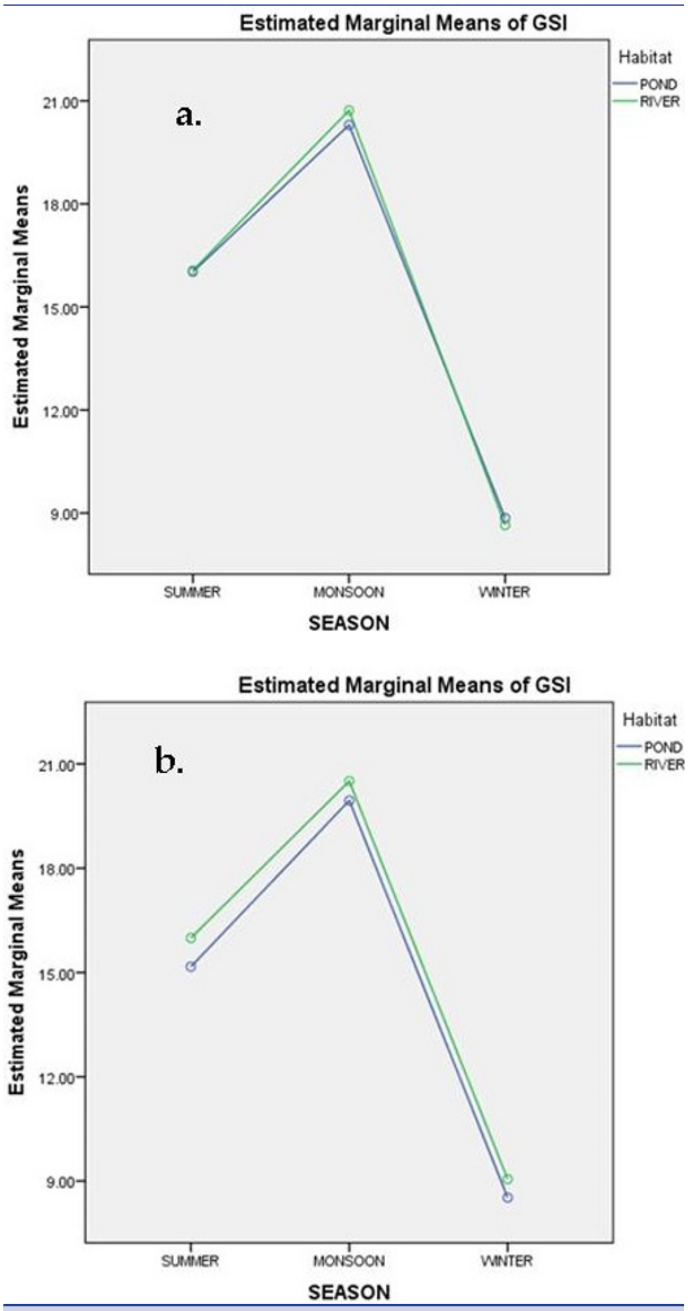

Figure 4. Seasonal variation of GSI for *P. atherinoides* in both habitats of Jhargram (a) and Paschim Medinipur (b) districts.

723 eggs/g of body weight for *Pachypterus khavalchor*. The study's measured fecundity values are lower than those from previous studies. The R^2 values between gonad weight and fecundity were measured as 0.850 and 0.9047 for Jhargram's lentic and lotic areas and as 0.738 and 0.921 for Paschim Medinipur's lentic and lotic areas, respectively (Figure 8). The R^2 values between body weight and fecundity were measured as 0.640 and 0.786 for Jhargram's lentic and lotic habitats and as 0.679 and 0.755 for Paschim Medinipur's lentic and lotic habitats, respectively (Figure 9). Fecundity was positively linearly correlated with gonad weight and body weight in both the studied habitats. The same

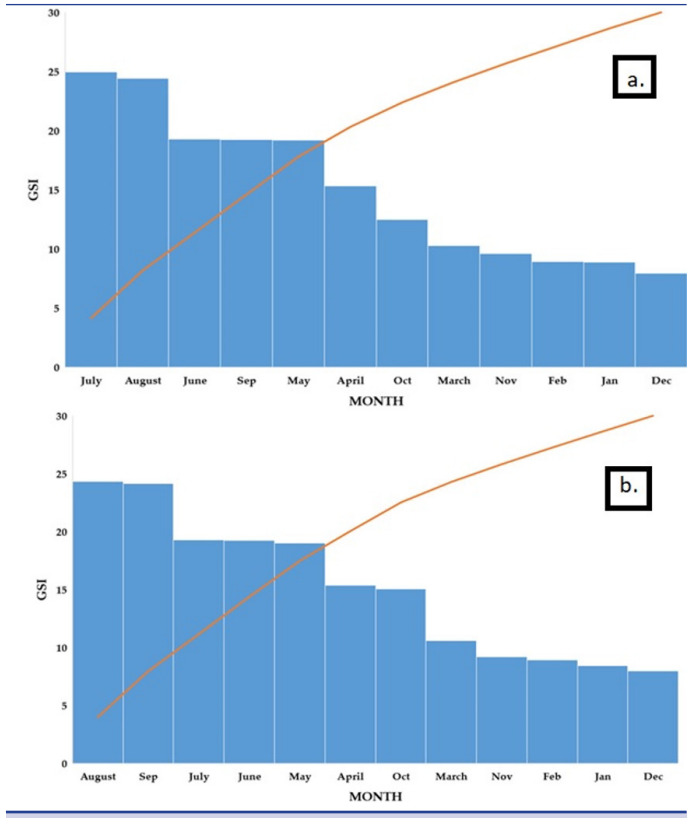

Figure 5. Monthly variation of GSI for *P. atherinoides* in the Jhargram district's: a.) ponds; b.) rivers.

result was observed by Hossain et al. (2019) and Paul et al. (2021) for the same species and by Islam and Das (2006), Siddique et al. (2008), Islam et al. (2011), Malla & Banik (2015), Gosavi et al. (2020), and Mawa et al. (2022) for other catfish species. This study has divided gonad maturity into 5 stages for *P. atherinoides*.

Ovary developmental stages (Figure 10):

Immature (Stage 1): Ovaries are thin, colorless, and transparent. Small and filiform, they only occupy one-third of the abdominal space. Oocytes are exceedingly tiny and invisible to the naked eye.

Maturing (Stage 2): Ovaries are pale white/cream in color, with minor granulation discernible to the naked eye. Half of the abdominal cavity is taken up by enlargement signs. Small ova, visible with the naked eye, begin to appear.

Mature (Stage 3): Ovaries are whitish-yellow in color and have a greater anterior expansion than Stage 2. Two-thirds of the abdominal cavity is lobular. The ova have a distinct yolk.

Ripe (Stage 4): Yellowish, voluminous, and with blood vessels; occupies two-thirds of the abdominal cavity, with a loosening of the previously compact arrangement of ova.

Spent (Stage 5): Flaccid, wrinkled, displaying a smaller size than Stage 4; ovaries become drastically smaller in both length and weight and show a sac-like look with a pale white tint.

Testis developmental stages (Figure 11):

Immature (Stage 1): Testis are thin, threadlike, transparent, and white in color, with unorganized tubules.

Maturing (Stage 2): Testis appear slightly longer and slightly more bulbous. The appearance of an indistinct vasa differentia.

Mature (Stage 3): Testis become large and creamy white in color. The Ductus difference is distinct. All spermatogenic cells with the maximum concentration of spermatids are present in seminiferous tubules.

Ripe (Stage 4): Testis are mature and more than two-thirds of the abdominal cavity in size. Spermatozoa-filled seminiferous tubules are easily discharged from the testis with a bit of pressure.

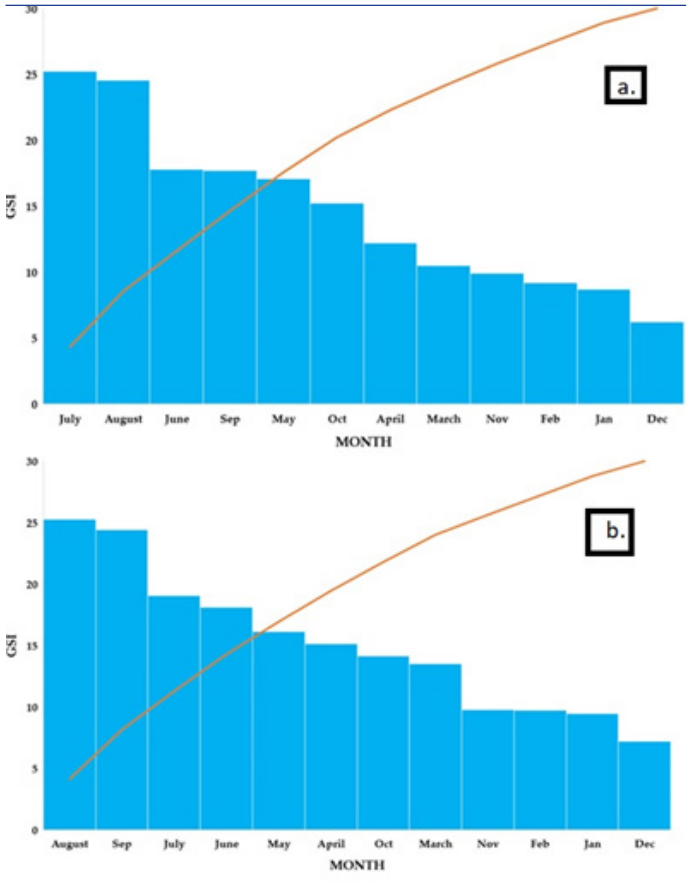

Figure 6. Monthly variation of GSI for *P. atherinoides* in Paschim Medinipur district's: a.) ponds; b.) rivers.

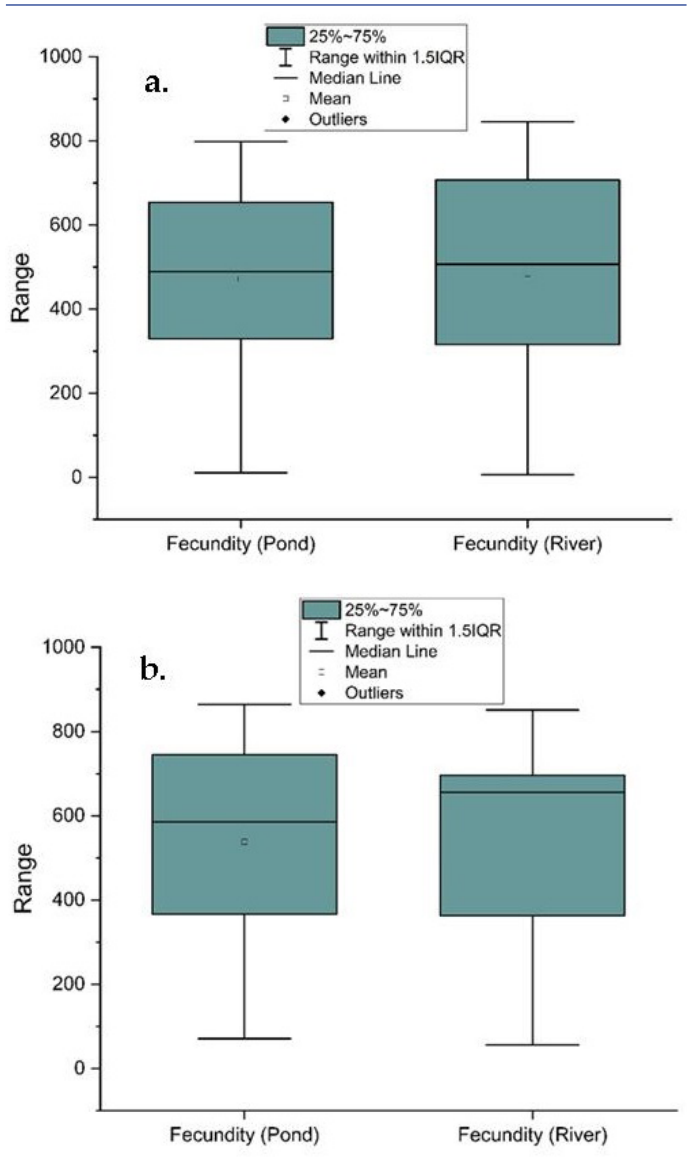

Figure 7. Fecundity of *P. atherinoides* in both habitats of (a) the Jhargram and (b) Paschim Medinipur districts.

Table 2. Total weight, gonad weight, and fecundity of *P. athertenoides* in both habitats of the Jhargram and Paschim Medinipur districts

District	Total Weight(g)				Gonad weight (g)				Fecundity			
	Min	Max	M	SD	Min	Max	M	SD	Min	Max	M	SD
JP	4.44	6.57	5.79	0.659	0.21	2.12	1.264	0.50	65.0	798	482.7	203.02
JR	4.31	6.85	5.90	0.748	0.15	2.41	1.250	0.57	65.0	845	492.6	236.91
MP	4.23	6.77	5.93	0.712	0.15	2.30	1.311	0.56	56.0	851	526.9	243.32
MR	4.36	6.83	5.97	0.738	0.15	2.30	1.319	0.56	71.0	864	538.3	245.12

N = 104; JP = Jhargram Pond; JR = Jhargram River; MP = Paschim Medinipur Pond; MR = Paschim Medinipur River

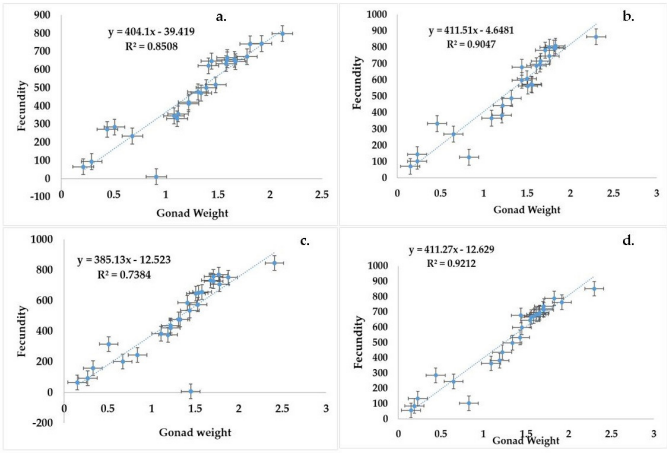

Figure 8. Relationship between fecundity and gonad weight for *P. atherinoides* in theJhargram (a &b) and Paschim Medinipur (c & d) districts; a and c are ponds; b and d are rivers.

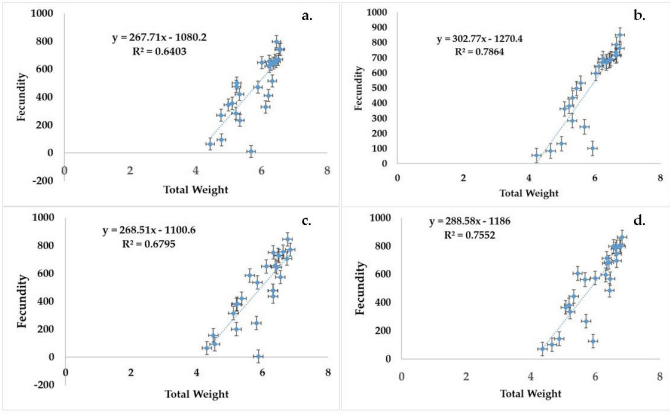

Figure 9. Relationship between fecundity and total weight for *P. atherinoides* in Jhargram (a & b) and Paschim Medinipur (c & d) districts; a and c are ponds; b and d are rivers

Spent (Stage 5): Flaccid gonads fill over half of the abdominal cavity in the spent (Stage 5) condition. Regression of the testis occurs. The cells seem to merge. High amount of primary and secondary spermatocytes.

During the study of ovary development in the lentic habitats (ponds), Stage 1 was noticed from November-March, with a peak in December and a minimum in May. Stage 2 was noticed from November-June, with a peak in April and a minimum in November. Stage 3 was noticed from March-September, with a peak in June and a minimum in September. Stage 4 was noticed from April-September, with a peak in July and a minimum in April. Stage 5 was noticed from June to November, with a peak in October and minimum in June. In the lotic habitats (rivers), Stage 2 of ovary development was noticed from November-July, with a peak in March and a minimum in July. Stage 4 was noticed from April-September, with a peak in July and a minimum in April. Stages 1, 3, and 5 were observed to have the same periods as those in lentic habitats (Figure 12).

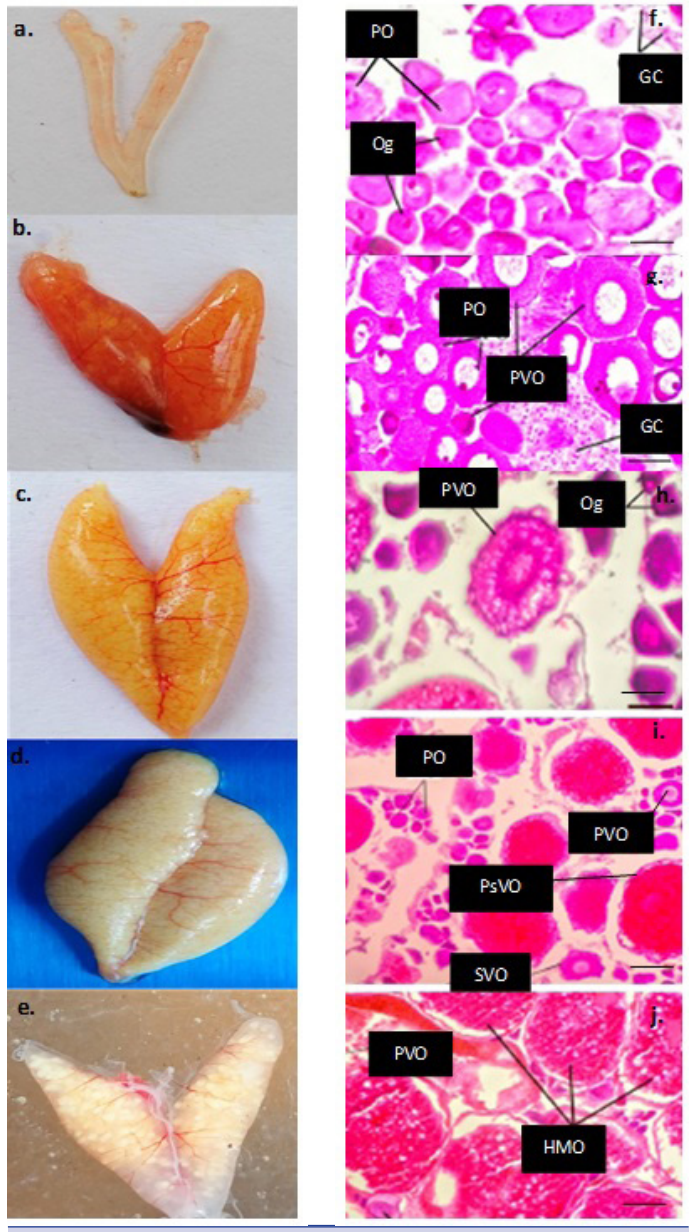

Figure 10. External (a-e) and microscopic (f-j) ovary of *P. atherinoides*. Stage 1 (a & f); Stage 2 (b & g); Stage 3 (c & h); Stage 4 (d & i); Stage 5 (e & j). GC = germ cells, Og = oogonia, PO = primary oocyte, PVO = primary vitellogenic oocyte, PsVO = post-vitellogenic oocyte, SVO = secondary vitellogenic oocyte, HMO = hydrated mature oocyte. Scale bar is at 100 µm.

For the study of testis development in the lentic habitats, Stage 1 was noticed from November-May, with a peak in December and a minimum in May. Stage 2 was noticed from November-June, with a peak in April and a minimum in November. Stage 3 was noticed from February-December, with a peak in June and a minimum in December. Stage 4 was noticed from April-October, with its peak in July and minimum in April. Stage 5 was noticed from June-October, with a peak in October and minimum in June. For the lotic habitats (rivers), Stage 1 of testis

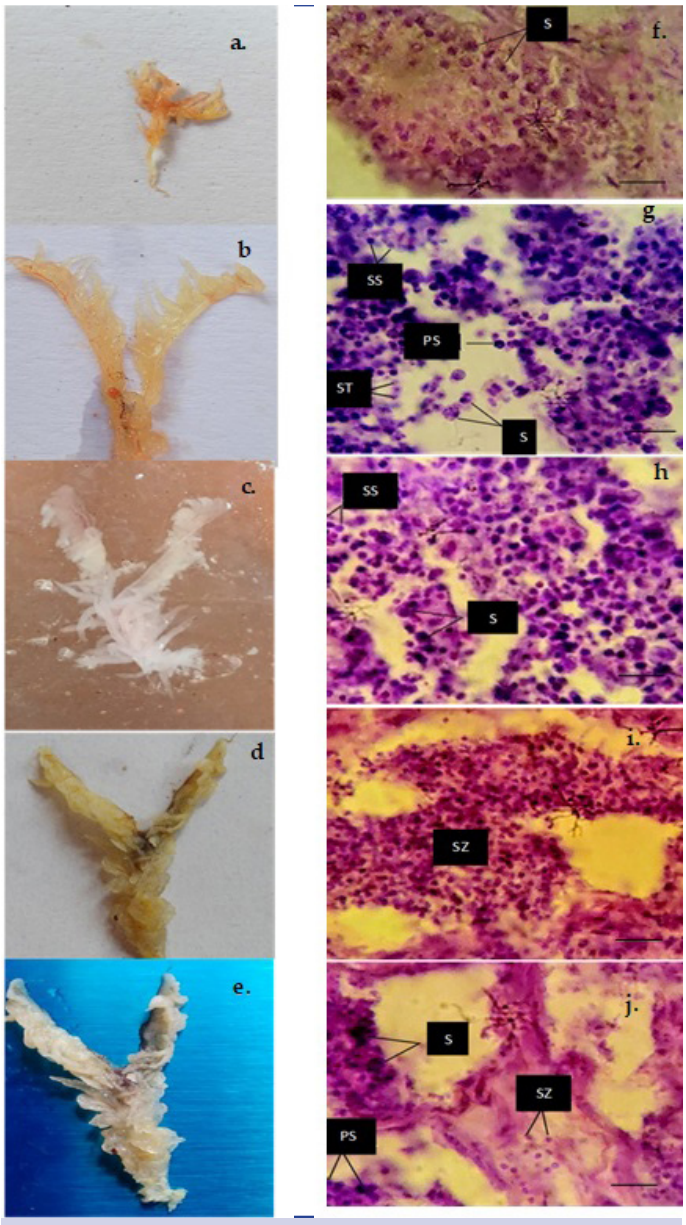

Figure 11. External (a-e) and microscopic (f-j) testes of P. atherinoides. Stage 1 (a & f); Stage 2 (b & g); Stage 3 (c & h); Stage 4 (d & i); Stage 5 (e & j). S = spermatogonia, PS = primary spermatocytes, SS = secondary spermatocytes, ST = spermatids, SZ = spermatozoa. Scale bar is at 100 µm.

development was noticed from November-May, with a peak in January and a minimum in May. Stage 2 was noticed from November-July, with the high in April and low in July. Stage 3 was noticed from February-December, with a peak in June and a minimum in February. Stage 4 was noticed from March-October, with the high in August and low in March. Stages 1, 3, and 5 were also observed to be similar to those in the pond habitats (Figure 13). The results for GSI, gonad development, and fecundity depict *P. atherinoides* to breed once a year between June-September.

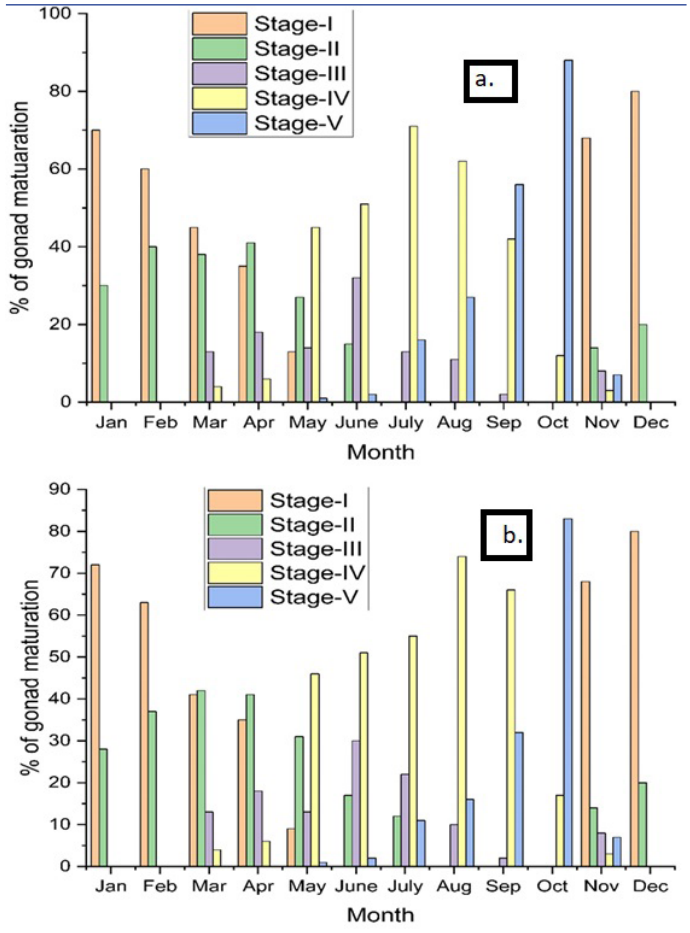

Figure 12.Monthly ovary maturation stages of *P. atherinoides* in both districts: a.) ponds; b.) rivers.

CONCLUSION

The breeding season of *P. atherinoides* has been determined in the study area to occur between June-September. Hence, the study suggests that government fishery managers take certain restrictions when capturing this species during their peak breeding season between June-September. The study's findings can be valuable tools for developing management and protection strategies for conservation and captive propagation. The knowledge from the current study may be applied for better managing *P. atherinoides* in India and for future conservation strategies, as well as for adopting this species as a possible candidate for commercial aquaculture.

Acknowledgement: The authors are indebted to the Principal of Raja Narendra Lal Khan Women's College (A) and the departmental faculties and staff members of PG Zoology for their constant inspiration and help in conducting sustainable research work for the benefit of science and society.

Conflict of Interest: The authors declare no conflicts of interest.

Ethics Committee Approval: Ethical approval was obtained from IAEC (Approval no. 18/IAEC (05)/RNLKWC/2019; dated: July 27, 2019).

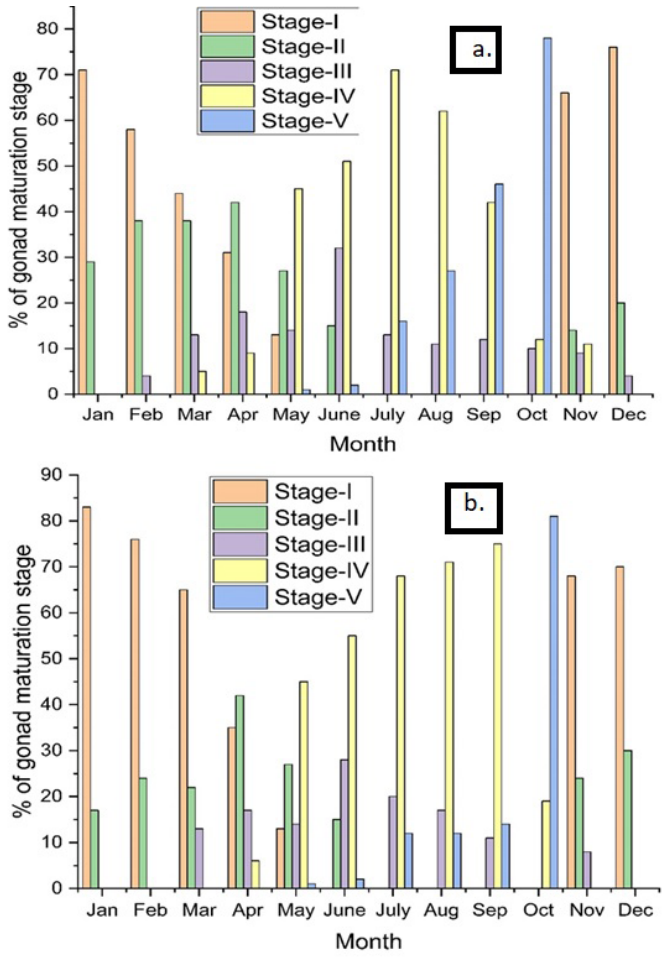

Figure 13.Monthly testis maturation stages of *P. atherinoides* in both districts; a.) ponds; b.) rivers.

Financial Disclosure: This study received no financial support from any funding source.

REFERENCES

Bagenal, T. B. (1978). Age and growth. Methods for assessment of fish production in fresh waters, *International Biological Programme*, Handbook no. 3. Blackwell, Oxford, 101-136.

Chanda, A. & Jana, A. (2021). A comparative review on freshwater fish fauna between West Bengal and Odisha, two middle-east Indian states. *Journal of Fisheries*, *9*(3), 93302-93302. https://doi.org/10.17017/j.fish.369

Chattopadhyay, S., Nandi, S., & Saikia, S. K. (2014). Mouth morphometry and architecture of freshwater cat fish *Mystus vittatus* Bloch (1974) (Siluriformes, Bagridae) in relation to its feeding habit. *Journal of Scientific Research*, *6*(1), 169-174. http://dx.doi.org/10.3329/jsr.v6i1.16369

Dasgupta, M. (2004). Relative length of the gut of some freshwater fishes of West Bengal in relation to food and feeding habits. *Indian Journal of Fisheries, 51*, 381-384.

Dewan, S., & Doha, S. (1979). Spawning and fecundity of certain pond fishes [viz. *Chela phula*, *Esomus danrica*, *Amblypharyngodon mola*, *Aplocheilus panchax*, *Ambassis nama*, *Glossogobius giuris* and *Colisa lalius*]. *Bangladesh Journal of Agriculture*, *4*(1), 1-8

Gopalan, C., Rama Sastry, B.V. & Bala Subramanian, S.C. (2004). Nutritive value of Indian foods, *National Institute of Nutrition*, ICMR, Hyderabad, 45-67.

Gogoi, B., Das, D. N., & Saikia, S. K. (2020). Feeding Ecology of *Pachypterus atherinoides* (Actinopterygii; Siluriformes; Schilbeidae): A Small Freshwater Fish from Floodplain Wetlands of Northeast India. Croatian Journal of Fisheries: Ribarstvo, 78(3), 105-120. https://doi.org/10.2478/cjf-2020-0011

Gosavi, S. M., Tapkir, S. D., Kumkar, P., & Kharat, S. S. (2020). The reproductive biology of *Pachypterus khavalchor* (Kulkarni, 1952) in the Panchaganga River of the Western Ghats, a biodiversity hotspot in India. *Asian Fisheries Science*, *33*, 79-90. https://doi.org/10.33997/j.afs.2020.33.2.001

Gomes, I. D., & Gerson Araújo, F. (2004). Reproductive biology of two marine catfishes (Siluriformes, Ariidae) in the Sepetiba Bay, Brazil. *Revista de Biologia Tropical*, *52*(1), 143-156.

Gupta, S. (2015). An Overview on Feeding and Breeding Biology of *Mystus tengara*, A Freshwater Catfish of Indian Subcontinent. *World*, *7*(3), 195-197. https://doi.org/10.5829/idosi.wjfms.2015.7.3.94217

Gupta, S., & Banerjee, S. (2013). Studies on reproductive biology of *Mystus tengara* (Ham.-Buch., 1822), a freshwater catfish of West Bengal, India. *International Journal of Aquatic Biology*, *1*(4), 175-184.

Heins, D. C., & Baker, J. A. (1988). Egg sizes in fishes: Do mature oocytes accurately demonstrate size statistics of ripe ova?. *Copeia*, *1988*(1), 238-240. https://doi.org/10.2307/1445942

Hossian, S., Bhattacharya, S., Rahman, M. A., Islam, R., Yesmin, R., Sume, S. A., & Moniruzzaman, M. (2019). Fecundity estimation of Indian Potasi, *Neotropius atherinoides* in Bangladesh. *Research in Agriculture Livestock and Fisheries*, *6*(3), 421-429. https://doi.org/10.3329/ralf.v6i3.44808

Howaida, R. G., Roger, T. H., Mahmoud, H. H., & Salah, G. (1998). Maturation, fecundity and seasonality of reproduction of two commercially valuable cuttlefish, *Sepia pharaonis* and S. dollfusi, in the Suez Canal. *Fisheries Research*, *36*(2-3), 99-115. https://doi.org/10.1016/S0165-7836(98)00107-6

Islam, M. K., & Das, M. (2006). Fecundity of Gulsha *Mystus cavasius* (Hamilton) from Brahmaputra and Kongsa rivers. *Journal of the Bangladesh Agricultural University*, *4*(2),347-355.https://doi.org/10.22004/ag.econ.276566

Islam, S. S., Shah, M. S., & Rahi, M. L. (2011). Study of fecundity and induced breeding of *Mystus vittatus*. *Bangladesh Journal of Zoology*, *39*(2), 205-212.

Jana, A., Sit, G., & Chanda, A. (2021A). Ichthyofaunal diversity of River Kapaleswari at Paschim Medinipur district of West Bengal, India. *Flora and Fauna*, *27*(1), 113-124. https://doi.org/10.33451/florafauna.v27i1pp113-124

Jana, A., Sit, G., & Chanda, A. (2021B). Record of hill stream catfish *Glyptothorax telchitta* (Hamilton-Buchanan, 1822) from Paschim Medinipur district, West Bengal, India. *Acta Biologica Sibirica*, *7*, 317-325. https://doi.org/10.3897/abs.7.e70963

Jana, A., Sit, G., & Chanda, A. (2024). Seasonal Feeding biology of catfish, *Pachypterus atherinoides* (Bloch, 1794) with Special reference to lentic and lotic ecosystem. *Aquatic Sciences and Engineering*, 39(2), 95-105. https://doi.org/10.26650/ASE20231412574

Jana, A., Sit, G., Das, P., Chanda, A. & Sahu, S. K. (2022A). Seasonal Length-Weight Relationships and Condition Factors of *Mystus tengara* (Hamilton, 1822) in Two Habitats. *Aquatic Sciences and Engineering*, 37(4), 205-211. https://doi.org/10.26650/ASE202221159748

Jana, A., Sit, G., Chanda, A., Nayak, S. & Sahu, S. K. (2022B). Seasonal length-weight relationships and condition factors of *Pachypterus atherinoides* (Bloch, 1794) in two habitats. *Ecology, Environment and Conservation*; 28, S515-S521. http://doi.org/10.53550/EEC.2022.v28i06s.0083

Jana, A., Sit, G., & Chanda, A. (2024). Seasonal Feeding biology of catfish, *Pachypterus atherinoides* (Bloch, 1794) with Special reference

to lentic and lotic ecosystem. *Aquatic Sciences and Engineering*, 39(2), 95-105. https://doi.org/10.26650/ASE20231412574

Lagler, K. F. (1956). Enumeration of fish eggs. *fresh water fishery biology. 2nd Edn. WM Brown company publishers. Dubuque*, 106-110.

Malla, S., & Banik, S. (2015). Reproductive biology of an endangered catfish, *Ompok bimaculatus* (Bloch, 1794) in the lotic waterbodies of Tripura, North-East India. *International Journal of Fisheries and Aquatic Studies*, *2*(4), 251-260.

Mawa, Z., Hossain, M. & Hasan, M. (2022). Life history traits of *Mystus vittatus* in the Ganges River, Bangladesh: recommendation for its sustainable management considering climate change. *International Journal of Biometeorol, 66***,** 927–943. https://doi.org/10.1007/s00484-022-02249-7

Menon, A.G.K. (1999). Check list - fresh water fishes of India. Record in Zoological Survey of India, Miscellaneous Publication, Occasional Paper No. 175.

Paul, B. and Chanda, A. 2017. A checklist of small indigenous freshwater fish fauna of undivided Paschim Medinipur, West Bengal, India. International Journal of Current Trends In Science And Technology, 7(11), 20393-20400. https://doi.org/10.15520/ctst. v7i11.149

Paul, S. K., Habib, M. A., Ali, M. N., Majumdar, P. R. and Saha, D. (2021). Meristic and morphometric characteristics relationships, condition factor and breeding biology of Indian potashi (*Neotropius atherinoides*) in the Adjacent River of Chalon Beel, Bangladesh. *Survey in Fisheries Sciences*. 8(1), 47-64. https://doi.org/10.17762/sfs.v8i1.67

Sahil, Mogalekar, H.S., Chandran, S., Nayak, .S.K., Kumar, S. & Singh, S.K. (2023). Length-weight relationship and condition factor of selected small Cyprinid fishes from river Burhi Gandak in North Bihar, India. Journal of the Inland Fisheries Society of India, 54(1), 27-32. https://epubs.icar.org.in/index.php/JIFSI/article/view/132388

Shankar, D. S., & Kulkarni, R. S. (2005). Somatic condition of the fish, *Notopterus notopterus* (Pallas) during different phases of the reproductive cycle. *Journal of Environmental Biology*, *26*(1), 49-53.

Siddique, M. A. B., Hasan, A. N. G. M., Islam, A. B. M. S., Ali, M. H., & Hossain, M. S. (2008). Study on the fecundity indices of two indigenous fish species from the Padma River. *Journal of Agroforestry and Environment*, *2*(2), 71-74.

Sit, G., Jana, A. & Chanda, A. (2020). Diversity of small indigenous freshwater ornamental fish under genus Puntius from Purba Medinipur, Paschim Medinipur and Jhargram districts of West Bengal, India. *Advances in Zoology and Botany*, *8*(4), 334-341. https://doi.org/10.13189/azb.2020.080405

Sit, G., Jana, A., Das, P., Chanda, A. & Sahu, S. K. (2022A). Induced breeding and embryonic development of indigenous ornamental fish *Puntius terio* (Hamilton, 1822). *Indian Hydrobiology*, 21(2), 43-51.

Sit, G., Jana, A., Chanda, A. & Sahu, S. K. (2022B). Record of zipper loach *Paracanthocobitis botia* (Hamilton 1822), an ornamental fish from Paschim Medinipur, West Bengal, India. *Journal of Fisheries*, *10*(3), 103401-103401. https://doi.org/10.17017/j.fish.355

Sit, G., Jana, A., Chanda, A. & Sahu, S. K. (2023A). Dose Selection for Induced Breeding and Larval Development of Indigenous Ornamental Fish *Puntius chola* (Hamilton, 1822). *Aquatic Sciences and Engineering*, 38(1), 62-67. https://doi.org/10.26650/ASE20221177106

Sit, G., Jana, A., Chanda, A. & Sahu, S. K. (2023B). Record of zipper loach *Paracanthocobitis mackenziei* (Chaudhuri, 1910), an indigenous ornamental fish from West Bengal, India. *Journal of Fisheries*, *11*(2), 112402-112402. https://doi.org/10.17017/j.fish.477

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EISSN 2602-473X

AQUATIC SCIENCES AND ENGINEERING

Aquat Sci Eng 2024; 39(3): 165-171 • DOI: https://doi.org/10.26650/ASE20241465764

Research Article

The Effect of Temperature on Growth and Odour Production in Three Cyanobacteria Species

Zuhal Zengin[1,2], Reyhan Akcaalan[1]

Cite this article as: Zengin, Z., Akcaalan, R. (2024). The effect of temperature on growth and odour production in three cyanobacteria species. *Aquatic Sciences and Engineering, 39(3)*, 165-171. DOI: https://doi.org/10.26650/ASE20241465764

ABSTRACT

Taste and odour episodes associated with geosmin and 2-methylisoborneol (MIB) produced by Cyanobacteria are common problems affecting drinking water supplies. However, it is difficult to find the source and species responsible for taste and odour production. The aim of this study was to investigate the effect of temperature, one of the most important environmental factors, on the growth and odour production of *Microcoleus* sp. MCAS-MC01 and compare the results with those of two other odour-producing cyanobacteria (*Oscillatoria* sp. UHCC 0332 and *Phormidium* sp. NIVA-CYA 7) isolated and kept in culture collections from geographically different areas. *Microcoleus* sp. MCAS-MC01 is a newly isolated geosmin and MIB producer from Türkiye. After complaints arose from consumers in a nearby city, samples were taken and a cyanobacterium, *Microcoleus* sp., was isolated from the samples and grown in Z8 medium. Experiments were conducted at 20°C and 25°C for 10 weeks, and the growth and MIB-geosmin concentrations of cultures were monitored weekly. The geosmin concentration reached a maximum 65.73 µg/l and the maximum MIB concentration was 626.65 µg/l in the study. Higher temperature had a significant positive effect on MIB levels in *Microcoleus* sp. MCAS-MC01 and geosmin levels in *Phormidium* sp. NIVA-CYA7 ($p<0.05$). On the other hand, the temperature did not affect the growth of all three strains ($p>0.05$). The results showed that taste and odour problems are species-specific, and in some species, they are stimulated by an increase in temperature. This study contributes to the understanding of taste and odour problems in relation to temperature.

Keywords: Cyanobacteria, Geosmin, 2-methylisoborneol, *Microcoleus*, *Oscillatoria*, *Phormidium*

ORCID IDs of the author:
Z.Z. 0000-0002-6560-6789;
R.A. 0000-0002-0756-8972

[1]Department of Marine and Freshwater Resources Management, Faculty of Aquatic Sciences, Istanbul University, Istanbul, Türkiye

[2]Istanbul University Institute of Sciences, Istanbul, Türkiye

Submitted:
05.04.2024

Revision Requested:
23.04.2024

Last Revision Received:
27.04.2024

Accepted:
06.05.2024

Correspondence:
Zuhal Zengin
E-mail:
zuhal.t@istanbul.edu.tr

INTRODUCTION

Climate change and eutrophication are a growing global issue and cause an increase in the occurrence of taste- and odour-producing cyanobacteria, which are particularly significant in drinking water reservoirs (Akcaalan et al. 2022; Mantzouki et al. 2018). Even if hazardous chemicals are not always the source of aquatic taste and odour, the drinking water sector suffers adverse effects as a result (Watson 2004), since customers consider taste and odour as the main measure of the safety of drinking water and complain at levels as low as 10 ng/l geosmin and MIB (Zamyadi et al. 2015). Geosmin and MIB are produced by members of certain groups of benthic and pelagic aquatic microorganisms found in freshwater ecosystems (Watson & Jüttner 2019). Furthermore, there are a few additional biological sources that are often overlooked, especially those that originate from drinking water treatment plants and terrestrial ecosystems (Jüttner & Watson 2007).

Water utilities rarely monitor benthic cyanobacteria, although there is growing evidence that they represent a significant source of taste and odour problems (Gaget et al. 2022). Benthic cyanobacteria grow in different matrices such as sediments, biofilms, and floating mats, and they can detach and colonise in treatment plants (Gaget et al. 2020). However, a systematic under-

standing of how benthic cyanobacteria contribute to taste and odour episodes is still lacking (Gaget et al. 2022). To date, some benthic taste and odour-producing cyanobacteria from *Microcoleus, Oscillatoria, Phormidium, Heteroleibleinia, Leibleinia, Plectonema, Nostoc, Tychonema, Kamptonema, Jaaginema, Lyngbya, Leptolyngbya,* and *Symplocastrum* genera have been reported as geosmin or MIB producers (Watson et al. 2016). A total of 132 cyanobacterial strains from 21 genera and 72 cyanobacterial strains from 13 genera have been reported for geosmin and MIB production, respectively (Devi et al. 2021).

Consumer complaints are an important step in coping with taste and odour problems in drinking water. After increasing complaints about the taste and odour from a nearby city, samples were taken to measure geosmin and MIB in the drinking water reservoir. Some samples from benthic mats at the entrance of treatment plants were also taken, and the odour-producing cyanobacterium were isolated. The species was identified as *Microcoleus* sp. MCAS-MC01, which is the first isolate to produce MIB and geosmin in Türkiye. Furthermore, to reveal the effect of temperature on growth and taste and odour production, a study was conducted together with two different cyanobacteria strains (*Oscillatoria* sp. UHCC 0332 and *Phormidium* sp. NIVA-CYA 7) isolated from geographically different areas to understand the production of these metabolites in different cyanobacteria strains.

MATERIALS AND METHODS

Cyanobacteria strains, culture conditions, and sampling procedures

Three benthic filamentous cyanobacteria (*Microcoleus* sp., *Oscillatoria* sp. and *Phormidium* sp.) were used in this study. *Microcoleus* sp. MCAS-MC01 (Microalgae Culture Collection of Faculty of Aquatic Sciences, İstanbul University) was isolated from a drinking water treatment plant. The isolate was identified using molecular and morphological methods according to Strunecký et al. (2013). *Oscillatoria* sp. UHCC 0332 was obtained from the HAMBI Microbial Culture Collection, University of Helsinki. It is a known geosmin and MIB producer. *Phormidium* sp. NIVA-CYA 7 was obtained from the Norwegian Culture Collection of Algae, NORCCA. It is a known geosmin producer. All strains were cultured in Z8 medium at 20°C and 25°C with a 12 h light:12 h dark period for 10 weeks, and sampling was performed at weekly intervals. The lower temperature was selected as 20°C because cyanobacterial dominance generally occurs at higher (>20°C) temperatures in aquatic ecosystems (Ozbayram et al. 2022; Robarts & Zohary, 1987). The higher selected temperature was 25°C because the benthic cyanobacteria growth rate is generally optimum around 25°C in natural ecosystems and the 5°C temperature change could have a different effect on different cyanobacterial species (Mantzouki et al. 2018). To homogenise the aggregated cultures before sampling, the cultures were sonicated from the 2nd week. The growth of the three cyanobacteria strains was estimated by measuring the optical density at 750 nm (OD750) (Ernst et al. 2005).

Quantitative analysis of odour production

Geosmin and MIB analysed by Headspace-Solid Phase Microextraction (HS-SPME) coupled Gas Chromatography-Mass Spectrometry (GC-MS) according to a standard protocol (Kaloudis et al., 2016). The GC oven temperature was started at 50°C and reached 250°C (12°C/min). Helium was used as the mobile phase (1 ml/min). The commercial standards of geosmin and MIB (Sigma CRM47525) were used for qualitative and quantitative analyses. Geosmin and MIB were quantified according to their m/z ratios (95 for MIB and 112 for geosmin) using the selected ion mode. Qualitative m/z values for ions were 112 and 126 for geosmin and 95, 108, and 135 for MIB.

Statistics

The t-test in "rstatix" package (Kassambara, 2023) in R software was used to analyse the temperature effect on growth and odour (geosmin and MIB) concentration at a 95% confidence level (ver. 4.3.1) (R Core Team 2024). Spearman correlation with the level of significance as $p<0.001$, $p<0.05$, and $p<0.01$ was used to analyse the correlations between growth and odour concentration (geosmin and MIB) in Jamovi software (ver.2.3.1) (Jamovi project 2022). One-way ANOVA analysis was conducted to analyse temporal variance of geosmin and MIB concentrations at a 99% confidence level in Jamovi.

RESULTS AND DISCUSSION

Odour production was observed in all three cyanobacteria species for 10 weeks at different concentrations. The temperature had no statistically significant effect on growth ($p>0.05$) indicating these two temperatures (20°C and 25°C) did not have a dramatic effect on the growth of the three cyanobacteria strains.

It was observed that the cultures generally started to lose their colour after the 9th week of the experiment (Figure 1). *Microcoleus* sp. MCAS-MC01 and *Oscillatoria* sp. UHCC 0332 cultures started to die earlier at 20°C than at 25°C. On the other hand, *Phormidium* sp. NIVA-CYA 7 culture started to lose its colour earlier at 25°C than at 20°C (Figure 1).

Our results showed that temperature had a significant positive

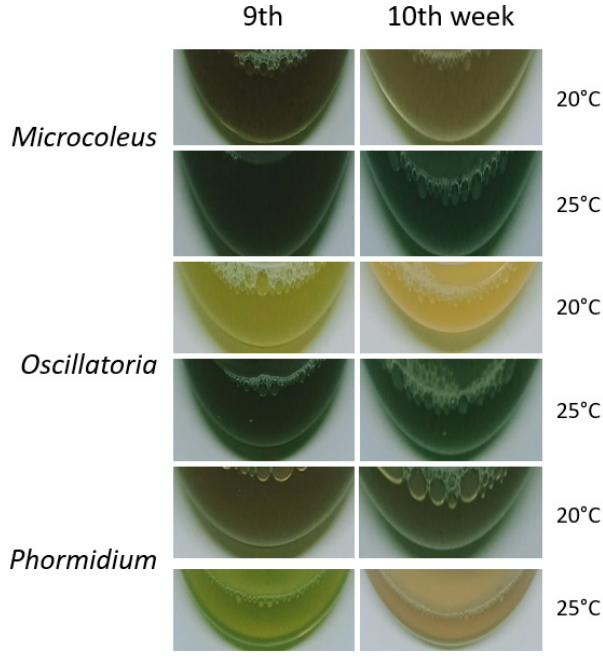

Figure 1. Colour changes in cultures.

effect on MIB production in *Microcoleus* sp. ($t_{(11.85)}$=-2.82, p<0.05) and geosmin production in *Phormidium* sp. ($t_{(10.47)}$=-2.19, p<0.05). However, there was no significant effect on geosmin production in the other two strains and MIB production in *Oscillatoria* sp. (p>0.05) (Figure 2).

The maximum geosmin concentration was detected in the 8th week at both temperatures reaching 63.11 µg/l at 20°C (Figure 2a) and 65.73 µg/l at 25°C in *Microcoleus* sp. (Figure 2b). Geosmin production followed an increasing trend until the 8th week and started to decrease afterwards (Figure 2). The optical density values increased at both temperatures during the experiment. Although geosmin production did not differ with temperature, the geosmin concentration of *Microcoleus* sp. MCAS-MC01 culture was dramatically higher than the threshold limits (10 ng/l) and reported environmental concentrations (Qiu et al. 2023). *Microcoleus* species are ubiquitous and form thick mats in different habitats, including freshwater ecosystems, a street flowerbed in an urbanised area, garden soil, and aquaculture areas (Tee et al. 2021; Churro et al. 2020; Alghanmi et al. 2018). For example, an episodic taste and odour problem related to the geosmin producer *Microcoleus* sp. was reported in a trout farming area, which was linked to the appearance of earthy/musty off-flavour and poor water quality during the water recirculation period (Robin et al. 2006). However, there are a limited number of isolated and identified geosmin/MIB-producing *Microcoleus* species: *Microcoleus pseudautumnalis* and *M. autumnalis* (formerly *Phormidium autumnale*) from Japan, *Microcoleus vaginatus* CCALA 145 (formerly *Phormidium autumnale*) from Switzerland, *Microcoleus vaginatus* from Iraq, *M. asticus* from Portugal and *Microcoleus* sp. from USA (Teneva et al. 2023; Churro et al. 2020; Niiyama & Tuji, 2019; Alghanmi et al. 2018; Oikawa et al. 2015; Izaguirre & Taylor, 1995). Therefore, our newly isolated geosmin and MIB producer strain from Türkiye makes a major contribution to research on taste and odour producers from *Microcoleus* genus, demonstrating a wide geographical distribution.

MIB levels at 25°C were significantly higher than those at 20°C in *Microcoleus* sp. ($t_{(11.85)}$=-2.82, p<0.05) reaching 626.65 µg/l at 25 °C in the 5th week (Figure 3b) and 320.48 µg/l at 20°C in the 8th week (Figure 3a). MIB production increased until the 8th and 5th weeks at 20°C and 25°C, respectively, and started to decrease in the following weeks (Figure 3). In line with our result, Alghanmi et al. (2018) also found maximum MIB concentrations at 25°C in *Microcoleus vaginatus* compared with 10°C and 33°C. Similarly, Microcoleus *autumnalis* (formerly *P.autumnale*) produces more MIB at high water temperatures above 20°C (Oikawa et al. 2015). In our study, the maximum total MIB level of our strain (626.65 µg/l) was significantly higher than the MIB concentrations measured in *M. vaginatus* (135.8 ng/l) and *Lyngbya* sp. (260 µg/l) (Izaguirre & Taylor, 1995). The results show that our isolate, *Microcoleus* sp., can respond to increasing temperatures by producing higher concentrations of the odour compound (MIB) and by surviving longer in the environment. Our strain is isolated from a water treatment plant that supplies drinking water to more than 200.000 people. These results are critical for determining water quality management strategies to be implemented in water treatment plants. Water authorities may encounter the problem intermittently in their drinking water systems, especially in summer months, and it may be necessary to implement appropriate treatment systems, such as ozonation or activated carbon, to be used when odour episodes occur (Oikawa et al. 2015).

Temperature had no significant effect on geosmin and MIB production in *Oscillatoria* sp. (p>0.05). The maximum geosmin production was 125.86 µg/l at 25°C and 90.8 µg/l at 20°C in the 9th week, followed by a significant decrease in the geosmin concentrations at both temperatures (Figure 4). Geosmin concentration increased from the 4th week at 20°C until the 9th week, and following this, the level of geosmin started to decrease (Figure 4a), whereas at 25°C, the trend over time was not as pronounced (Figure 4b).

On the contrary, maximum MIB concentrations were significantly higher than geosmin at both temperatures and measured as 1339 µg/l at 25 °C (9th week) and 768.69 µg/l at 20°C (8th week) (Figure 5). A significant MIB increase was observed at 20°C from the 4th week until the 8th week, following which the level of MIB

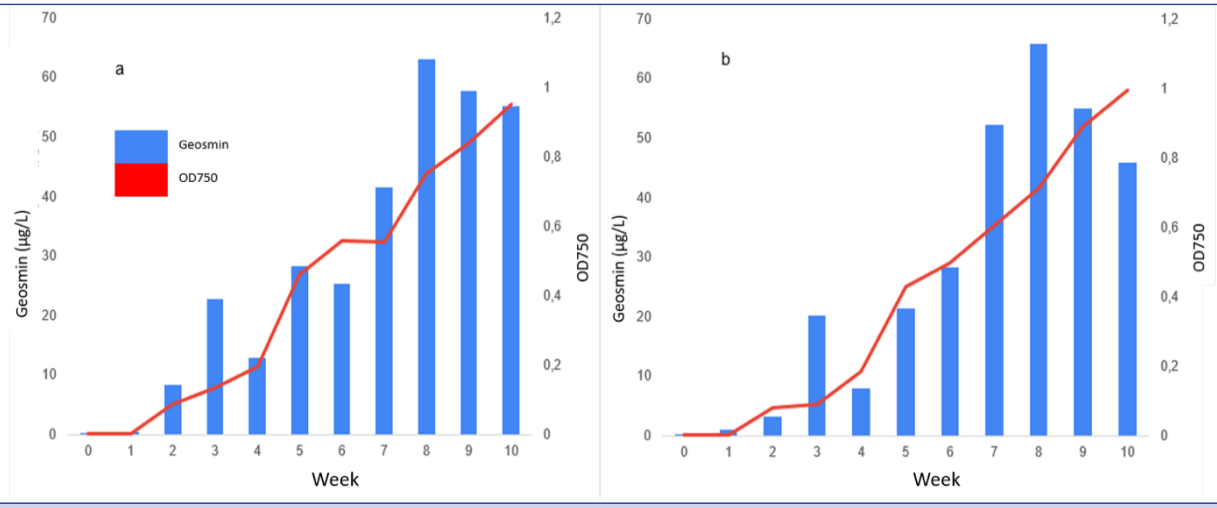

Figure 2. Comparison of geosmin production and growth in *Microcoleus* sp. at a) 20°C and b) 25°C.

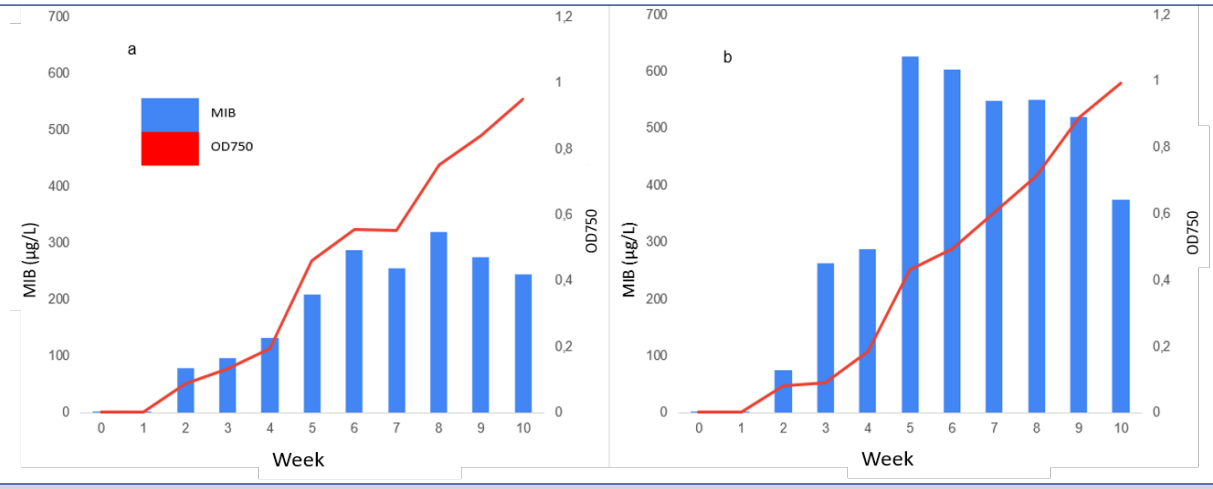

Figure 3. Comparison of MIB production and growth in *Microcoleus* sp. at a) 20°C and b) 25°C.

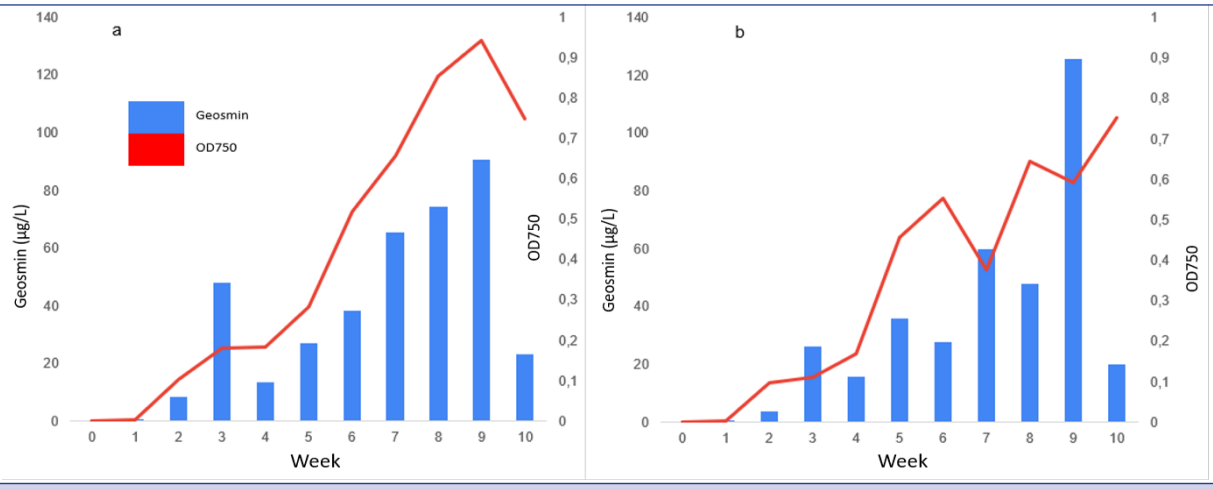

Figure 4. Comparison of geosmin production and growth in *Oscillatoria* sp. at a) 20°C and b) 25°C.

started to decrease (Figure 5a). Although there was not a distinct MIB trend at 25°C over time (Figure 5b), *Oscillatoria* sp. showed better growth at that temperature, consistent with the literature (Sivonen 1990, Cai et al. 2017). Chu et al. (2007) found that *Oscillatoria* sp. became dominant over the common cyanobacterium *Microcystis* sp. at temperatures below 25°C, whereas the contrary occurred at temperatures of 30°C and above. When there is competition between these two cyanobacteria species in natural ecosystems, temperature could be an important driving factor, and *Oscillatoria* sp., an earthy and mouldy odour producer, may have an advantage over *Microcystis* sp., which does not produce such odours, at 25°C and below. The information observed in the experimental studies also supports our understanding of the odour production dynamics in aquatic ecosystems.

The concentrations of geosmin and MIB in *Oscillatoria* sp. varied greatly at both temperatures. Although these metabolites utilise common metabolic pathways in cyanobacteria, their production levels may differ (Jüttner & Watson, 2007). However, since the removal of MIB from water is more difficult compared with geosmin, high levels of MIB can cause a very high economic loss for treatment plants (Zamyadi et al., 2015). The temperature could triggered odour production in various cyanobacteria species. Our results showed that *Oscillatoria* sp. produced both metabolites in higher concentrations at 25 °C than at 20 °C. In line with our results, Cai et al. (2017) also found that the maximum geosmin production in *Oscillatoria limosa* occurred at 25 °C.

Higher temperatures resulted in higher geosmin production in *Phormidium* sp. (Figure 6). The maximum geosmin production was measured at 25°C as 62.06 µg/l in the 7th week (Figure 6b). Our results are in line with *Phormidium amoenum* isolate from South Australia, which had a maximum geosmin concentration measured as 49 µg/l at 25°C (Li et al. 2012). Additionally, *Dolichospermum smithii* could also produce a maximum total geosmin concentration of 19.82 µg/l at 25°C (Shen et al. 2022). In our study, the maximum geosmin production at 25°C was three times higher than that at 20°C (19.02 µg/l at 20°C, 62.06 µg/l at 25°C) in *Phormidium* sp. The geosmin concentration started to increase from the 5th week until the 7th week and started to decrease the following weeks at 25°C (Figure 6b). On the other hand, there was no significant change over time, with maximum production

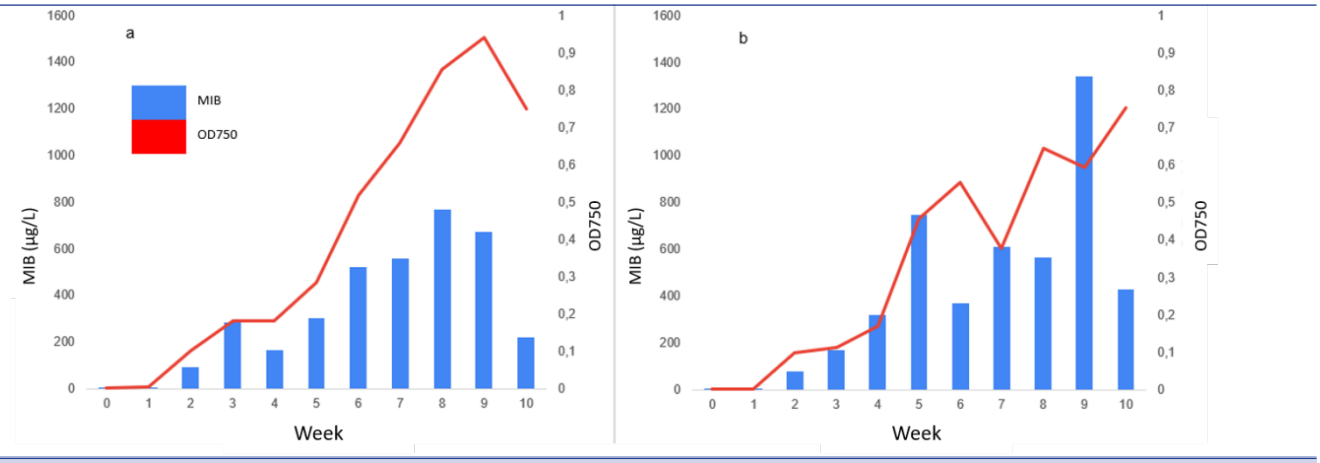

Figure 5. Comparison of MIB production and growth in *Oscillatoria* sp. at a) 20°C and b) 25°C.

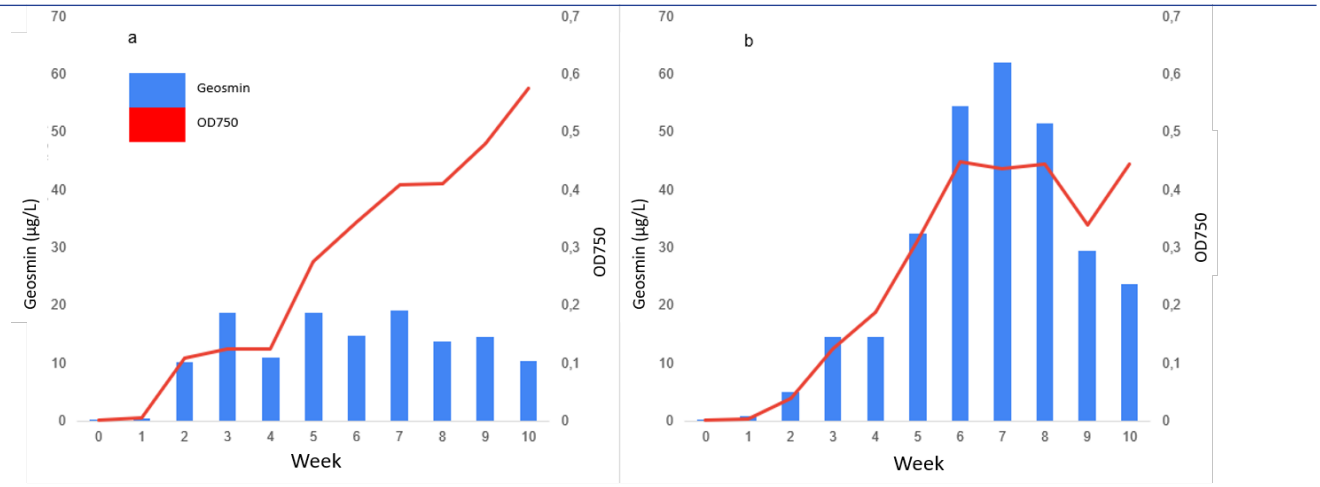

Figure 6. Comparison of geosmin production and growth in *Phormidium* sp. at a) 20°C and b) 25°C.

at week 7 being slightly higher than in other weeks at 20°C (Figure 6a). However, the growth curve of the strain maintained at 20°C continued to increase throughout the experiment. Fujimoto et al. (1997) found that *Phormidium* sp. could become dominant over *Microcystis* sp. at 20°C and a high N:P ratio, whereas the advantage shifted to *Microcystis* sp. at 25°C. Therefore, the competitive advantage of *Phormidium* sp. and associated odour production at relatively lower temperatures in aquatic environments should be considered in water quality management.

These three odour-producing cyanobacteria, *Microcoleus* sp. MCAS-MC01, *Oscillatoria* sp. UHCC 0332 and *Phormidium* sp. NIVA-CYA 7, showed similar thermotolerance profiles at 20°C and 25°C. Comparing the total geosmin production capacity, *Oscillatoria* sp. UHCC 0332 showed highest capacity reaching up 125.86 µg/l, *Microcoleus* sp. MCAS-MC01 reaching up 63.11 µg/l, and *Phormidium* sp. NIVA-CYA 7 to 62.06 µg/l (Figure 7). Regarding MIB production capacity, *Oscillatoria* sp. UHCC 0332 showed higher capacity by reaching up 1339 µg/l in comparison to the highest concentration measured as 626.65 µg/l in *Microcoleus* sp. (Figure 8).

Geosmin variation was significant over time in *Microcoleus* sp., *Oscillatoria* sp., *and Phormidium* sp. at both 20°C and 25°C (Figure 7). Comparing the temporal variation of geosmin, the concentration was significantly different for each week at both 20°C and 25°C in all strains except for *Phormidium* sp. (Figure 7).

Comparing temporal variation of MIB concentration, each week was significantly different in *Microcoleus* sp. and *Oscillatoria* sp. at both 20°C and 25°C (Figure 8).

Geosmin had a strong positive correlation with growth in *Microcoleus* sp. at both temperatures ($r = 0.903$ for 20°C and $r=0.842$ for 25°C respectively, $p < 0.001$), while MIB had only a strong correlation with growth at 20°C ($r=0.840$ $p<0.001$). At 25°C, a moderate positive correlation between MIB and growth ($r = 0.588$ $p<0.05$) was detected . Similarly, both geosmin and MIB had a strong positive correlation with growth in *Oscillatoria* sp. at both temperatures ($r = 0.794$, $p < 0.01$ for geosmin at 20°C and $r=0.612$, $p<0.05$ at 25°C; $r = 0.842$, $p < 0.01$ for MIB at 20°C and $r=0.721$, $p<0.05$ at 25°C) (Figure 4 and Figure 5), which was consistent with the results of previous studies (Shen et al. 2022, Jüttner & Watson 2007). Shen et al. (2022) highlighted that tem-

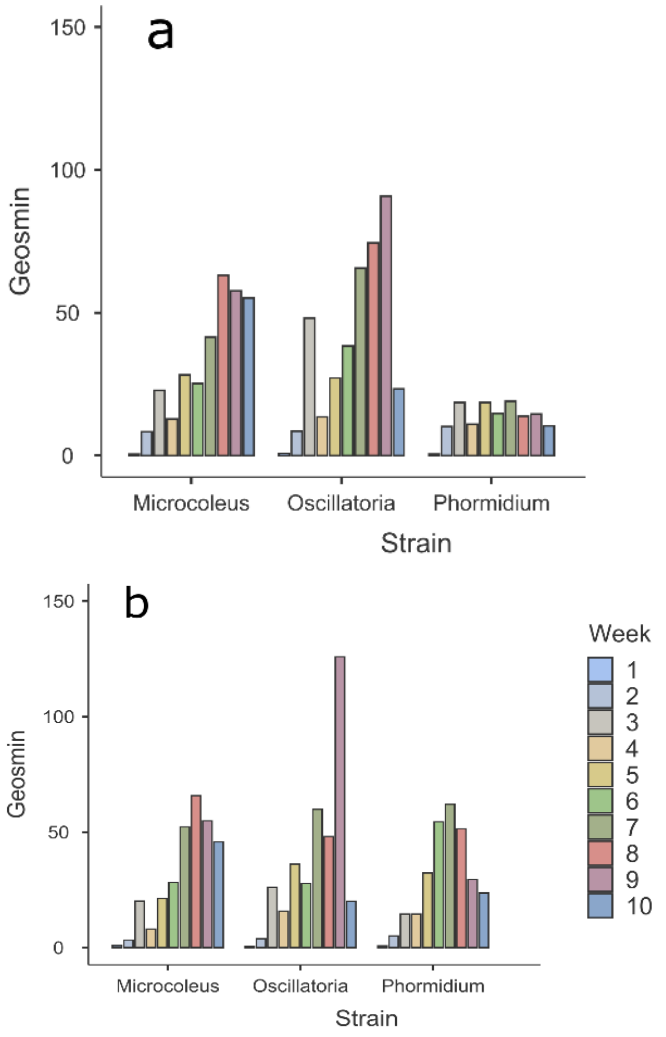

Figure 7. Comparison of total geosmin concentration in three strains (a. 20°C, b. 25°C).

perature can increase the potential of geosmin/MIB synthesis. We also found 20°C - 25°C temperature range is generally favourable for growth and odour production in cyanobacteria. Geosmin had no correlation with growth in *Phormidium* sp. at 20°C ($r = 0.274$ $p > 0.05$) (Figure 6a), whereas it had a strong correlation at 25°C ($r = 0.851$ $p < 0.001$) (Figure 6b). We can conclude that geosmin production in *Phormidium* sp. at 20°C may be suppressed because of a possible competition for the essential common substrates with Chl-*a* synthesis, as we found growth continued while geosmin concentration had a generally stationary trend at 20°C (Shen et al. 2021; Wang & Li, 2015).

CONCLUSION

Our results highlight the important role of the strain-specific response to temperature changes. Furthermore, the production of geosmin and MIB compounds differed under different temperatures in our strain *Microcoleus* sp. MCAS-MC01 and *Phormidium* sp. NIVA CYA7. We found that a 5°C temperature difference had a dramatic effect on the odour production of different cyanobacterial strains. The total

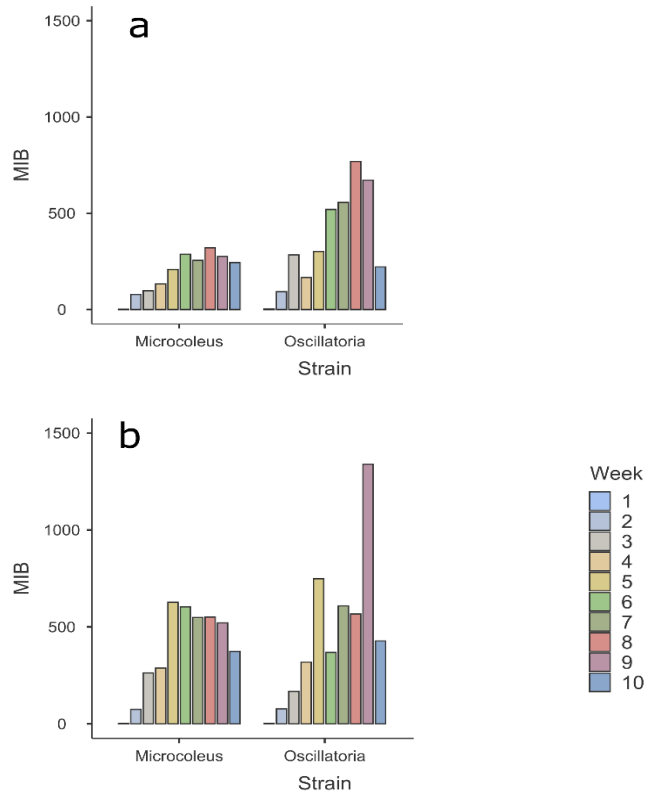

Figure 8. Comparison of total MIB concentration in three strains (a. 20°C, b. 25°C).

odour concentrations without comparing intra- and extracellular components were measured in this study. Further studies are needed to address whether the temperature had a mechanism to increase intracellular odour production or to affect the release of odour compounds outside the cell. In addition to *in vitro* studies, to predict the effects of changing environmental conditions on odour production in natural ecosystems, it is also important to determine the levels of odour compounds using *in situ* studies, such as mesocosm experiments to analyse other biotic and abiotic triggers.

Conflict of Interest: The author has no conflicts of interest to declare.

Ethics committee approval: Ethics committee approval is not required.

Financial disclosure: This study was funded by the Istanbul University Research Fund (Project numbers: 35783 and 37896)

REFERENCES

Akcaalan, R., Devesa-Garriga, R., Dietrich, A., Steinhaus, M., Dunkel, A., Mall, V., ... & Kaloudis, T. (2022). Water taste and odor (T&O): Challenges, gaps and solutions from a perspective of the WaterTOP network. *Chemical Engineering Journal Advances, 12*, 100409. https://doi.org/10.1016/j.ceja.2022.100409

Alghanmi, H. A., Fo'ad, M. A., & Al-Taee, M. M. (2018). Effect of light and temperature on new cyanobacteria producers for geosmin and 2-methylisoborneol. *Journal of Applied Phycology, 30*(1), 319-328. https://doi.org/10.1007/s10811-017-1233-0

Cai, F., Yu, G., Zhang, K., Chen, Y., Li, Q., Yang, Y., Xie, J., Wang, Y., & Li, R. (2017). Geosmin production and polyphasic characterization of *Oscillatoria limosa* Agardh ex Gomont isolated from the open canal of a large drinking water system in Tianjin City, China. *Harmful Algae, 69*, 28–37. https://doi.org/10.1016/J.HAL.2017.09.006

Churro, C., Semedo-Aguiar, A. P., Silva, A. D., Pereira-Leal, J. B., & Leite, R. B. (2020). A novel cyanobacterial geosmin producer, revising Geo A distribution and dispersion patterns in Bacteria. *Scientific Reports, 10*(1), 8679. https://doi.org/10.1038/s41598-020-64774-y

Devi, A., Chiu, Y. T., Hsueh, H. T., & Lin, T. F. (2021). Quantitative PCR based detection system for cyanobacterial geosmin/2-methylisoborneol (2-MIB) events in drinking water sources: Current status and challenges. *Water research, 188*, 116478. https://doi.org/10.1016/j.watres.2020.116478

Ernst, A., Deicher, M., Herman, P. M., & Wollenzien, U. I. (2005). Nitrate and phosphate affect cultivability of cyanobacteria from environments with low nutrient levels. *Applied and environmental microbiology, 71*(6), 3379-3383. https://doi.org/10.1128/AEM.71.6.3379-3383.2005.

Fujimoto, N., Sudo, R., Sugiura, N., & Inamori, Y. (1997). Nutrient-limited growth of Microcystis aeruginosa and Phormidium tenue and competition under various N: P supply ratios and temperatures. *Limnology and Oceanography*, *42*(2), 250-256. https://doi.org/10.4319/lo.1997.42.2.0250

Gaget, V., Hobson, P., Keulen, A., Newton, K., Monis, P., Humpage, A. R., ... & Brookes, J. D. (2020). Toolbox for the sampling and monitoring of benthic cyanobacteria. *Water research, 169*, 115222. https://doi.org/10.1016/j.watres.2019.115222

Gaget, V., Almuhtaram, H., Kibuye, F., Hobson, P., Zamyadi, A., Wert, E., & Brookes, J. D. (2022). Benthic cyanobacteria: A utility-centred field study. *Harmful Algae, 113*, 102185. https://doi.org/10.1016/j.hal.2022.102185

Izaguirre, G., & Taylor, W. D. (1995). Geosmin and 2-methylisoborneol production in a major aqueduct system. Water science and technology, 31(11), 41-48. https://doi.org/10.1016/0273-1223(95)00454-U

The jamovi project (2022). jamovi. (Version 2.3) [Computer Software] (https://www.jamovi.org)

Jüttner, F., & Watson, S. B. (2007). Biochemical and ecological control of geosmin and 2-methylisoborneol in source waters. *Applied and environmental microbiology, 73*(14), 4395-4406. https://doi.org/10.1128/AEM.02250-06

Kaloudis, T., Triantis, T. M. , & Hiskia, A. (2016). Determination of Geosmin and 2-Methylisoborneol in Water by HS-SPME-GC/MS. In J. Meriluoto, L. Spoof, & G. A. Codd (Eds.), *Handbook of Cyanobacterial Monitoring and Cyanotoxin Analysis (1st ed.)*. John Wiley & Sons, Ltd. https://doi.org/10.1002/9781119068761

Kassambara (2023) Pipe-friendly Framework for Basic Statistical Tests in R (https://rpkgs.datanovia.com/rstatix/)

Li, Z., Hobson, P., An, W., Burch, M. D., House, J., & Yang, M. (2012). Earthy odor compounds production and loss in three cyanobacterial cultures. *Water research, 46*(16), 5165-5173. https://doi.org/10.1016/j.watres.2012.06.008

Mantzouki, E., Lürling, M., Fastner, J., de Senerpont Domis, L., Wilk-Woźniak, E., Koreivienė, J., ... & Warming, T. P. (2018). Temperature effects explain continental scale distribution of cyanobacterial toxins. *Toxins, 10*(4), 156. https://doi.org/10.3390/toxins10040156

Niiyama, Y., & Tuji, A. (2019). *Microcoleus pseudautumnalis* sp. nov. (Cyanobacteria, Oscillatoriales) producing 2-methylisoborneol. *Bulletin of the National Museum of Nature and Science, 45*, 93–101, https://cir.nii.ac.jp/crid/1523388080253362688

Oikawa, T., Tsunoda, T., Nakahigashi, H., Shimoriku, M., Kanami, T., & Kimura, S. (2015). Musty odor producing benthic cyanobacteria in the Tama River (Japan) and identification of species by genetic analysis. *Journal of Water Supply: Research and Technology—AQUA, 64*(7), 839-846. https://doi.org/10.2166/aqua.2015.068

Ozbayram, E.G., Camur, D., Koker, L., Oguz Cam, A., Akcaalan, R., Albay, M. (2022). The effects of climate change on aquatic ecosystems in relation to human health. *Aquatic Sciences and Engineering, 37*(3), 123-128. https://doi.org/10.26650/ASE20221057160

Qiu, P., Zhang, Y., Mi, W., Song, G., & Bi, Y. (2023). Producers and drivers of odor compounds in a large drinking-water source. *Frontiers in Ecology and Evolution, 11*, 1216567. https://doi.org/10.3389/fevo.2023.1216567

R Core Team (2024) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria (https://www.R-project.org)

Robarts, R. D., & Zohary, T. (1987). Temperature effects on photosynthetic capacity, respiration, and growth rates of bloom-forming cyanobacteria. *New Zealand journal of marine and freshwater research, 21*(3), 391-399. https://doi.org/10.1080/00288330.1987.9516235

Robin, J., Cravedi, J. P., Hillenweck, A., Deshayes, C., & Vallod, D. (2006). Off flavor characterization and origin in French trout farming. *Aquaculture*, *260*(1-4), 128-138. https://doi.org/10.1016/j.aquaculture.2006.05.058

Shen, Q., Wang, Q., Miao, H., Shimada, M., Utsumi, M., Lei, Z., ... & Shimizu, K. (2022). Temperature affects growth, geosmin/2-methylisoborneol production, and gene expression in two cyanobacterial species. *Environmental Science and Pollution Research, 29*, 12017–12026. https://doi.org/10.1007/s11356-021-16593-5

Shen, Q., Shimizu, K., Miao, H., Tsukino, S., Utsumi, M., Lei, Z., ... & Akiba, M. (2021). Effects of elevated nitrogen on the growth and geosmin productivity of *Dolichospermum smithii*. *Environmental Science and Pollution Research, 28*, 177-184. https://doi.org/10.1007/s11356-020-10429-4

Sivonen, K. (1990). Effects of light, temperature, nitrate, orthophosphate, and bacteria on growth of and hepatotoxin production by *Oscillatoria agardhii* strains. *Applied and environmental microbiology, 56*(9), 2658. https://doi.org/10.1128/aem.56.9.2658-2666.1990

Strunecký, O., Komárek, J., Johansen, J., Lukešová, A., & Elster, J. (2013). Molecular and morphological criteria for revision of the genus *Microcoleus* (Oscillatoriales, C yanobacteria). *Journal of phycology, 49*(6), 1167-1180. https://doi.org/10.1111/jpy.12128

Tee, H. S., Wood, S. A., Bouma-Gregson, K., Lear, G., & Handley, K. M. (2021). Genome streamlining, plasticity, and metabolic versatility distinguish co-occurring toxic and nontoxic cyanobacterial strains of Microcoleus. *Mbio*, *12*(5), e02235-21 https://doi.org/10.1128/mbio.02235-21

Teneva, I., Belkinova, D., Paunova-Krasteva, T., Bardarov, K., Moten, D., Mladenov, R., & Dzhambazov, B. (2023). Polyphasic characterisation of *Microcoleus autumnalis* (Gomont, 1892) Strunecky, Komárek & JR Johansen, 2013 (Oscillatoriales, Cyanobacteria) using a metabolomic approach as a complementary tool. *Biodiversity Data Journal, 11*. https://doi.org/10.3897/BDJ.11.e100525

Wang, Z., & Li, R. (2015). Effects of light and temperature on the odor production of 2-methylisoborneol-producing *Pseudanabaena* sp. and geosmin-producing *Anabaena ucrainica* (cyanobacteria). *Biochemical Systematics and Ecology, 58*, 219-226. https://doi.org/10.1016/j.bse.2014.12.013

Watson, S., & Jüttner, F. (2019). Biological production of taste and odour compounds. Taste and Odour in Source and Drinking Water: Causes, Controls, and Consequences. London, UK, IWApublishing, 63-112. https://doi.org/10.2166/9781780406664_0063

Watson, S. B., Monis, P., Baker, P., & Giglio, S. (2016). Biochemistry and genetics of taste-and odor-producing cyanobacteria. *Harmful Algae, 54*, 112-127. https://doi.org/10.1016/j.hal.2015.11.008

Watson, S. B. (2004). Aquatic taste and odor: a primary signal of drinking-water integrity. *Journal of Toxicology and Environmental Health, 67*(20-22), 1779-1795. https://doi.org/10.1080/15287390490492377

Zamyadi, A., Henderson, R., Stuetz, R., Hofmann, R., Ho, L., & Newcombe, G. (2015). Fate of geosmin and 2-methylisoborneol in full-scale water treatment plants. *Water research, 83*, 171-183. https://doi.org/10.1016/j.watres.2015.06.038

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EISSN 2602-473X

AQUATIC SCIENCES AND ENGINEERING

Aquat Sci Eng 2024; 39(3): 172-178 • DOI: https://doi.org/10.26650/ASE20241397385

Research Article

Phenotypic and Genotypic Antibiotic Resistance of *Staphylococcus warneri* and *Staphylococcus pasteuri* Isolated from Stuffed Mussels

Dilek Kahraman Yılmaz[1], Nermin Berik[2]

Cite this article as: Kahraman Yılmaz, D., & Berik, N. (2024). Phenotypic and genotypic antibiotic resistance of *Staphylococcus warneri* and *Staphylococcus pasteuri* isolated from stuffed mussels. *Aquatic Sciences and Engineering, 39(3)*, 172-178. DOI: https://doi.org/10.26650/ASE20241397385

ABSTRACT

This study aimed to assess the presence of Staphylococcus species in stuffed mussel samples sold in Çanakkale province and to determine the antibiotic resistance of the isolates. A total of 246 stuffed mussel samples were examined, and two different *Staphylococcus* (*S. warneri* and *S. pasteuri*) were isolated from 12.19% of the samples. Among the Staphylococcus isolates, *S. warneri* was detected at a rate of 73.33% and *S. pasteuri* at 26.67%. Bacterial isolates (N=30) were examined for their resistance to amoxicillin/clavulanic acid (30 µg), ampicillin (10 µg), erythromycin (15 µg), clindamycin (10 µg), vancomycin (30 µg), oxacillin (5 µg), tetracycline (30 µg), doxycycline hydrochloride (30 µg), chloramphenicol (30 µg), streptomycin (10 µg), gentamicin (10 µg), kanamycin (30 µg), nalidixic acid (30 µg), ciprofloxacin (5 µg), meropenem (10 µg), imipenem (10 µg), sulfamethox/ trimethoprim (25 µg), cefotaxime (30 µg), cephalothin (30 µg), ceftriaxone (30 µg), and levofloxacin (5 µg) antibiotics. Resistance to amoxicillin/clavulanic acid and erythromycin was found in all *S. warneri* isolates. In addition, all S. pasteuri isolates were found to be resistant to amoxicillin/clavulanic acid. In *S. warneri* isolates, at least three resistance genes (*BlaTEM, tetB-6, tetK-8*) and up to eight resistance genes (*BlaTEM, tetB-6, tetK-8, strA-strB, aphAI-IAB, ermC*) were identified. All *S. pasteuri* isolates exhibited blaTEM, strA-strB, and aphAI-IAB resistance genes. In conclusion, it was determined that antibiotic-resistant *S. warneri* and/or *S. pasteuri* contaminates stuffed mussel samples. This study will serve as a valuable resource for enhancing monitoring strategies in stuffed mussel production. Further studies should be conducted to determine whether the products are suitable for food safety. Therefore, it is necessary to clarify the disease effects and mechanisms of the pathogens identified in stuffed mussels.

Keywords: Stuffed mussel, *Mytilus galloprovincialis*, *S. warneri*, *S. pasteuri*, Public health, antibiotic resistance

ORCID IDs of the author:
D.K.Y. 0000-0002-9626-5446;
N.B. 0000-0003-3015-8688

[1]Department of Marine Biology, Faculty of Marine Sciences and Technology, Çanakkale Onsekiz Mart University, Çanakkale, Turkiye

[2]Department of Fisheries and Processing Technology, Faculty of Marine Sciences and Technology, Çanakkale Onsekiz Mart University, Çanakkale, Turkiye

Submitted:
28.11.2023

Revision Requested:
22.12.2023

Last Revision Received:
05.01.2024

Accepted:
02.02.2024

Online Published:
07.06.2024

Correspondence:
Nermin Berik
E-mail:
nberik@yahoo.com

INTRODUCTION

Consumers directly consume ready-to-eat foods without requiring processing, such as heat treatment (WHO, 1996; Mosupye & von Holy, 1999). Those prepared by street vendors and sold especially on the streets or other public places are also defined as ready-to-eat foods and beverages (FAO, 1997). Ready-to-eat meals are encountered in various presentations and packaging in markets, restaurants, and many other areas, with street foods being the most prevalent. Street foods play a significant role in meeting a substantial part of our daily meal needs and are preferred by everyone, regardless of age or profession. Street foods can be classified as seafood, animal-derived products, pastries, sweets, fruits, and other gastronomic products (Demir et al., 2017). Stuffed mussels, especially in coastal cities, are among the most consumed street foods. Ready-to-eat foods are preferred for reasons such as easy accessibility, quick preparation, affordability, and compatibility with different taste preferences.

The most significant danger regarding street food is the uncertainty regarding its suitability for consumption in terms of food safety. The safety of foods depends on various common factors, ranging from the quality of raw materials to food processing and storage practises. Most vendors may expose the meals to conditions that are not suitable for food safety, such as "cross-contamination, unhygienic storage, and poor time-temperature" conditions (Lucca and Torres, 2006; Mepba et al., 2007). Products produced and sold without considering food safety can lead to situations that endanger human health. Bacteria that contaminate food due to various contamination sources and cause epidemic diseases in humans are among the primary concerns. In addition to causing various infections and diseases, these bacteria are becoming increasingly resistant to antibiotics, leading to challenging-to-treat illnesses. The World Health Organisation (WHO, 2017) has declared antibiotic-resistant bacteria as the "greatest and most urgent global threat" because as resistance to antibiotics increases, bacteria develop multiple antibiotic resistance. Therefore, antibiotics used for treatment do not yield successful results.

Stuffed mussels, generally prepared using *Mytilus galloprovincialis*, are ready-to-eat meals that are commonly sold on the streets in countries with coastlines along Türkiye and the Mediterranean. Throughout the processing, preparation, and cooking of stuffed mussels, they can be susceptible to human-induced contamination, and during the sales process, they may be exposed to environmental factors such as air pollution and insects. The high pH and nutrient profile of stuffed mussels create a favourable environment for microbial growth. Street vendors typically sell stuffed mussels in open-air environments on tables for 6-8 hours or even longer (Kişla and Üzgün 2008). Consumers use the mussel shell itself as a spoon, remove the stuffing, and consume it. In this case, if the mussel shell is contaminated, it becomes a source of risk when it comes into contact with the mouth. Leftover stuffed mussels at the end of the day are sometimes stored inappropriately in refrigerators (+4 °C), often at unsuitable ambient temperatures, to be put up for sale again the next day.

Studies conducted in Türkiye have reported that stuffed mussels may contain *Escherichia coli, Staphylococcus spp., Bacillus cereus, Vibrio alginolyticus, Listeria monocytogenes*, yeast, mold, and anaerobic bacteria (Bingol et al., 2008; Durgun, 2013; Üzgün, 2015; Karademir, 2018; Güngörür, 2019). However, to the best of our knowledge, there is no detailed study examining the phenotypic and genotypic antibiotic resistances of *S. warneri* and/or *S. pasteuri* species isolated from stuffed mussels. *Staphylococcus warneri* has been isolated from various sources, including fermented foods, humans, and numerous animal species (Becker et al., 2014). Studies in the literature have reported the isolation of *S. warneri* from sea cucumbers (Kim et al., 2017) and marine fish flesh (Regecova et al., 2014). *Staphylococcus pasteuri* has been reported to be present in various foods, including goat milk (Chesneau et al., 1993), Italian sausages (Rantsiou et al., 2005), and retail beef (Bhargava et al., 2014), as well as in drinking water (Faria et al., 2009). However, no literature is available regarding the isolation of *S. warneri* and *S. pasteuri* from ready-to-consume stuffed mussels.

S. pasteuri (Petti et al., 2008; Savini et al., 2008; Ramnarain et al., 2019; Morfin-Otero et al., 2012; Sánchez et al., 2013; Savini et al., 2009a; Savini et al., 2009b) and *S. warneri* (Incani et al., 2010; Dimitriadi et al., 2014; Gelman et al., 2022; Hoque et al., 2023a; Hoque et al., 2023b; Louail et al., 2023; Si et al., 2024) were appeared in reports of different diseases that could be associated with human. This study aims to investigate the presence of *S. warneri* and *S. pasteuri*, which are known to cause significant health problems in the respiratory, skin, digestive systems, etc., in humans, in stuffed mussels sold in Çanakkale and their resistance to antibiotics.

MATERIALS AND METHODS

Materials

In the scope of this study, stuffed mussels offered for sale in open and closed spaces in the market of Çanakkale Province were used as the material. Mussel samples (N=246) were collected from a total of 20 locations, with 10 from open spaces (street vendors) and 10 from closed spaces (restaurants, etc.), during the peak consumption seasons of spring (May), summer (June to August), and autumn (September-October) (Table 1). Ready-to-consume stuffed mussel samples were transported to the microbiology laboratory within 30 min in styrofoam boxes at +4°C.

Methods

Isolation and identification of bacteria

For microbiological analyses, 10 g of mussel samples were homogenised in 90 ml of peptone water for 1 min. Decimal dilutions (10^{-1} to 10^{-6}) were prepared from the homogenate. From these dilutions, inoculations were made using the spread plate and pour plate methods. Mannitol Salt Phenol Red Agar (Merck 105404) was used as the culture medium. The culture plates were incubated 35°C for 3 days in the incubator to allow for the development of microorganisms. After the bacteria were purified from the Mannitol Salt Agar medium where they grew, classical methods (colony type, morphology, gramme +/- characteristics, oxidase, catalase, H_2S, indole, etc.) were used for genus-level identification. The isolates were grown for stocks in Brain Heart Infusion (Merck 110493) liquid medium. Subsequently, they were stored at -80°C in cryogenic tubes containing 30% glycerol until further molecular identification and antibiotic susceptibility testing were performed.

Species identification of the bacterial isolates

For DNA (Deoxyribonucleic acid) isolation, the EurX GeneMATRIX Bacterial & Yeast DNA isolation kit from Poland was utilised

Table 1. Sampling plan for the study.

Seoson/ Year	Locations/ Count	Count of the Sample
Spring/2021	Street vendors/2	15
Spring/2021	Restaurants/2	15
Summer/2021	Street vendors/5	60
Summer/2021	Restaurants/5	60
Autumn/2021	Street vendors/3	48
Autumn/2021	Restaurants/3	48

(https://eurx.com.pl/docs/manuals/en/e3580.pdf). The quantity and purity of the obtained DNA after isolation were assessed using the Scientific Nanodrop 2000 device (USA) through spectrophotometric measurements conducted at Thermo.

In the PCR (Polymerase Chain Reaction) study, target gene regions for species identification were amplified using the universal primers 27F–1492R. The primer sequences used were 27F 5' AGAGTTTGATCMTGGCTCAG 3' and 1492R 5' TACGGYTACCTTGTTACGACTT 3' (Lane, 1991). The PCR mixture (35 µL) included approximately 1 PCR buffer, 1.5 mM MgCl2, 0.2 mM dNTP, 100 ng template DNA, 0.3 µM of each primer, and nuclease-free water. PCR amplification was performed using a thermal cycler (Kyratec, Geumcheon gu, Seoul Korea) with the following parameters: 95°C for 5 min (initial denaturation), 30 cycles of 95°C for 45 s (denaturation), 57°C for 45 s (annealing), 72°C for 60 s (extension), and a final step at 72°C for 5 min (final extension). Then, 10 µl of the PCR sample was loaded on a 1.5% agarose gel in 1 TAE containing ethidium bromide, and electrophoresis was performed for 90 min at 100 V. PCR products were cleaned and sequenced by BM Labosis (Ankara, Turkey) using the universal primers 27F–1492R. Sequence editing was performed using BioEdit (Bioedit v7.0.0). 16S sequences were compared against all GenBank *S. warneri* and *S. pasteuri* sequences using the BLASTN search at http://blast.ncbi.nlm.nih.gov/.

Antibiogram tests for *Staphylococcus* isolates

The Kirby-Bauer disc diffusion test was employed to determine the antibiotic resistance of the bacteria (Bauer et al., 1966). The analysis execution and interpretation of test results adhered to the standards set by the Clinical and Laboratory Standards Institute (CLSI, 2015; CLSI, 2017). The bacterial strains stored in the freezer were initially cultured at least twice in tryptic soy (TS) medium. Subsequently, the bacterial isolates were transferred to Mueller-Hinton (MH) solid medium. After successful growth, colonies displaying the best development were selected, and their density was adjusted to 0.5 McFarland in liquid medium (MH). From the adjusted-density liquid medium (0.5 McF), bacterial transfers were made to an appropriate solid culture medium using a sterile cotton swab. After bacterial inoculation on solid culture media, antibiotic discs [amoxicillin/clavulanic acid (30 µg), ampicillin (10 µg), erythromycin (15 µg), clindamycin (10 µg), vancomycin (30 µg), oxacillin (5 µg), tetracycline (30 µg), doxycycline hydrochloride (30 µg), chloramphenicol (30 µg), streptomycin (10 µg), gentamicin (10 µg), kanamycin (30 µg), nalidixic acid (30 µg), ciprofloxacin (5 µg), meropenem (10 µg), imipenem (10 µg), sulfamethox/ trimethoprim (25 µg), cefotaxime (30 µg), cephalothin (30 µg), ceftriaxone (30 µg), and levofloxacin (5 µg) were placed on the culture medium for the disc diffusion test. The plates were then incubated at 35°C for 24 h. The resulting zone diameters were measured using a ruler and interpreted according to the CLSI standards (CLSI, 2015; CLSI, 2017).

Identification of antibiotic resistance genes in bacterial isolates

For the analysis of bacterial antibiotic resistance genes, the EurX GeneMATRIX DNA Isolation Kit from Poland was used to isolate DNA from 30 samples. After DNA isolation, the quantity and purity of the obtained DNA were assessed through spectrophotometric measurements using a Thermo Scientific Nanodrop 2000 device (USA). The contents of the PCR cocktail were adjusted to 20 µL as 1 PCR buffer, 2 mM $MgCl_2$, 0.2 mM dNTP mix, 0.5 µM of each forward and reverse primer, 2 U Taq polymerase, DNA template, and PCR grade distilled water. Amplification results obtained through PCR (kyratec thermocycler) were run on a 1.0% agarose gel prepared in 1 TAE buffer and subjected to electrophoresis at 100 V for 60 min. The gel was then visualised under UV light using an ethidium bromide dye. The size of the amplicons was estimated using 50- and 100-pb DNA size markers. The primer sequences used are presented in Table 2.

RESULTS

Identification of the bacterial species

From 246 mussel samples obtained under different selling conditions, 30 *Staphylococcus* isolates were obtained through culture. Through 16S rRNA analysis, it was determined that the 30 bacterial isolates had a high similarity (≥99.12 - 100%) with species registered in the GenBank database. Eight of the isolated species were identified as *Staphylococcus pasteuri*, and 22 were identified as *Staphylococcus warneri*. In this study, Other staphylococcus members were not isolated, except *S. pasteuri* and *S. warneri*.

Antibiotic resistance findings

The phenotypic and genotypic antibiotic resistance results of the 30 isolates obtained from stuffed mussel samples are presented in Table 3. All *S. warneri* isolates demonstrated resistance to amoxicillin/clavulanic acid and erythromycin among the tested antibiotics. Additionally, seven *S. warneri* isolates exhibited intermediate sensitivity to tetracycline and streptomycin, whereas eight were found to have intermediate sensitivity to tetracycline antibiotics. In *S. warneri* isolates, at least three resistance genes (*BlaTEM, tetB-6, tetK-8*), and up to eight resistance genes (*BlaTEM, tetB-6, tetK-8, strA-strB, aphAI-IAB, ermC*) were identified (Table 3).

In this study, it was found that all *S. pasteuri* isolates were resistant to amoxicillin/clavulanic acid. Eight isolates of S. *pasteuri* were determined to have intermediate sensitivity to ampicillin and streptomycin antibiotics. Resistance genes *blaTEM, strA-strB, and aphAI-IAB* were identified in all *S. pasteuri* isolates.

DISCUSSION

In the present study, it was determined that antibiotic-resistant *S. warneri* and *S. pasteuri* contaminate stuffed mussel samples. Similarly, In Italy, samples taken from the preparation counters of ready-to-eat meal companies revealed the isolation of *S. pasteuri* and *S. warneri*. These isolates were reported to be resistant to antibiotics at rates of 83.3% and 42.9%, respectively, among Staphylococcus species (Marino et al., 2011). In Türkiye, *S. warneri* was isolated from fish gills sold at counters, whereas *S. pasteuri* was isolated from the skin and gills (Çoban & Yaman, 2023).

Similar to the present study, antibiotic resistance of *S. warneri* isolated from seafood was reported in a previous study. Isolates of *S. warneri* from sea fish meat have been reported to be resistant to penicillin, ampicillin, tetracycline, erythromycin, and/or

Table 2. List of primers for the detection of antimicrobial resistance genes.

Targeted gene			Sequence (5'-3')	Amplicon (pb)	References
β-lactamases	bla_{TEM}	F	CATTTCCGTGTCGCCCTTATTC	800	Dallenne et al. 2010
		R	CGTTCATCCATAGTTGCCTGAC		
	$bla_{S}Hv$	F	AGCCGCTTGAGCAAATTAAAC	710	
		R	ATCCCGCAGATAAATCACCAC		
	$bla_{C}rx$-*M*	F	CGCTTTGCGATGTGCAG	550	Paterson et al. 2003
		R	ACCGCGATATCGTTGGT		
	mecA	F	GTGAAGATATACCAAGTGATT	150	Alfatemi et al. 2014
		R	ATGCGCTATAGATTGAAAGGAT		
Tetracycline	*tetA*	F	GTAATTCTGAGCACTGTCGC	950	Sengeløv et al. 2003
		R	CTGCCTGGACAACATTGCTT		
	tetB	F	CTCAGTATTCCAAGCCTTTG	400	Sunde and Sørum, 2001
		R	CTAAGCACTTGTCTCCTGTT		
	tetE	F	GTGATGATGGCACTGGTCAT	1100	Sengeløv et al. 2003
		R	CTCTGCTGTACATCGCTCTT		
	tetK	F	TATTTTGGCTTTGTATTCTTTCAT	1150	Trzcinski et al. 2000
		R	GCTATACCTGTTCCCTCTGATAA		
	tetM	F	ACAGAAAGCTTATTATATAAC	171	Aminov et al. 2001
		R	TGGCGTGTCTATGATGTTCAC		
Aminoglycoside resistance	*strA-strB*	F	TATCTGCGATTGGACCCTCTG	540	Sunde and Sørum 2001
		R	CATTGCTCATCATTTGATCGGCT		
	aphAI-IAB	F	AAACGTCTTGCTCGAGGC	460	Frana et al. 2001
		R	CAAACCGTTATTCATTCGTGA		
	aac(3)-IIa	F	ATGGGCATCATTCGCACA	750	Dai et al. 2010
		R	TCTCGGCTTGAACGAATTGT		
	aac(6')-Ib	F	TTGCGATGCTCTATGAGTGGCTA	480	Katalin, 2000
		R	CTCGAATGCCTGGCGTGTTT		
Macrolides	*ermA*	F	GTTCAAGAACAATCAATACAGAG	420	Lina et al. 1999
		R	GGATCAGGAAAAGGACATTTTAC		
	ermB	F	CCGTTTACGAAATTGGAACAGGTAAAGGGC	360	
		R	GAATCGAGACTTGAGTGTGC		
	ermC	F	GCTAATATTGTTTAAATCGTCAATTCC	570	
		R	GGATCAGGAAAAGGACATTTTAC		

oxacillin, whereas *S. pasteuri* isolates have been reported to be resistant to penicillin, oxacillin, ampicillin, and/or erythromycin antibiotics (Regecová et al., 2014). Moreover, isolates of *S. warneri* from fermented sausage products on the market were reported to be resistant to ampicillin, erythromycin, kanamycin, penicillin G, cefalotin, and cefoxitin antibiotics, whereas *S. pasteuri* was reported to be resistant only to erythromycin (Geniş & Tuncer, 2018). In a study conducted by Çavdar et al. in 2022, *S. warneri* was isolated from hospital water tanks, showers, and taps, whereas *S. pasteuri* was isolated only from the water tank.

S. warneri causes disease in rainbow trout in Türkiye and shows resistance to gentamicin, oxacillin, colistin, oxytetracycline, tylosin, spectinomycin, ampicillin, clindamycin, and erythromycin antibiotics (Diler et al., 2023).

To date, there is limited information regarding the presence of antibiotic resistance genes in isolated species of *S. pasteuri* and *S. warneri* from aquatic products. For example, *S. pasteuri* isolated from Jeotgal has been reported to be resistant to linezolid, penicillin, and trimethoprim antibiotics, with trimethoprim resistance associated with carrying the dfrA gene (Jeong and Lee, 2015).

CONCLUSION

In this study, resistance to amoxicillin/clavulanic acid and erythromycin was observed in all *S. warneri* isolates. In addition, all *S. pasteuri* isolates were found to be resistant to amoxicillin/clavulanic acid. In *S. warneri* isolates, the identified resistance genes include *BlaTEM, tetB-6, tetK-8, strA-strB, aphAI-IAB,* and *ermC*, whereas in *S. pasteuri* isolates, the identified resistance genes are *blaTEM, strA-strB,* and *aphAI-IAB*. Consequently, this study will provide a valuable reference for enhancing monitoring strategies in stuffed mussel production. In further studies, the level of public health risk posed by the product should be determined. Therefore, it is necessary to clarify the pathogenic potential and mechanisms of the pathogens identified in stuffed mussels.

Table 3. *Staphylococcus* isolates identification results and phenotypic/genotypic antibiotic resistance profiles.

No	Species	Similarity*	Antibiotics	Resistance Gene					
1	*Staphylococcus warneri*	MK737139 (99.78%)	E, AMC, TE*, S*	bla_{TEM}	*tetB-6*	*tetK-8*	*strA-strB*	*aphAI-IAB*	*ermC*
2	*Staphylococcus warneri*	MT453899 (99.93%)	E, AMC, TE*	bla_{TEM}	*tetB-6*	*tetK-8*			
3	*Staphylococcus warneri*	MW527395 (100%)	E, AMC, TE	bla_{TEM}	*tetB-6*	*tetK-8*	*ermC*		
4	*Staphylococcus pasteuri*	MT539733 (99.92%)	AMC, AMP*, S*	bla_{TEM}	*strA-strB*	*aphAI-IAB*			
5	*Staphylococcus pasteuri*	MF429378 (99.72%)	AMC, AMP*, S*	bla_{TEM}	*strA-strB*	*aphAI-IAB*			
6	*Staphylococcus warneri*	MT328647 (100%)	E, AMC, TE	bla_{TEM}	*tetB-6*	*tetK-8*	*ermC*		
7	*Staphylococcus pasteuri*	OR976013 (99.12%)	AMC, AMP*, S*	bla_{TEM}	*strA-strB*	*aphAI-IAB*			
8	*Staphylococcus warneri*	MF681861 (99.14%)	E, AMC, TE*, S*	bla_{TEM}	*tetB-6*	*tetK-8*	*strA-strB*	*aphAI-IAB*	*ermC*
9	*Staphylococcus warneri*	KY218866 (99.13%)	E, AMC, TE*	bla_{TEM}	*tetB-6*	*tetK-8*			
10	*Staphylococcus warneri*	KR809427 (99.14%)	E, AMC, TE*, S*	bla_{TEM}	*tetB-6*	*tetK-8*	*strA-strB*	*aphAI-IAB*	*ermC*
11	*Staphylococcus warneri*	ON386170 (99.14%)	E, AMC, TE*, S*	bla_{TEM}	*tetB-6*	*tetK-8*	*strA-strB*	*aphAI-IAB*	*ermC*
12	*Staphylococcus warneri*	KT906680 (99.95%)	E, AMC, TE	bla_{TEM}	*tetB-6*	*tetK-8*	*ermC*		
13	*Staphylococcus warneri*	MW148447 (99.55%)	E, AMC, TE	bla_{TEM}	*tetB-6*	*tetK-8*	*ermC*		
14	*Staphylococcus warneri*	MN512291 (99.15%)	E, AMC, TE*	bla_{TEM}	*tetB-6*	*tetK-8*			
15	*Staphylococcus warneri*	KX826983 (99.15%)	E, AMC, TE*	bla_{TEM}	*tetB-6*	*tetK-8*			
16	*Staphylococcus warneri*	MZ043864 (99.14%)	E, AMC, TE	bla_{TEM}	*tetB-6*	*tetK-8*	*ermC*		
17	*Staphylococcus warneri*	LN794823 (99.15%)	E, AMC, TE	bla_{TEM}	*tetB-6*	*tetK-8*	*ermC*		
18	*Staphylococcus pasteuri*	KJ486553 (99.15%)	AMC, AMP*, S*	bla_{TEM}	*strA-strB*	*aphAI-IAB*			
19	*Staphylococcus warneri*	MF681861 (99.20%)	E, AMC, TE*, S*	bla_{TEM}	*tetB-6*	*tetK-8*	*strA-strB*	*aphAI-IAB*	*ermC*
20	*Staphylococcus pasteuri*	KR809418 (99.70%)	AMC, AMP*, S*	bla_{TEM}	*strA-strB*	*aphAI-IAB*			
21	*Staphylococcus warneri*	MG800691 (99.20%)	E, AMC, TE*, S*	bla_{TEM}	*tetB-6*	*tetK-8*	*strA-strB*	*aphAI-IAB*	*ermC*
22	*Staphylococcus warneri*	MZ043864 (99.20%)	E, AMC, TE	bla_{TEM}	*tetB-6*	*tetK-8*	*ermC*		
23	*Staphylococcus warneri*	MK737139 (99.15%)	E, AMC, TE*	bla_{TEM}	*tetB-6*	*tetK-8*			
24	*Staphylococcus warneri*	MW527395 (99.98%)	E, AMC, TE*, S*	bla_{TEM}	*tetB-6*	*tetK-8*	*strA-strB*	*aphAI-IAB*	*ermC*
25	*Staphylococcus warneri*	MT328647 (99.82%)	E, AMC, TE*	bla_{TEM}	*tetB-6*	*tetK-8*			
26	*Staphylococcus pasteuri*	OR517214 (99.73%)	AMC, AMP*, S*	bla_{TEM}	*strA-strB*	*aphAI-IAB*			
27	*Staphylococcus pasteuri*	KP261074 (99.56%)	AMC, AMP*, S*	bla_{TEM}	*strA-strB*	*aphAI-IAB*			
28	*Staphylococcus warneri*	KP860608 (99.13%)	E, AMC, TE*	bla_{TEM}	*tetB-6*	*tetK-8*			
29	*Staphylococcus warneri*	KP261060 (99.13%)	E, AMC, TE*	bla_{TEM}	*tetB-6*	*tetK-8*			
30	*Staphylococcus pasteuri*	KT582293 (99.37%)	AMC, AMP*, S*	bla_{TEM}	*strA-strB*	*aphAI-IAB*			

*Intermediate, **Accession numbers and Sequence result (similarity with 16S rRNA), AMC: amoxicillin/clavulanic acid, AMP: ampisilin, E: erythromycin, TE: tetracycline, S: streptomycin

Acknowledgments: The authors thank Çanakkale Onsekiz Mart University Scientific Research Projects Coordination Department for supporting this project (FBA-2020-3368).

Conflict of Interest: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethics Committee Approval: Ethics committee approval is not required. Both authors declare that this study does not include any experiments with human or animal subjects.

Financial Disclosure: This study was financially supported by Çanakkale Onsekiz Mart University Scientific Research Projects Coordination Department (project code: FBA-2020-3368).

REFERENCES

Bauer, A. W., Kirby, W. M. M., Sherris, J. C., & Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology*, *45*(4_ts), 493-496. https://doi.org/10.1093/ajcp/45.4_ts.493

Becker, K., Heilmann, C., & Peters, G. (2014). Coagulase-negative staphylococci. *Clinical Microbiology Reviews*, *27*(4), 870-926. https://doi.org/10.1128/CMR.00109-13

Bhargava, K., & Zhang, Y. (2014). Characterization of methicillin-resistant coagulase-negative staphylococci (MRCoNS) in retail meat. *Food Microbiology*, 42, 56-60. https://doi.org/10.1016/j.fm.2014.02.019

Bingol, E. B., Colak, H., Hampikyan, H., & Muratoglu, K. (2008). The microbiological quality of stuffed mussels (Midye Dolma) sold in Istanbul. *British Food Journal*, 110, 1079-1087. https://doi.org/10.1108/00070700810917992

Çavdar, G., Köroğlu, M., Ölmez, M., Şahin, E. Ö., & Altindiş, M. (2022). Hastane İçme ve Kullanma Sularının Mikrobiyolojik Analizi; Sakarya. *Online Türk Sağlık Bilimleri Dergisi*, *7*(3), 346-355.

Chesneau, O., Morvan, A., Grimont, F., Labischinski, H., & El Solh, N. (1993). *Staphylococcus pasteuri sp.* nov., isolated from human, animal, and food specimens. *International Journal of Systematic and Evolutionary Microbiology*, *43*(2), 237-244. https://doi.org/10.1099/00207713-43-2-237

CLSI (2015). Clinical and Laboratory Standards Institute (CLSI). Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard—Tenth Edition. CLSI document M07-A10 (ISBN 1-56238-987-4 [Print]; ISBN 1-56238-988-2 [Electronic]). Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087 USA, 2015.

CLSI (2017). Clinical and Laboratory Standards Institute (CLSI). *Performance Standards for Antimicrobial Susceptibility Testing.* 27th ed. CLSI supplement M100 (ISBN 1-56238-804-5 [Print]; ISBN 1-56238-805-3 [Electronic]). Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087 USA, 2017.

Çoban, E. P., & Yaman, F. (2023). Biofilm Formation and Prevention of Bacteria Isolated from Fish and Fish Stalls. *Acta Aquatica Turcica*, 19(1), 21-34. https://doi.org/10.22392/actaquatr.1116548

Demir, Ş., Akdağ, G., Sormaz, Ü. & Özata, E. (2018). Sokak lezzetlerinin gastronomik değeri: İstanbul sokak lezzetleri. *Güncel Turizm Araştırmaları Dergisi*, 2(Ek.1), 589-601.

Diler, Ö., Yilmaz, H. E., Çağatay, İ. T., Naziroğlu, M., Öznur, Ö. Z. İ. L., & Şeydanur, K. A. N. (2023). Gökkuşağı Alabalıklarında (*Oncorhynchus mykiss*) *Staphylococcus warneri*'nin Teşhis ve Histopatolojisi. *Acta Aquatica Turcica*, 19(3), 277-288. https://doi.org/10.26453/otjhs.1006312

Dimitriadi, D., Charitidou, C., & Charvalos, E. (2014). Urinary tract infection due to beta-lactams-resistant Staphylococcus warneri: a case report. *The International Arabic Journal of Antimicrobial Agents*, *4*(4).

Durgun, S. (2013). İzmir'de açıkta satılan midye dolmaların mikrobiyolojik açıdan incelenmesi. Celal Bayar Üniversitesi / Fen Bilimleri Enstitüsü / Gıda Mühendisliği Anabilim Dalı, Yüksek Lisans Tezi, Manisa.

FAO, 1997. Food and Agriculture Organization of the United Nations. Street foods. FAO food and nutrition paper 63. Report of an FAO technical meeting on street foods, Calcutta, India, 6 to 9 November 1995. Food and Agriculture Organization of the United Nations, Rome.

Faria, C., Vaz-Moreira, I., Serapicos, E., Nunes, O. C., & Manaia, C. M. (2009). Antibiotic resistance in coagulase negative staphylococci isolated from wastewater and drinking water. *Science of the Total Environment*, 407(12), 3876-3882. https://doi.org/10.1016/j.scitotenv.2009.02.034

Gelman, R., Potruch, A., Oster, Y., Ishay, Y., Gur, C., Beeri, R., & Strahilevitz, J. (2022). Native aortic valve Staphylococcus warneri endocarditis after COVID-19 infection: a case report and a review of literature. *Apmis*, *130*(5), 270-275.

Geniş, B., & Tuncer, Y. (2018). Determination of antibiotic susceptibility and decarboxylase activity of coagulase-negative *Staphylococcus* and *Macrococcus caseolyticus* strains isolated from fermented Turkish sausage (sucuk). *Journal of Food Processing and Preservation*, *42*(1), e13329. https://doi.org/10.1111/jfpp.13329

Güngörür, N.M. (2019). İstanbul'da satışa sunulan midye dolmaların mikrobiyolojik kalitesinin incelenmesi. İstanbul Aydın Üniversitesi / Fen Bilimleri Enstitüsü / Gıda Güvenliği ve Beslenme Anabilim Yazım alanları gerektiği kadar uzatılabilir Dalı, Yüksek Lisans Tezi, İstanbul.

Hoque, M. N., Faisal, G. M., Das, Z. C., Sakif, T. I., Al Mahtab, M., Hossain, M. A., & Islam, T. (2023). Genomic Features and Pathophysiological Impact of a Multidrug-Resistant Staphylococcus warneri Variant in Murine Mastitis. *Microbes and Infection*, 105285.

Hoque, M. N., Moyna, Z., Faisal, G. M., & Das, Z. C. (2023). Whole-Genome Sequence of the Multidrug-Resistant Staphylococcus warneri Strain G1M1F, Isolated from Mice with Mastitis. *Microbiology Resource Announcements*, *12*(5), e00275-23.

Incani, R. N., Hernández, M., Cortez, J., González, M. E., & Dorel Salazar, Y. (2010). Staphylococcus warneri meningitis in a patient with Strongyloides stercoralis hyperinfection and lymphoma: first report of a case. *Revista do Instituto de Medicina Tropical de São Paulo*, *52*, 169-170.

Jeong, D. W., & Lee, J. H. (2015). Safety assessment of coagulase-negative staphylococci from jeotgal, a Korean high-salt-fermented seafood. *Microbiology and Biotechnology Letters*, 43(1), 84-90. https://doi.org/10.4014/mbl.1501.01001

Karademir, F. (2018). İstanbul'da tüketime sunulan midye dolmaların mikrobiyolojik kalitesinin belirlenmesi. İstanbul Üniversitesi / Sağlık Bilimleri Enstitüsü / Besin Hijyeni ve Teknolojisi Anabilim Dalı, Yüksek Lisans Tezi, İstanbul.

Kim, T. Y., Lee, J. J., Kim, B. S., & Choi, S. H. (2017). Whole-body microbiota of sea cucumber (Apostichopus japonicus) from South Korea for improved seafood management. *Journal of Microbiology and Biotechnology*, 27(10), 1753-1762. https://doi.org/10.4014/jmb.1707.07067

Kişla, D., & Üzgün, Y. (2008). Microbiological evaluation of stuffed mussels. *Journal of Food Protection*, *71*(3), 616-620. https://doi.org/10.4315/0362-028X-71.3.616

Lane, DJ. (1991). 16S/23S rRNA sequencing. In: Stackebrant E., Goodfellow M. (eds.) Nucleic acid techniques in bacterial systematics. John Wiley & Sons Ltd. London, UK, pp 115–75.

Louail, R., Florin, F., Bernard, S., Michaud, J. B., Breton, J., Achamrah, N., ... & Ribet, D. (2023). Invasion of intestinal cells by Staphylococcus warneri, a member of the human gut microbiota. *Gut Pathogens*, *15*(1), 4.

Lucca, A., & da Silva Torres, E. A. F. (2006). Street-food: the hygiene conditions of hot-dogs sold in São Paulo, Brazil. *Food Control*, *17*(4), 312-316. https://doi.org/10.1016/j.foodcont.2004.11.005

Marino, M., Frigo, F., Bartolomeoli, I., & Maifreni, M. (2011). Safety-related properties of staphylococci isolated from food and food environments. *Journal of Applied Microbiology*, *110*(2), 550-561. https://doi.org/10.1111/j.1365-2672.2010.04909.x

Mepba, H. D., Achinewhu, S. C., Aso, S. N., & Wachukwu, C. K. (2007). Microbiological quality of selected street foods in Port Harcourt, Nigeria. *Journal of Food Safety*, *27*(2), 208-218. https://doi.org/10.1111/j.1745-4565.2007.00073.x

Morfin-Otero, R., Martínez-Vázquez, M. A., López, D., Rodríguez-Noriega, E., & Garza-González, E. (2012). Isolation of rare coagulase-negative isolates in immunocompromised patients: Staphylococcus gallinarum, Staphylococcus pettenkoferi and Staphylococcus pasteuri. *Annals of Clinical & Laboratory Science*, *42*(2), 182-185.

Mosupye, F. & Von Holy, A. (1999). Microbiological quality and safety of ready to eat street-vended foods in Johannesburg, South Africa. *Journal of Food Protection*, *62*, 1278-1284. https://doi.org/10.4315/0362-028x-62.11.1278

Petti, C. A., Simmon, K. E., Miro, J. M., Hoen, B., Marco, F., & Chu, V. H. (2008). International collaboration on endocarditis-microbiology investigators genotypic diversity of coagulase-negative staphylococci causing endocarditis: a global perspective. *J Clin Microbiol*, *46*(5), 1780-1784.

Ramnarain, J., Yoon, J., & Runnegar, N. (2019). Staphylococcus pasteuri infective endocarditis: A case report. *IDCases*, *18*, e00656.

Rantsiou, K., Iacumin, L., Cantoni, C., Comi, G., & Cocolin, L. (2005). Ecology and characterization by molecular methods of Staphylococcus species isolated from fresh sausages. *International Journal of Food Microbiology*, *97*(3), 277-284. https://doi.org/10.1016/j.ijfoodmicro.2004.02.018

Regecová, I., Pipová, M., Jevinová, P., Marušková, K., Kmeť, V., & Popelka, P. (2014). Species identification and antimicrobial resistance of coagulase-negative staphylococci isolated from the meat of sea fish. *Journal of Food Science*, *79*(5), M898-M902. https://doi.org/10.1111/1750-3841.12429

Sánchez, E., Donat, E., Ribes-Koninckx, C., Fernández-Murga, M. L., & Sanz, Y. (2013). Duodenal-mucosal bacteria associated with celiac disease in children. *Applied and environmental microbiology*, *79*(18), 5472-5479.

Savini, V., Bianco, A., Catavitello, C., Balbinot, A., Pompilio, A., Piccolomini, R., & D'Antonio, D. (2009a). Meticillin-heteroresistant Staphylococcus pasteuri from an apheresis platelet product. *Journal of medical microbiology*, *58*(11), 1527-1528.

Savini, V., Catavitello, C., Carlino, D., Pompilio, A., Piccolomini, R., & Di Bonaventura, G. (2009b). Staphylococcus pasteuri bacteraemia in a leukemic patient. *Journal of Clinical Pathology*.

Savini, V., Catavitello, C., Pompetti, F., Passeri, C., Di Zacomo, S., Esattore, F., & D'Antonio, D. (2008). Contamination of a donated platelet unit by Staphylococcus pasteuri. *Journal of Infection*, *57*(6), 494-496.

Si, W., Li, M., Wang, K., Li, J., Xu, M., Zhou, X., ... & Tian, Z. (2024). Staphylococcus warneri strain XSB102 exacerbates psoriasis and promotes keratinocyte proliferation in imiquimod-induced psoriasis-like dermatitis mice. *Archives of Microbiology*, *206*(1), 3.

US FDA. (2001). Bacteriological analytical manual, 8th edition, revision A. In R. W. Benett & G. A. Lancett (Eds.). Rockville, MD, USA: AOAC International.

Üzgün, Y. (2005). İzmir›in çeşitli semtlerinde satışa sunulan midye dolmaların mikrobiyolojik kalite kontrolü. Ege Üniversitesi / Fen Bilimleri Enstitüsü / Su Ürünleri Avlama ve İşleme Teknolojisi Anabilim Dalı, Yüksek Lisans Tezi, İzmir.

WHO (1996). World Health Organisation. Essential safety requirements for street-vended foods (Revised Edition). WHO/FAO Food Safety Unit - Division of Food and Nutrition, Geneva.

WHO (2017). World Health Organization guidelines on use of medically important antimicrobials in food-producing animals.

İSTANBUL UNIVERSITY PRESS

EISSN 2602-473X

AQUATIC SCIENCES AND ENGINEERING

Aquat Sci Eng 2024; 39(3): 179-188 • DOI: https://doi.org/10.26650/ASE20231367517

Review Article

Effect of Curry Leaf (*Murraya koenigii*) as Edwardsiellosis Treatment on Gourami Fish (*Osphronemus goramy*)

Arif Syaifurrisal[1], Suciyono[2], Muhammad Browijoyo Santanumurti[3,4], Arief Prajitno[5], Mohammad Fadjar[1], Farid Mukhtar Riyadi[1], Annisa Isti Fauziyyah[1], Agus Pariawan[6], Muyassar H. Abualreesh[3]

Cite this article as: Syaifurrisal, A., Suciyono, Santanumurti, M.B., Prajitno, A., Fadjar, M., Riyadi, F.M., Fauziyyah, A.I., Pariawan, A. & Abualreesh, M.H. (2024). Effect of Curry Leaf (*Murraya koenigii*) as Edwardsiellosis Treatment on Gourami Fish (*Osphronemus goramy*). *Aquatic Sciences and Engineering, 39(3)*, 179-188. DOI: https://doi.org/10.26650/ASE20231367517

ORCID IDs of the author:
A.S. 0009-0004-8305-8415;
S.S. 0000-0003-0361-5089;
M.B.S. 0000-0003-0846-9447;
A.P. 0009-0001-4568-013X;
M.F. 0000-0002-2318-1698;
F.M.R. 0009-0000-6522-5372;
A.I.F. 0009-0006-4390-0641;
A.P. 0009-0006-4984-1961;
M.H.A. 0000-0003-3170-3609

[1]Magister Study Program of Aquaculture, Faculty of Fisheries and Marine Sciences, Brawijaya University, Ketawanggede, Lowokwaru Sub-District, Malang, East Java, Indonesia

[2]Aquaculture Study Program, School of Health and Natural Science (SIKIA), Universitas Airlangga, Banyuwangi, East Java, Indonesia

[3]Department of Marine Biology, Faculty of Fisheries Sciences, King Abdulaziz University, Jeddah 21589, Kindom of Saudi Arabia

[4]Department of Aquaculture, Faculty of Fisheries and Marine, Universitas Airlangga. Jl. Mulyorejo, Kampus C UNAIR, Mulyorejo, Surabaya, East Java, Indonesia

[5]Aquaculture Study Program, Aquaculture Master Program, Faculty of Fisheries and Marine Science, Brawijaya University, Jl. Veteran, Malang, Indonesia

[6]Aquaculture Study Program, Faculty of Fisheries and Marine, Universitas Airlangga. Jl. Mulyorejo, Kampus C UNAIR, Mulyorejo, Surabaya, East Java, Indonesia

Submitted:
03.10.2023

Revision Requested:
21.12.2023

Last Revision Received:
13.02.2024

Accepted:
05.03.2024

Online Published:
29.04.2024

Correspondence:
Muhammad Browijoyo Santanumurti
E-mail:
m.browijoyo.s@fpk.unair.ac.id

ABSTRACT

The primary objective of this study was to explore the potential of *Murraya koenigii* as a natural alternative to synthetic antimicrobial agents for the treatment of edwardsiellosis in gourami fish (*Osphronemus goramy*). This research employed a combination of both experimental and descriptive methods. The research followed a two-stage approach. In the first stage, antibacterial compounds were extracted and characterized from *M. koenigii* leaves, followed by an assessment of their antibacterial activity against *Edwardsiella tarda*. The second stage involved applying the crude extract of *M. koenigii*, known for its antibacterial properties, to gourami fish infected with *E. tarda*. The findings revealed that *M. koenigii* predominantly contains alkaloids, terpenoids, saponins, flavonoids, and tannins, all of which exhibit antibacterial activity. The application of the crude *M. koenigii* extract had a substantial impact on the hematological profile of the infected gourami fish. The most effective dose in treating Edwardsiellosis in gourami fish was found to be 600 mg/L, as it significantly improved various hematological parameters, including hematocrit, hemoglobin, erythrocyte count, leukocyte count, lymphocytes, monocytes, and neutrophils. Additionally, histopathological analysis showed improvements in tissue conditions, with lower scores for degeneration, congestion, and necrosis. Furthermore, the survival rate of gourami fish reached its highest at 83.33% when treated with the 600 mg/L dose of *M. koenigii* extract. These results suggest the potential of *M. koenigii* as an effective alternative for the treatment of Edwardsiellosis in gourami fish. However, further research is needed to assess the practical application of *M. koenigii* extract in gourami fish culture.

Keywords: Gouramy Fish, Bacterial Fish Diseases, Edwardsiellosis, Fish Diseases Treatment, *Murayya koenigii*

INTRODUCTION

Gourami fish (*Osphronemus goramy*) is a freshwater fish species that has been cultured in the Southeast Asian region; its maintenance cost is relatively low, and it has adaptability with low dissolved oxygen content. Gourami fish also has a relatively stable high selling price (2.28 USD/kg) (Al-Baiquni, 2019). However, an intensive maintenance strategy to pursue production targets evokes damage to the immune system and susceptibility to diseases which evokes high economic losses if not resolved (Abdel-Latif et al., 2020).

Edwardsiella tarda is a species of the Gram-negative bacteria, which poses a significant challenge to the aquaculture industry. It is responsible for causing edwardsiellosis, a disease that has been identified as a major threat to various economically valuable fish species globally (Li et al., 2019).

This pathogen has led to substantial losses in the aquaculture sector, with significant impacts observed in countries such as the United States of America, Japan, and various European and Asian countries. In scenarios where fish are raised in ponds with limited water circulation, losses due to *E. tarda* infections can be particularly severe, sometimes reaching as high as 50% (Kole et al., 2017).

The effective treatment of edwardsiellosis, caused by *E. tarda* infection, has been achieved through the use of antibiotics such as tetracycline and kanamycin. However, the widespread and inappropriate use of antibiotics has led to significant problems. These challenges encompass the development of antibiotic resistance in *E. tarda* and the potential presence of antibiotic residues in human populations (Xu et al., 2019). As highlighted by Harikrishnan et al. (2020), the use of antibiotics and chemical agents in aquaculture can also pose risks, including concerns related to water toxicity, public health, and environmental damage.

One potential solution to address the aforementioned challenges involves the utilization of a natural bioactive crude extract derived from curry leaves, *Murraya koenigii*, which contains antibacterial compounds. *M. koenigii* is a plant belonging to the Rutaceae family and is commonly found in tropical and subtropical regions. Extensive phytochemical analyses of *M. koenigii* have revealed a wide range of natural compounds, including alkaloids, sesquiterpenes, essential oils, and alkenes (Patil et al., 2024). Different parts of the *M. koenigii* plant exhibit various biological activities, including anti-inflammatory, antioxidative, nephroprotective, hepatoprotective, anti-listerial, and antibacterial properties (Ma et al., 2019). To gain a deeper understanding of the bioactive components of *M. koenigii*, researchers conducted GC-MS (Gas Chromatography-Mass Spectrometry) analysis. They investigated the chemical composition of the ethanol extract of *M. koenigii* using GC-MS and compared the mass spectra of the identified compounds in the extract to the National Institute of Standards and Technology (NIST) library (Azhagu et al., 2021). The analysis of the ethanol extract of *M. koenigii* revealed the presence of two primary compounds: 1-methyl-pyrrolidine-2-carboxylic acid, which constituted 69.00% of the extract, and ethyl α-glucopyranoside, making up 13.36% of the extract (Salikutty et al., 2012).

Numerous investigations have explored the potential antibacterial properties of *M. koenigii*, but its specific use as an antibacterial agent against *in vitro* antibacterial activity was investigated by Syaifurrisal et al. (2021) and its application in the treatment of fish has never been explored. It is imperative to initiate research and studies focused on the identification of active compounds within *M. koenigii* with antibacterial properties. Furthermore, these studies should investigate the feasibility of employing these compounds as potential drug candidates for the treatment of *E. tarda* infections in gourami fish. This research should encompass comprehensive hematological and histopathological examinations to determine the potential therapeutic effects.

MATERIAL AND METHODS

Experimental fish and bacteria

Experimental gourami fish samples (7-10 cm) were provided from fish farms located in Tulungagung, East Java, Indonesia. To conduct antibacterial activity testing, a pure culture of *E. tarda* was acquired from BUSKIPM Jakarta. The culture and revitalization of *E. tarda* bacteria were facilitated using TSB (Tryptic Soy Broth) as the growth medium. The LD_{50} (Lethal Dosage 50) test was carried out to determine the density and time required for bacteria to kill 50% of the experimental fish (Rattanachaikunsopon & Phumkhachorn, 2010). The LD_{50} test uses a pure culture of *E. tarda* bacteria with a density 10^9 cells/ml, 10^8 cells/ml, 10^7 cells/ml, 10^6 cells/ml, and 10^5 cells/ml. The *E. tarda* used in the study had a density of 2.53×10^7 cells/mL, as determined by the LD_{50} results. In this test, fish were put into treatment containers with a stocking density of 10 fish/container.

Antibacterial activity testing was carried out using a disc test to examine the inhibitory power of the crude extract of *M. koenigii* leaves on *E. tarda* bacteria. According to Ulmursida et al., (2017) in testing how big the impact of giving the extract is and if the diffusion method shows positive results, the clear zone area results will be obtained by reducing the diameter of the clear zone by the diameter of the paper disc (6 mm). *E. tarda* cell damage was analyzed by comparing SEM photos (normal conditions and after the bacteria were treated with crude extract of *M. koenigii* leaves so that a picture of bacterial cell wall damage was visible. According to Nursidika et al., (2014), determining the antimicrobial mechanism can be done using the SEM method. The working principle of SEM is the creation of images based on the detection of new (secondary) or reflected electrons that appear or emerge from the sample surface when the sample surface is scanned using an electron beam.

Experimental design

To infect experimental fish with *E. tarda* bacteria, the experimental fish were soaked in a pure culture of *E. tarda*. Soaking was carried out in an aquarium measuring 30 x 30 x 30 cm with a density of 10 fish in 20 liters of water at a temperature of 27-30°C. Observations of fish death were then carried out for 96 hours, according to the statement of Wulandari et al. (2014), since this was the time commonly used in short-scale bioassays. This research employed a combination of both experimental and descriptive methods. The experimental approach was employed to obtain quantitative data for this study, while the descriptive method was utilized to comprehensively depict the natural bioactive components found in *M. koenigii* and their effects on the hematological and histopathological aspects of gourami fish. In this study, various treatments were administered, including negative control (K-) infected fish that were not treated, positive control (K+) normal fish that were not infected, as well as doses of 500 mg/L, 600 mg/L, 650 mg/L, and 700 mg/L (three repetitions, 10 fish each). The selection of these dosages was based on prior *in vitro* research conducted by Syaifurrisal et al. (2021).

Extraction of *M. koenigii*

The *M. koenigii* leaves utilized in this study were collected from the Ampel Religious Tourism area of Surabaya, Indonesia, and extracted with ethanol as the maceration solvent. To ascertain the presence of active compounds in the extract, several tests were conducted by the methodology outlined by Dewi et al. (2019). These tests encompassed phytochemical screening, UV-Vis (Ultraviolet-Visible) analysis, and FTIR (Fourier-transform in-

frared) spectroscopy. The primary objective of phytochemical screening was to detect the presence of active compounds in the plant extract such as saponins, tannins, flavonoids, alkaloids, and terpenoids, within the crude extract of *M. koenigii* leaves.

The extract was then also tested for LC_{50} (Lethal Concentration) to determine the concentration of the extract that could cause 50%, to determine a dose that was safe and did not cause death in experimental fish (Suciati et al., 2012). In this test, fish were put into treatment containers with a stocking density of 10 fish/container. Then the aquarium is filled with extract according to the treatment. The behavior, number of fish that died, and the time were recorded. The test was repeated if fish in the control treatment experienced mortality above 10% (Taufik & Setiadi, 2012).

Histopathology analysis

Edwardsiellosis can be observed with symptoms such as lesions in muscle tissue, skin, and internal organs, such as kidney tissue (Harikrishnan et al., 2020). Kidney tissue can be an important organ in the study of pathogenic mechanisms and immune responses in fish (Rauta et al., 2012; Bailey et al., 2020). The relationship between the severity of histological changes due to bacterial infection and the immune response can be elucidated by the study of these organs. Some studies report kidney tissue is used as histopathology analysis for edwardsiellosis in fish (Cheng et al., 2020; Kalindamar et al., 2020). To determine the characteristics of kidney tissue, histopathological observations of gourami fish were carried out. The staining method used Haematoxylin Eosin previously fixed with 10% formalin and calculations were used to assess the impact of *M. koenigii* extract on edwardsiellosis, as described by Maftuch et al. (2015). Also, the normal and pathological semi-quantitative approach was used to compare normal tissue samples with tissue that had been affected by the pathogen. This involved determining the extent of staining in stained areas and manually calculating the percentage of affected tissue, following the method outlined by Maftuch et al. (2015). This histopathological analysis was performed with three replications, and for each treatment, five distinct views were examined to ensure a comprehensive assessment.

Blood analysis

Hematological observations encompassed the assessment of several parameters, including hematocrit value, hemoglobin levels, erythrocyte count, leukocyte count, monocyte count, lymphocyte count, and neutrophil count, following the methodology outlined by Maftuch (2018). These observations were conducted by extracting blood from the lateral line of the gourami fish using a 1 ml syringe, which had been preloaded with 0.5 µL of EDTA as an anticoagulant. The blood cell count was then determined. This test aimed to evaluate the condition of infected gourami fish after the administration of *M. koenigii* extract. The blood analysis was performed with three repetitions and monitored before infection, after infection, and after treatment.

Survival rate

To assess the effectiveness of *M. koenigii* in treating Edwardsiellosis, the survival rate was calculated with the formula given by Kasnir et al. (2023). The research spanned 7 days for survival rate assessment, involving three replications for each treatment group and a sample of 10 fish in each replication.

$$SR = \frac{Nt}{No} x\ 100\%$$

Notes:

SR: Survival Rate (%)

Nt: The quantity of fish at a specific time point t (individual)

No: The initial count of fish at the start of the experiment (individual)

Data analysis

In this study, data analysis was carried out in a completely randomized design (CRD) using the Statistical Package for the Social Sciences (SPSS) 25.0 for Windows software application.

RESULTS AND DISCUSSION

Active compound present in *M. koenigii*

The phytochemical tests conducted on the crude extract of *M. koenigii* leaves are summarized in Table 1. The results of the phytochemical screening indicated that the crude extract derived from *M. koenigii* leaves contained various active compounds, including flavonoids, alkaloids, saponins, terpenoids, and tannins. These outcomes are consistent with the findings reported by Fauziah et al. (2014), who also identified alkaloids, steroids, flavonoids, tannins, and phenolic compounds as the natural bioactive constituents present in the crude extract of *M. Koenigii*.

Table 1. Phytochemical Test Results.

Active Compound	Result	Description
Flavonoid	+	Formed pink color
Saponin	+	Formed non-permanent foam
Alkaloid		
- Dragendroff	+	An orange precipitate formed
- Meyer	+	A white precipitate formed
- Buchardat	+	A brown precipitate formed
Tannin	+	A blackish brown color is formed
Terpenoid		
- Steroid	-	Formed a bluish green color
- Triterpenoid	+	A brownish-orange color is formed

To validate the presence of the active constituents identified in the phytochemical test results, further analysis of the crude extract from *M. koenigii* was conducted using both a UV-Vis spectrophotometer (Table 2) and an FTIR (Fourier-transform infrared) spectrophotometer (Table 3). The UV-Vis observations indicated that the dominant compounds in the extract were derived from the alkaloid group (with a peak at 3.893), saponin group (peaking at 3.824), and terpenoid group (peaking at 4.016). These groups exhibited notably high absorbance values. Conversely, flavonoids (with a peak at 0.022) and tannins (peaking at 1.590) displayed comparatively lower absorbance values. According to Hammado and Illing (2013), absorbance values can provide in-

sights into the concentration of bioactive compounds within the extract. A higher absorbance value suggests that the absorption band contains specific compounds with a stronger absorption affinity. Furthermore, the FTIR observations unequivocally confirmed the presence of compounds such as flavonoids, alkaloids, saponins, terpenoids, and tannins.

Table 2. UV-Vis peak point data from *M. koenigii* extract.

Long Waveform (nm)	Absorbance	Compound	Literature
662.0	0.022	Flavonoid	(Obaseki et al., 2017)
294.9	1.590	Tanin	(Lestari & Sidik, 2013)
220.0	3.480	Alkaloid	(Hammado & Illing, 2013)
217.0	3.577	Alkaloid	(Hammado & Illing, 2013)
213.0	3.893	Alkaloid	(Hammado & Illing, 2013)
211.0	3.824	Saponin	(Amanati et al., 2017)
205.1	4.016	Terpenoid	(Agustini et al, 2019)
202.1	3.879	Terpenoid	(Agustini et al., 2019)

Table 3. FTIR spectrophotometer wavelength absorption data from *M. koenigii* extract.

FTIR wavelength (cm^{-1})	Functional groups	Group
1453.868	N-H	Alkaloid (Hammado & Illing, 2013)
1627.321	C=O	Alkaloid (Aksara et al, 2013)
1453.868	C-H alifatik	Alkaloid (Hammado & Illing, 2013)
1163.814	N-H bending	Alkaloid (Aksara et al, 2013)
1069.868	N-H bending	Alkaloid (Aksara et al, 2013)
928.175	Tekuk C- H	Alkaloid (Santi, 2010)
2924.574	-OH	Terpenoid (Agustini et al., 2019)
2855.169	C-H alifatik	Terpenoid (Agustini et al., 2019)
1713.556	C=O	Terpenoid (Agustini et al., 2019)
1314.393	C-O-H	Tanin (Sari et al., 2015)
679.341	C-H *bending*	Tanin (Sari et al., 2015)
583.419	C-H *bending*	Tanin (Sari et al., 2015)
1209.989	C-O	Saponin (Bintoro et al, 2017)
835.125	C-H	Flavonoid (Ningrum et al, 2017)

Histopathology analysis

The histopathological images presented in Figure 1 offer valuable insights. The kidney tissue of healthy fish appears normal, showing no signs of cellular damage. The cross-section of the network, including the glomerulus and tubular tissue exhibits a healthy state. The distal tubule tissue and hematopoietic tissue also display normal conditions, devoid of any damage. This contrasts with infected fish, where congestion is apparent. The reddish color observed in kidney cells is indicative of congestion resulting from an increased presence of blood clots within the blood vessels, as explained by Parameswari et al. (2013). In severe cases, congestion can lead to ruptured blood vessels, ultimately causing cell death or necrosis, characterized by changes in cell nuclei, where the nuclei become denser and darker (a condition known as pyknosis) (Abdelfadeel et al., 2023). Coagulative necrosis, described by Adinata et al. (2012), involves densely packed and fixed protoplasmic cells that can still be observed under a microscope.

Takashima and Hibiya (1995) define necrosis as a state of reduced tissue activity marked by the progressive loss of cell components, accompanying cell degeneration in organisms. In histopathological terms, the use of *M. koenigii* is shown to inhibit *E. tarda* infection in gourami fish. Many prior studies have explored the application of *M. koenigii* in treating pathogenic bacteria such as *Escherichia coli, Aeromonas hydrophila, Citrobacter freundii, Vibrio parahaemolyticus, Vibrio vulnificus, Staphylococcus aureus, Streptococcus agalactiae*, and *Streptococcus anginosus* in fish *in vitro* (Heny et al., 2011; Najiah et al., 2011). However, this research is the first to demonstrate the effectiveness of *M. koenigii* in treating *E. tarda* infection in gourami fish. Notably, kidney necrosis was observed to increase in average scores at the 650 mg/L and 700 mg/L treatments, indicating that the extract reached its optimal efficacy for treatment following *E. tarda* infection. Doses exceeding this optimal point appeared to result in more severe kidney tissue damage.

According to Kumar et al. (2012), proximal tubular epithelial cells are highly susceptible to the effects of toxic substances. This vulnerability arises because toxic substances tend to accumulate at higher levels in the proximal tubules due to active processes of absorption and secretion in this region. Additionally, the proximal tubules have elevated levels of cytochrome P450 enzymes, which play a role in either detoxifying or activating toxic substances (Ge et al., 2022). Consequently, these tubules are often the primary targets of the adverse effects caused by toxic substances. Continuous exposure of the proximal convoluted tubule to toxic substances can result in cellular injury, ultimately leading to cell death, known as necrosis (Moore et al., 2021).

In the overall evaluation of the comparison to the positive control group, the treatment with *M. koenigii* extract appeared to have a positive impact on mitigating the extent of kidney necrosis (Figure 2). This positive effect can be attributed to the bioactive compounds present in *M. koenigii*'s crude extract, such as alkaloids and flavonoids, which can stimulate the fish's immune defense system. Previous studies have indicated that alkaloids and flavonoids possess antibacterial properties (Donadio et al., 2021; Yan et al., 2021). Alkaloids can disrupt bacterial cell membranes, inhibiting bacterial growth (Jubair et al., 2021), while flavonoids can cause physical membrane disruption and act as inhibitors of ATP synthase (Donadio et al., 2021).

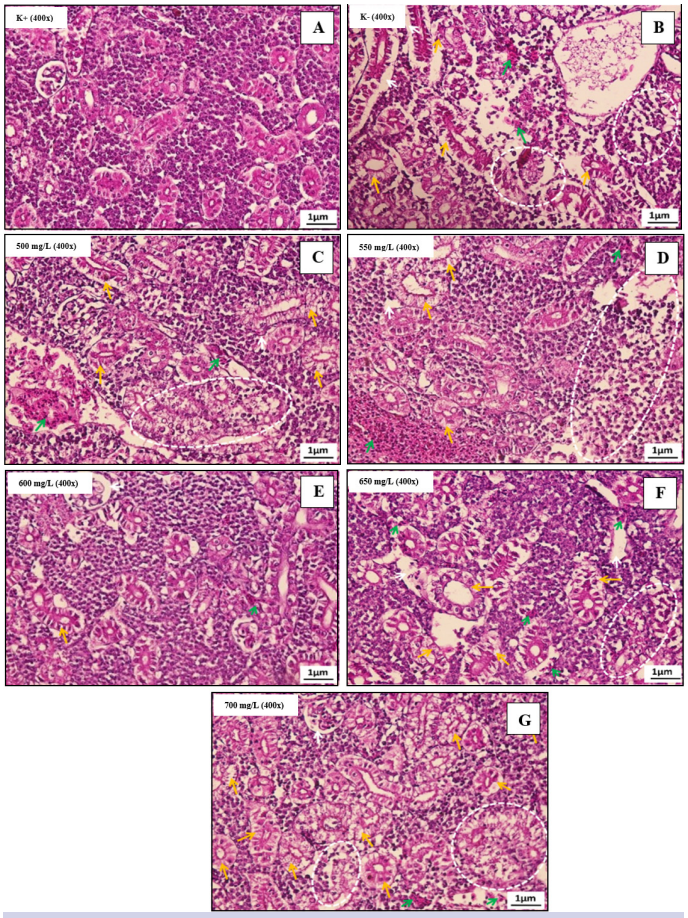

Figure 1. Kidney Histopathological Overview of Gourami Fish / 400x. (A) Normal (non-infected) /K+, (B) Infected fish (not-treated) / K-, (C) treated with 500 mg/L. (D) treated with 550 mg/L. (E) treated with 600 mg/L. (F) treated with 650 mg/L. (G) treated with 700 mg/L, (Yellow Arrow: Degeneration; Green Arrow: Congestion; White Circle: Necrotic Area)

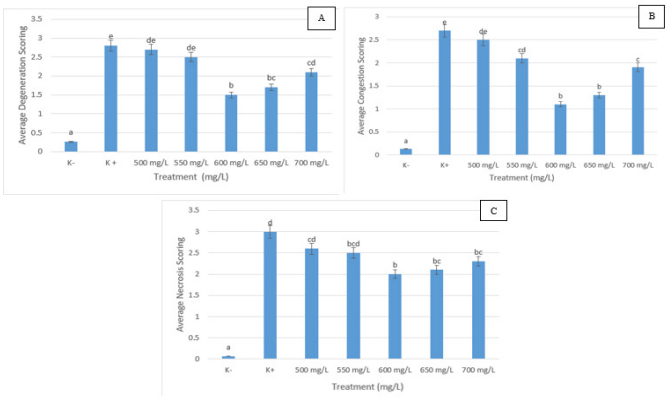

Figure 2. Histopathological Scoring Test Results (A) Degeneration, (B) Congestion, and (C) Kidney Necrosis of Gourami Fish.

The treatment with the lowest average score for abnormal histopathological findings was observed at a dose of 600 mg/L, which resulted in 1.5 for degeneration, 1.1 for congestion, and 2 for necrosis. This indicates that 600 mg/L was the most effective dosage of *M. koenigii* for treating edwardsiellosis in gourami fish. According to Kim & Yang (2018), employing natural active ingredients at the appropriate dosage can stimulate the immune system and enhance protection against infections. Interestingly, after the decrease in damage scores observed in the extract concentrations ranging from 500 mg/L to 600 mg/L, there was a noticeable increase in congestion at the 650 mg/L and 700 mg/L treatment levels. This suggests that the extract's effectiveness had reached its peak at the optimal dosage, and doses higher than this optimum could become toxic, causing more severe kidney tissue damage. At high doses, the secondary metabolites present in the crude extract of *M. koenigii* leaves can be toxic to gourami fish. According to Rand et al. (2015), as the dose of the extract administered increases, the damage to tissue cells also intensifies. This study also showed that no test animals succumbed during the treatment process because the density of bacteria and the concentration of the extract dose used had been through acute LD_{50} and LC_{50} toxicity tests to assess the acute safety of a drug or substance to be used.

SEM test (*Scanning Electron Microscopy*)

The SEM results offer insights into the effects of the treatment on the structural changes in bacterial cells (Figure 3). Figure 3A shows the morphology of *E. tarda* bacteria with no apparent damage to their cell walls, while Figure 3B presents morphological images of *E. tarda* bacteria that have experienced lysis due to instability in their cell walls. For bacterial fish pathogens, after entering the host body, the first step for inducing an infection is the attachment, colonization, and biofilm formation of the microorganism on the host cells and tissues which is generally linked to their cell wall proteins. According to Hussain et al (1997), accumulative growth on polymer surfaces resulting in biofilm formation; it was contributing to pathogenicity by reducing the efficacy of host defenses and antimicrobial killing. This instability disrupts bacterial metabolism, depletes ATP reserves, and decreases cell acidity. Several studies have elucidated this phenomenon, which occurs when antibacterial compounds induce changes or lysis in the protein structure, cell wall stability, and bacterial plasma membrane. This is a result of antibacterial compounds (bacteriostatic effect) binding to proteins through hydrogen bonds (Zhou et al., 2022). Alkaloid compounds, known for their antibacterial properties, can interfere with the formation of cross-bridges within the peptidoglycan constituent components of bacterial cells. This interference prevents the complete formation of the cell wall layer, ultimately leading to the removal of the bacterial cells from the host's cell surface as explained by Ernawati and Sari (2015). Antimicrobials are bacteriostatic if they only inhibit bacterial growth as compound application continues. However, if it is stopped or finished, bacterial growth will increase again (Soelama et al., 2015).

Hematology

The collected data included measurements of hematocrit, hemoglobin, and erythrocyte levels, as depicted in Figure 4. The

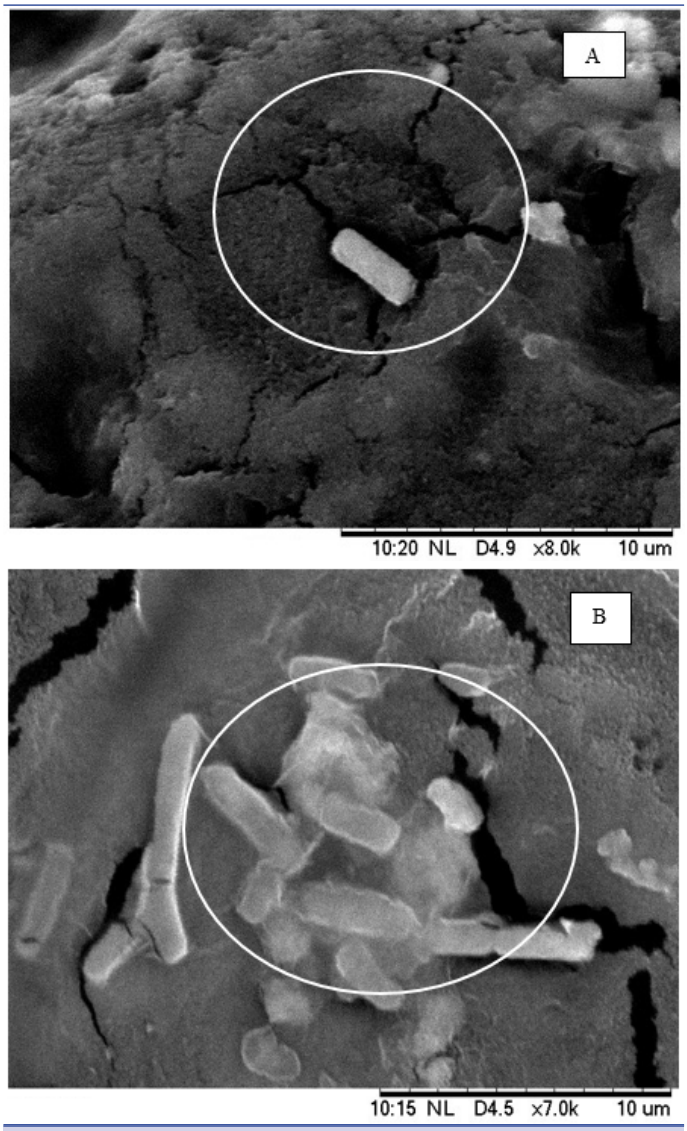

Figure 3. Morphology of *E. tarda* bacteria after SEM test (Lysis) (A) Without treatment, (B) With treatment of *M. koenigii*.

optimal hematocrit value was achieved in the treatment with a dose of 600 mg/L, reaching 32.67%. Regression analysis revealed that the crude extract of *M. koenigii* had a 73% positive effect on improving hematocrit values, which were notably lower after infection, at 19.50%. Similarly, the hemoglobin level showed a significant increase in the 600 mg/L treatment, reaching 4.504 g%. *M. koenigii* demonstrated a 78% effect in improving hemoglobin values, which had declined to 2.652 g% after infection. The highest increase in erythrocyte values was also observed in the treatment with an extract concentration of 600 mg/L, reaching 3.27 x 10^6 cells/mm^3. The crude extract of *M. koenigii* exhibited a remarkable 78% effect in enhancing erythrocyte values, which had dropped to 1.73 x 10^6 cells/mm^3 after infection. These findings indicate that the extract concentration of 600 mg/L was the most effective in increasing blood parameters after *E. tarda* infection in gourami fish. The increase in hematocrit, hemoglobin, and erythrocyte levels in the blood of gourami fish can be attributed to the bioactive properties of the extract, such as terpenoids. According to Sundaryono (2011), terpenoids can stimulate erythropoiesis, the process of erythrocyte formation in the bone marrow. The results of UV-Vis observations in this study showed that the dominant compounds in the extract came from the alkaloid group (with a peak of 3.893), the saponin group (peak of 3.824), and the terpenoid group (peak of 4.016). These groups showed very high absorption values.

According to Anderson and Siwick (1993), when fish are infected, their hematocrit levels tend to decrease. A hematocrit value below 20% indicates erythrocyte deficiency. Zorriezahra et al. (2010) stated that a low erythrocyte count and hematocrit level are indicative of anemia in fish. Hardi et al. (2011) also explained that an increase in total erythrocytes in fish blood indicates a homeostatic response in the fish's body. This response aims to produce more blood cells to replace erythrocytes that may have undergone lysis due to infection. Conversely, the decline in hematocrit, hemoglobin, and erythrocyte values at doses of 650 mg/L and 700 mg/L was attributed to excessive dosages causing stress in the fish. This stress was evident from the lower survival rates of the test fish at these two higher doses compared to the 600 mg dose. According to Awad and Awaad (2017), the more extracts used will not necessarily help treatment efforts due to bacterial infections. The active components contained in an extract are toxic to test animals if the concentration is too high. Using natural ingredients at the right dosage can stimulate the immune system and increase protection against infection.

Treatment with various doses of *M. koenigii* also caused different effects on the leukocyte, lymphocyte, monocyte, and neutrophil values of the fish samples (Figure 5). Following treatment with *M. koenigii*, there was a notable decrease in leukocyte values. The most effective dose observed during the study was 600 mg/L, which reduced leukocyte count from 15,7x10^4 cells/mm^3 to 9.6 x 10^4 cells/mm^3. Regression analysis revealed that the 600 mg/L dose of *M. koenigii* had a 61.59% positive effect on improving leukocyte values. This reduction in leukocyte count indicated that the *M. koenigii* extract played a role in the process of treating gourami fish after they were infected with *E. tarda* bacteria. According to Rieger & Barreda (2011), an increase in the number of leukocytes in the blood is an indication of leukocytes mounting an immune response to bacteria, while a decrease in leukocyte count signifies healing and the cessation of the inflammatory process.

Lymphocyte values showed a significant decrease to 48% after the fish were infected with bacteria. However, after treatment, lymphocyte values began to increase, with the most substantial increase occurring at an extract concentration of 600 mg/L, reaching a 68.88% increase. This rise in lymphocyte count following treatment indicated that the compounds present in the crude extract of *M. koenigii* could enhance the gourami fish's immune system in combating *E. tarda* bacterial infection. As stated by Kim & Yang (2018), natural ingredients, when used at the right dosage, can stimulate the immune system and enhance protection against infections.

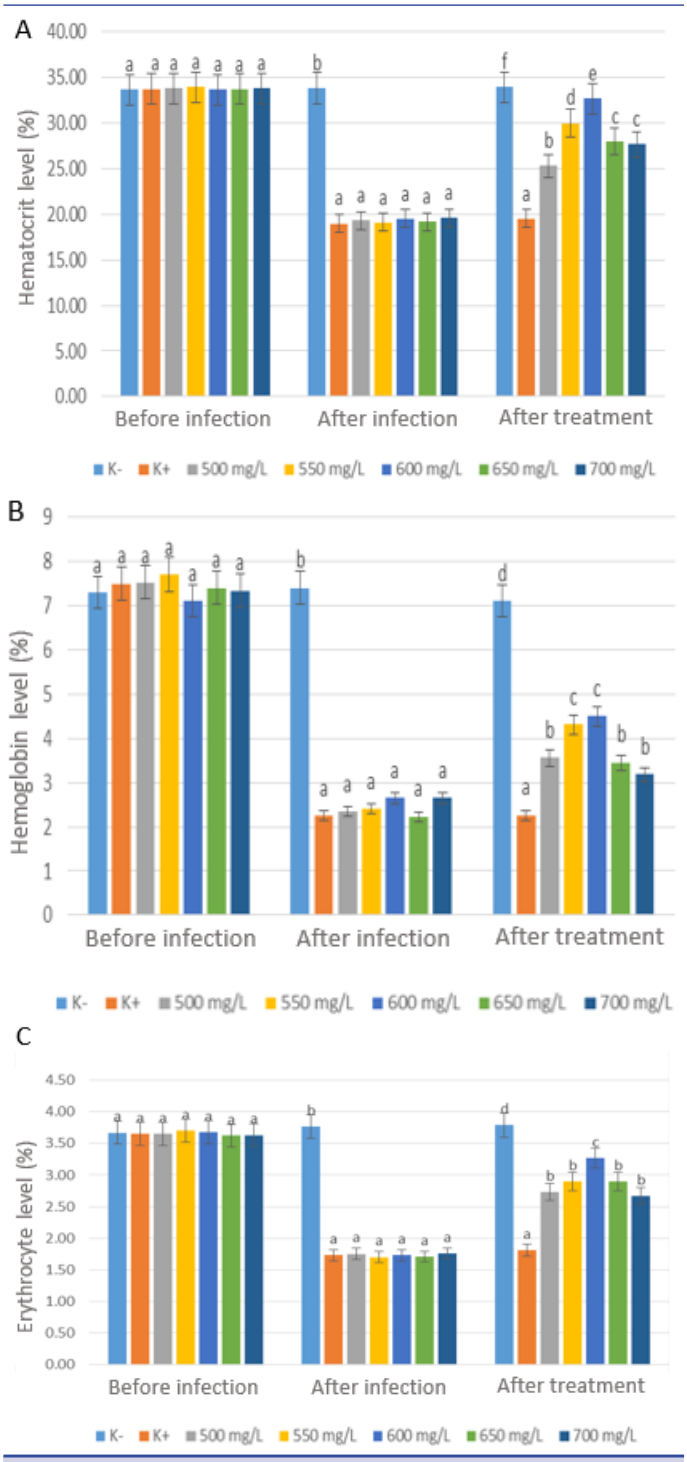

Figure 4. he value of hematocrit (A), hemoglobin (B), and erythrocytes (C) from gourami fish in this study. (K+) Normal (non-infected), (K-) Infected fish (not-treated).

Monocyte and neutrophil values showed a decrease after treatment with *M. koenigii* extract. The best reduction in monocyte values occurred at a concentration of 600 mg/L from 15% to 11.3%. Meanwhile, the highest neutrophil reduction activity occurred at a treatment concentration of 600 mg/L from 17% to 12.6%. The crude extract of *M. koenigii* leaves exhibited the capability to inhibit the growth of *E. tarda* bacteria, leading to a decline in the number of monocytes. This is consistent with Fujaya (2004), suggesting that monocytes leave the bloodstream and migrate to infected areas, where they phagocytize bacteria due to their superior phagocytic capabilities compared to neutrophils. Monocytes serve as macrophages, engaging in the immune system's function of engulfing and destroying pathogenic cells, microorganisms, and foreign substances.

At doses of 650 mg/L and 700 mg/L, the improvement in leukocyte, lymphocyte, monocyte, and neutrophil values was not optimal. This aligns with previous research findings, such as those by Awad & Awaad (2017), which suggest that increasing extract doses may not necessarily enhance treatment efficacy against bacterial infections. Active ingredients within an extract can inhibit pathogen replication and stimulate the innate immune system's defense mechanisms. According to Venkatalakshmi et al. (2016), active compounds like flavonoids can stimulate the release of adrenocorticotropic hormone (ACTH), which, in turn, triggers the adrenal glands to produce cortisol. This cortisol acts as an immunosuppressant. Batool et al. (2020) stated that curry leaf extract exhibited effective immunomodulation in experimental animals through antioxidant and immunosuppressant mechanisms, which are crucial in medicine for reducing an excessive immune response that can occur during infection.

Survival Rate

After the administration of *M. koenigii* extracts, the survival rate values were monitored in the experimental groups, and different values were achieved (Figure 6). The highest mean survival rate, at 83.33%, was observed in the 600 mg/L treatment group. This survival rate value closely approached that of the negative control group (not treated), which recorded a survival rate of 93.33%. According to Mulia (2012), providing a booster like an herbal extract can trigger an increase in antibody production because the test fish already possess immune memory, allowing the booster to generate a more robust immune response.

Conversely, the lowest mean survival rate was observed in the positive control treatment group (received infectious agent only and not treated), which recorded a survival rate of 23.33%. The decline in gourami fish survival rates in the 650 mg/L and 700 mg/L treatment groups indicated that the dosage administered had reached its maximum point. Setyani et al. (2018) explained that high mortality occurs when there is a substantial amount of organ damage resulting from bacterial infection and when the extract dosage is sufficient to have a severe impact on the organism. There was increased degeneration, congestion, and necrosis at treatment levels of 650 mg/L and 700 mg/L. This shows that the effectiveness of the extract has reached its peak at the optimal dose, and doses higher than the optimal dose can be toxic, causing more severe kidney tissue damage. At high doses, secondary metabolites contained in the crude extract of *M. koenigii* leaves can be toxic to gourami fish. Rand et al. (2015) explained that as the dose of extract given increases, tissue cell damage also increases.

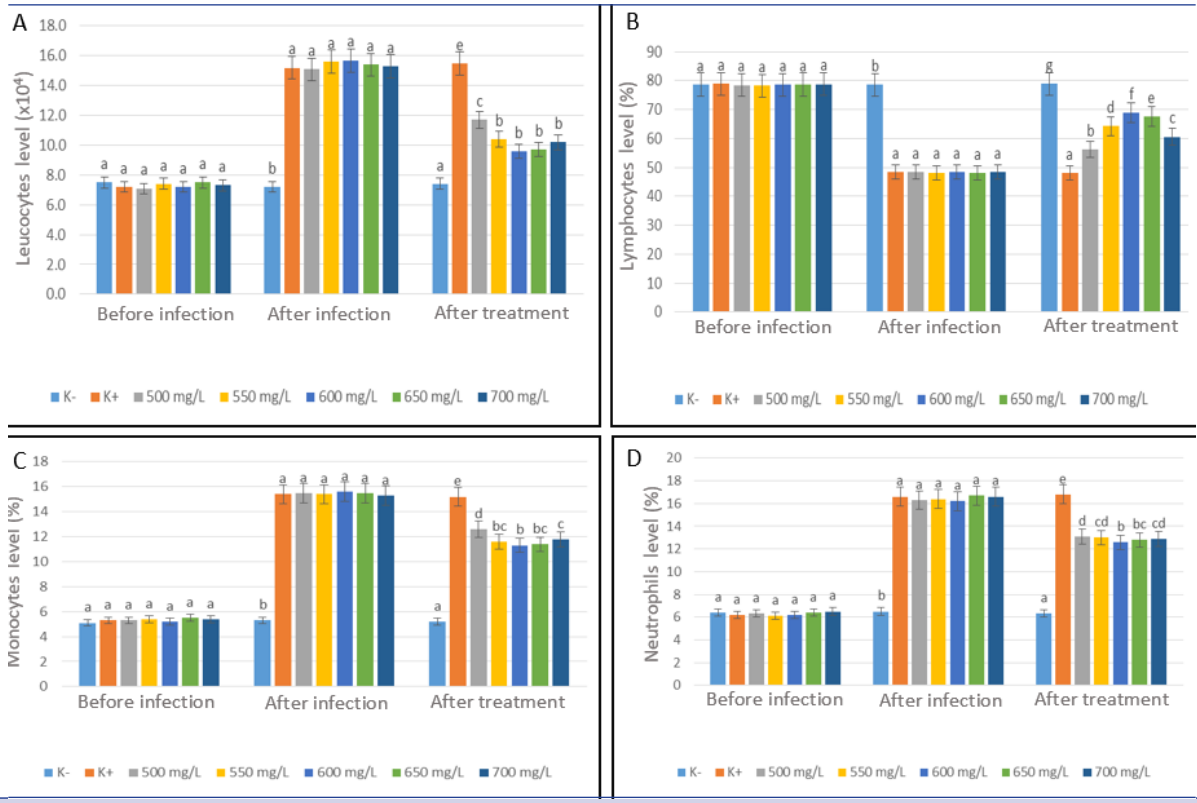

Figure 5. The value of leukocytes (A), lymphocytes (B), monocytes (C), and neutrophils (D) from gourami fish in this study. (K+) Normal (non-infected), (K-) Infected fish (not-treated).

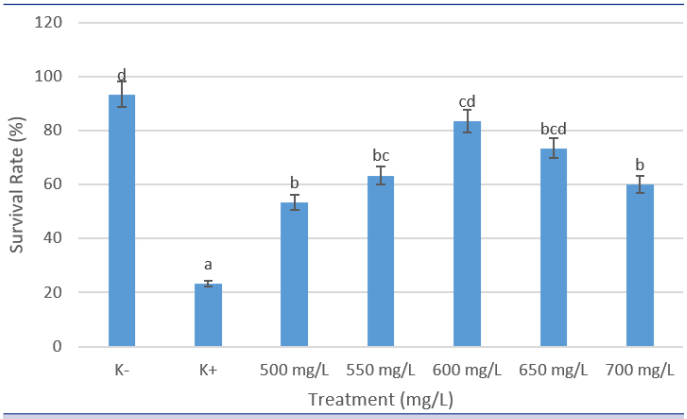

Figure 6. Survival Rate of Gourami Fish After Treatment.

CONCLUSION

In summary, the result of this study revealed that *M. koenigii* leaves are rich in alkaloids, terpenoids, saponins, flavonoids, and tannins so it provides an opportunity for usage as a natural product for the treatment of bacterial infections in fishes. The most effective dose for combating edwardsiellosis in gourami fish is 600 mg/L. This dose significantly influenced various hematological parameters, including hematocrit, hemoglobin, erythrocytes, leukocytes, lymphocytes, monocytes, and neutrophils. Additionally, it had a notable impact on histopathological aspects, with scores indicating mild degeneration, congestion, and necrosis.

To further advance the application of *M. koenigii* extract, additional research and experimentation should be conducted directly within aquaculture activities. This would help to better understand its potential benefits and feasibility in practical aquaculture settings.

Conflict of Interest: The authors declare no conflict of interest.

Ethics Committee Approval: This research does not harm the experimental animals used.

Acknowledgments: We would like to extend our appreciation to all individuals who contributed to this research.

REFERENCES

Abdelfadeel, K. F., Radwan, M., Gobran, A. M., & Reda, S. (2023). The role of omega-3 on diazepam treated adrenal cortex of the adult male albino rats (histological and immunohistochemical study). *Egyptian Journal of Histology*, *46*(2), 667-681. [CrossRef]

Abdel-Latif, H. M., Abdel-Tawwab, M., Khafaga, A. F., & Dawood, M. A. (2020). Dietary origanum essential oil improved antioxidative status, immune-related genes, and resistance of common carp (*Cyprinus carpio* L.) to *Aeromonas hydrophila* infection. *Fish & Shellfish Immunology*, *104*, 1-7. [CrossRef]

Adinata, M. O., Sudira, I. W., & Berata, I. K. (2012). The effect of the ashitaba (*Angelica keiskei*) leaves extract against histopathological kidney feature of male mice. *Buletin Veteriner Udayana*, *4*(2), 55-62.

Agustini, D. M., Yun, Y. F., Suprabawati, A., & Defara, S. M. (2020, February). Senyawa terpenoid dan aktivitas antidiabetes ekstrak n-heksana dan metanol *Hydrangea macrophylla*. *Prosiding Seminar*

Hasil Penelitian dan Pengabdian kepada Masyarakat Unjani Expo (Unex), 1(1) 27-30.

Aksara, R., Musa, W. J., & Alio, L. (2013). Identifikasi Senyawa Alkaloid Dari Ekstrak Metanol Kulit Batang. *Jurnal Entropi*, *8*(01), 514-519.

Al-Baiquni, B. J. (2019). Analysis of market structure-conduct-performance of gurame fish (*Osphonemus goramy*) in Kediri Regency. *Journal of Economic and Social of Fisheries and Marine*, 06(02), 134-148. [CrossRef]

Azhagu M. S., Vijayakumar, S., Sripriya, R., & Rajalakshmi, S. (2021). Phytochemical screening and GC–MS analysis of bioactive compounds present in ethanolic leaf extract *Murraya koenigii*. *Bulletin of Environment, Pharmacology and Life Sciences*, *10*, 158-164.

Awad, E., & Awaad, A. (2017). Role of medicinal plants on growth performance and immune status in fish. *Fish & Shellfish Immunology*, *67*, 40-54. [CrossRef]

Bailey, C., Holland, J. W., Secombes, C. J., & Tafalla, C. (2020). A portrait of the immune response to proliferative kidney disease (PKD) in rainbow trout. *Parasite Immunology*, *42*(8), e12730. [CrossRef]

Batool, S. A.K. Rashid, M.A. Hanif, M.A. Ayub & S. Memon. (2020). Chapter 14 - Curry Leaf. *Medicinal Plants of South Asia*. Elsevier. 179-190p [CrossRef]

Bintoro, A., Ibrahim, A. M., Situmeang, B., Kimia, J. K. S. T. A., & Cilegon, B. (2017). Analisis dan identifikasi senyawa saponin dari daun bidara (*Zhizipus mauritania* L.). *Jurnal Itekima*, *2*(1), 84-94.

Cheng, K., Tang, Q., Huang, Y., Liu, X., Karrow, N. A., & Wang, C. (2020). Effect of vitamin D3 on the immunomodulation of head kidney after *Edwardsiella ictaluri* challenge in yellow catfish (*Pelteobagrus fulvidraco*). *Fish & Shellfish Immunology*, *99*, 353-361. [CrossRef]

Dewi, A. F., Prajitno, A., & Yuniarti, A. (2019). Phytochemicals and the ability of *Plantago major* Linn. extract to inhibit the growth of *Aeromonas hydrophila*. *The Journal of Experimental Life Science*, *9*(2), 70-75. [CrossRef]

Donadio, G., Mensitieri, F., Santoro, V., Parisi, V., Bellone, M. L., De Tommasi, N., Izzo, V., & Dal Piaz, F. (2021). Interactions with microbial proteins driving the antibacterial activity of flavonoids. *Pharmaceutics*, *13*(5), 660. [CrossRef]

Ernawati, S. K., & Sari, K. (2015). Chemical Compound Content And Antibacterial Activity Of Avocado (*Persea americana* P. Mill) Peel Extract On *Vibrio alginolyticus* Bacteria. *Jurnal Kajian Veteriner*, *2*(3), 203-211. https://doi.org/10.35508/jkv.v3i2.1043.

Fauziah, F., Putri, N. N., & Firdus, F. (2014). The Effect of Curry Leaves (*Murayya Koenigii L.*) on Blood Glucose Levels In Alloxan Diabetic Mice (*Mus Musculus*). *Jurnal Natural*, *14*(1). 23-29.

Fujaya, Y. (2004). *Basic Fish Physiology of Fishery Technology Development*. Jakarta: Rineka Cipta.

Ge, J., Huang, Y., Lv, M., Zhang, C., Talukder, M., Li, J., & Li, J. (2022). Cadmium induced Fak-mediated anoikis activation in kidney via nuclear receptors (AHR/CAR/PXR)-mediated xenobiotic detoxification pathway. *Journal of Inorganic Biochemistry*, 227, 111682. [CrossRef]

Hammado, N. & Illing, I. (2013). Identification of alkaloid active compounds in the Lahuna plant (*Eupatorium odoratum*). *Journal of Dynamics*. *4*(2), 1-18.

Hardi, E. H., Sukenda, S., Harris, E., & Lusiastuti, A. M. (2011). Toksisitas produk ekstrasellular (ECP) *Streptococcus agalactiae* pada ikan nila (*Oreochromis niloticus*). *Jurnal Natur Indonesia*, *13*(3), 187-199. [CrossRef]

Harikrishnan, R., Devi, G., Paray, B. A., Al-Sadoon, M. K., Al-Mfarij, A. R., & Van Doan, H. (2020). Effect of cassic acid on immunity and immune-reproductive genes transcription in *Clarias gariepinus* against *Edwardsiella tarda*. *Fish & Shellfish Immunology*, *99*, 331-341. [CrossRef]

Heny, S., Sri, A., & Rayhan, A. (2011) In vitro ihbition of *Pseudomonas fluorescens* bacteria by using curry tree (*Murraya koenigii*) crude extract. *Research Journal of Medical Plant*, *5*(6), 772-778. [CrossRef]

Hidayati, D. A., Prajitno, A., Sulistyawati, T. D., Pratama, G., & Nilakandhi, T. Antibacterial activity of red galangal (*A. purpurata*) extract on the growth of *E. tarda* bacteria. *Journal of Aquaculture and Fish Health*, *12*(1), 127-134. [CrossRef]

Hussain, M., Herrmann, M., von Eiff, C., Perdreau-Remington, F. & Peters, G. (1997). A 140-kilodalton extracellular protein is essential for the accumulation of *Staphylococcus epidermidis* strains on surfaces. *Infection & Immunity*, 65, 519524. [CrossRef]

Jubair, N., Rajagopal, M., Chinnappan, S., Abdullah, N. B., & Fatima, A. (2021). Review on the antibacterial mechanism of plant-derived compounds against multidrug-resistant bacteria (MDR). *Evidence-Based Complementary and Alternative Medicine*, *2021*, 3663315. [CrossRef]

Kalindamar, S., Kordon, A. O., Abdelhamed, H., Tan, W., Pinchuk, L. M., & Karsi, A. (2020). *Edwardsiella ictaluri* evpP is required for colonisation of channel catfish ovary cells and necrosis in anterior kidney macrophages. *Cellular Microbiology*, *22*(3), e13135. [CrossRef]

Kasnir, M., Nisaa, K., Darmawan, A. B., Farizah, N., & Sudrajat, I. (2023). Potential Bioactive Compounds of *Melastoma malabathricum* Leaf Extract in Feed on Growth Performance and Survival Rate of Tilapia Fry in Brackish Water. *Journal of Aquaculture & Fish Health*, *12*(2), 207-215. [CrossRef]

Kawengian, S. A., Wuisan, J., & Leman, M. A. (2017). Uji daya hambat ekstrak daun serai (*Cymbopogon citratus* L) terhadap pertumbuhan *Streptococcus mutans*. *e-GiGi*, *5*(1). [CrossRef]

Kim, Y. R., & Yang, C. S. (2018). Protective roles of ginseng against bacterial infection. *Microbial Cell*, *5*(11), 472. [CrossRef]

Kole, S., Anand, D., Sharma, R., Tripathi, G., Makesh, M., Rajendran, K. V., & Bedekar, M. K. (2017). Tissue specific expression profile of some immune related genes in *Labeo rohita* to *Edwardsiella tarda* infection. *Fish & Shellfish Immunology*, *66*, 575-582. [CrossRef]

Kumar, V., Cotran, R. S., & Robbins, S. L. (2012). *Textbook of Pathology*. Jakarta: EGC.

Lestari, T., & Sidik, Y. (2013). Isolation and identification of tannin compounds from water extract of gading coconut bark (*Cocos nucifera* var. eburnea). *Jurnal Kesehatan Bakti Tunas Husada, 9*(1), 22-27. [CrossRef]

Li, W. R., Hu, Y. H., Jiang, S., & Sun, L. (2019). Global profiling and characterization of Japanese flounder (*Paralichthys olivaceus*) kidney microRNAs regulated by *Edwardsiella tarda* infection in a time-dependent fashion. *Fish & Shellfish Immunology*, *93*, 766-780. [CrossRef]

Ma, Q., Wei, R., Yang, M., Huang, X., Zhong, G., Sang, Z., Dong, J., Shu, J., Liu, J., Zhang, R., Yang, J., Wang, A., & Su, Y. (2019). Structures and biological evaluation of phenylpropanoid derivatives from *Murraya koenigii*. *Bioorganic Chemistry*, *86*, 159-165. [CrossRef]

Maftuch, M., Putri, V. D., Lulloh, M. H., & Wibisono, F. K. H. (2015). Studi ikan bandeng (*Chanos chanos*) yang dibudidayakan di tambak tercemar limbah kadmium (Cd) dan timbal (Pb) di Kalanganyar, Sidoarjo, Jawa Timur terhadap histopatologi hati, ginjal dan insang. *Journal of Environmental Engineering & Sustainable Technology*, *2*(2), 114-122. [CrossRef]

Maftuch, M. (2018). Hematological analysis of nile tilapia (*Oreochromis niloticus*) and striped catfish (*Pangasius hypophthalmus*) using hematology analyzer tool software at Fish Breeding Center Jojogan, Tuban, East Java. *Research Journal of Life Science*, *5*(2), 107-115. [CrossRef]

Moore, C. L., Savenka, A. V., & Basnakian, A. G. (2021). TUNEL assay: a powerful tool for kidney injury evaluation. *International Journal of Molecular Sciences*, 22(1), 412. [CrossRef]

Mulia, D. S. (2012). *Dumbo catfish vaccination*. Yogyakarta : Pustaka Pelajar.

Naeemi, A., Jamili, S., Shabanipour, N., Mashinchian, A., & Shariati Feizabadi, S. (2013). Histopathological changes of gill, liver and

kidney in Caspian kutum exposed to Linear Alkylbenzene Sulfonate.. *Iranian Journal of Fisheries Sciences, 12*(4), 887-897.

Najiah, M., Nadirah, M., Arief, Z., Zahrol, S., Tee, L. W., Ranzi, A. D., Amar, A. S., Laith, A. A., Mariam, M., Suzana, S., & Aida, R. J. (2011). Antibacterial activity of Malaysian edible herbs extracts on fish pathogenic bacteria. *Research Journal of Medicinal Plant*, *5*(6), 772-778. [CrossRef]

Ningrum, D. W., Kusrini, D., & Fachriyah, E. (2017). Uji aktivitas antioksidan senyawa flavonoid dari ekstrak etanol daun johar (*Senna siamea* lamk). *Jurnal Kimia Sains dan Aplikasi*, *20*(3), 123-129. [CrossRef]

Nursidika, P., Saptarini, O. & Rafiqua, N. (2014). Antimicrobial Activity of Ethanol Extract Fraction of Areca Fruit (*Areca catechu* L) on Methicillin Resistant Bacteria *Staphylococcus aureus*. *Bandung Medical Journal*, 46(2), 94-99. [CrossRef]

Obaseki, O. S., Olugbuyiro, J. A., De, D. K., & Kesinro, O. R. (2017). Analysis of UV spectra of some natural plant dyes applicable in fabrication of Grätzel cells. *Journal of Scientific and Engineering Research, 4*(9), 418-424.

Patil, R., Mandlik, S., & Mandlik, D. (2024). *Murraya koenigii* (Curry Tree): A review of its phytochemistry, ethnomedicinal uses, and pharmacology with respect to molecular mechanisms. *Current Traditional Medicine*, 10(5), 73-97. [CrossRef]

Parameswari, W., Sasanti, A. D., & Muslim. (2013). Bacterial population, histopathology, survival and growth of snakehead fish (*Channa striata*) seeds reared in media with the addition of probiotics. *Journal of Indonesian Swamp Aquaculture. 1*(1), 76-89. [CrossRef]

Rand, E. E. D., Smit, S., Beukes, M., Apostolides, Z., Pirk, C. W., & Nicolson, S. W. (2015). Detoxification mechanisms of honey bees (*Apis mellifera*) resulting in tolerance of dietary nicotine. *Scientific reports*, *5*(1), 11779. [CrossRef]

Rattanachaikunsopon, P., & Phumkhachorn, P. (2010). Assessment of synergistic efficacy of carvacrol and cymene against *Edwardsiella tarda* in vitro and in Tilapia (*Oreochromis niloticus*). *African Journal of Microbiology Research, 4*(5), 420-425.

Rieger, A. M., & Barreda, D. R. (2011). Antimicrobial mechanisms of fish leukocytes. *Developmental & Comparative Immunology*, *35*(12), 1238-1245. [CrossRef]

Rifa'i, R. A. R., Andayani, S., & Fadjar, M. (2023). Antibacterial ability of *Rhizophora mucronata* leaf extract against bacterial infections of *Edwardsiella tarda*. *The Journal of Experimental Life Science*, *13*(1), 12-16. [CrossRef]

Salikutty, J. (2012). *Handbook of herbs and spices*. Cambridge: Woodhead Publishing Limited.

Santi, S. R. 2010. Active anti-food compound from gadung tubers (*Dioscorea hipida* Dennst). *Jurnal Kimia, 4*(1), 71-78

Sariningsih, P, Rita, W. S., & Puspawati, N. M. (2015). Identifikasi dan uji aktivitas senyawa tanin dari ekstrak daun trembesi (*Samanea saman* (Jacq.) Merr) sebagai antibakteri *Escherichia coli* (*E. coli*). *Jurnal Kimia*, *9*(1), 27-34.

Setjianingsih. (2007). Characterization of three gourami strains using the morphometric truss method. *Indonesian Journal of Ichthyology, 7*(1), 23-30. https://doi.org/10.32491/jii.v7i1.220.

Setyani, R., Sarjito, A. H. C., & Haditomo. (2018). The effect of immersion ceremai leaf extract (*Phyllanthus acidus* [L] *skeels*) toward total erytrocyte and survival rate of *Cyprinus carpio* infected by *Aeromonas hydrophila*. *Journal of Aquaculture Management and Technology*, *7*(1), 114-119.

Soelama, H.J.J., Kepel, B.J., & Siagian. K.V. (2015). Test of Minimum Inhibitory Concentration (MIC) of Seaweed Extract (*Eucheuma cottonii*) as an Antibacterial Against *Streptococcus mutans*. *Journal e-GiGi (eG).* 3(2), 374-379. [CrossRef]

Suciati, A., Wardiyanto & Sumino. (2012). Effectiveness of *Rhizopora mucronata* leaf extract in inhibiting the growth of *Aeromonas salmonicida* and *Vibrio harveyi*. *E-Journal of Aquaculture Engineering and Technology.* 1(1), 1-8.

Sundaryono, A. (2011). Uji aktivitas senyawa flavonoid total dari *Gynura segetum* (lour) terhadap peningkatan eritrosit dan penurunan leukosit pada mencit (*Mus musculus*). *Jurnal Exacta*, *9*(2), 8-16.

Syaifurrisal, A., Prajitno, A., Fadjar, M., Riyadi, F. M., & Fauziyyah, A. I. (2021). *In vitro* analysis of antibacterial activities of curry leaf (*Murraya koenigii*) extract towards bacteria *Edwardsiella tarda*. *Journal of Fish Health and Aquaculture*. *10*(2), 221-228. [CrossRef]

Takashima, F., & Hibiya, T. (1995). *An atlas of fish histology: normal and pathological features*. New York: Gustav Fischer Verlag.

Taufik, I. & Setiadi, E. (2012). Toxicity and potential for bioaccumulation and bioelimination of the insecticide endosulfan in *Cyprinus carpio*. *Journal of Aquaculture Research*. 7(1), 131-143. [CrossRef]

Ulmursida, A., Ambariyanto, A. and Trianto, A. (2017). Antibacterial activity of mangrove *Avicennia marina* leaves extract against *Virgibacillus marismortui* and *Micrococcus luteus* bacteria. *AACL Bioflux*. 10(2), 372-380.

Venkatalakshmi, P., Vadivel, V, & Brindha, P. (2016). Role of phytochemicals as immunomodulatory agents: A review. *International Journal of Green Pharmacy*, *10*(1), 1-18. https://doi.org/10.22377/ijgp.v10i1.600

Wulandari, A., Prayitno, S. B., & Sarjito. (2014). Pathogenicity of isolate K14 isolated from African catfish (*Clarias gariepinus*) originating from Demak. *Journal of Aquaculture Management and Technology. 3*(2), 143-149.

Xu, D., Wang, J., Guo, C., Peng, X. X., & Li, H. (2019). Elevated biosynthesis of palmitic acid is required for zebrafish against *Edwardsiella tarda* infection. *Fish & Shellfish Immunology*, *92*, 508-518. [CrossRef]

Yan, Y., Li, X., Zhang, C., Lv, L., Gao, B., & Li, M. (2021). Research progress on antibacterial activities and mechanisms of natural alkaloids: A review. *Antibiotics*, *10*(3), 318. [CrossRef]

Zhou, J., Cai, Y., Liu, Y., An, H., Deng, K., Ashraf, M. A., Zou, L., & Wang, J. (2022). Breaking down the cell wall: Still an attractive antibacterial strategy. *Frontiers in Microbiology*, *13*, 952633. [CrossRef]

İSTANBUL UNIVERSITY PRESS

EISSN 2602-473X

AQUATIC SCIENCES AND ENGINEERING

Aquat Sci Eng 2024; 39(3): 189-199 • DOI: https://doi.org/10.26650/ASE20231388644

Review Article

An Overview of Gillnet and Trammel Net Size Selectivity in the Turkish Inland Fisheries

Mehmet Cilbiz[1], **Tuncay Ateşşahin**[2]

Cite this article as: Cilbiz, M., Ateşşahin, T. (2024). An overview of gillnet and trammel net size selectivity in the Turkish inland fisheries. *Aquatic Sciences and Engineering, 39(3)*, 189-199. DOI: https://doi.org/10.26650/ASE20231388644

ABSTRACT

This study compiled and remodelled length selectivity studies carried out on fish caught with gill nets and trammel nets in the inland fishery in Türkiye and evaluated them based on both the initial reproduction length and minimum landing size of species. The required data for the study were obtained through a literature review. 34 selectivity studies in total were identified, and 26 (76.5%) of them were carried out with gill net while the remaining 8 (23.5%) were conducted with trammel net. 24 of the studies were carried out according to *SELECT* (Share Each Length-class's Catch Total) and 10 of them according to Holt (1963). In conclusion, it was found that minimum conservation reference size (*MCRS*) values determined by the Ministry in the inland fishery in Türkiye were substantially greater than lengths at first maturity (*LFMs*) of species and that fishing gears used for fishing typically tended to fish mature individuals above *MCRS*. The major problem in terms of management is that different ideal mesh sizes are applied to different fish species that are in the same fishing place. Set nets with an ideal-mesh size for a species has a potential of catching individuals below *LFM* of other species in the same fishing grounds. It is thought that this problem can only be resolved with the Ecosystem Approach to Fisheries Management (*EAFm*).

Keywords: Inland fisheries, Length at first maturity, Minimum conservation reference size, Small scale fisheries, Sustainable fishery

ORCID IDs of the author:
M.C. 0000-0002-7686-7570;
T.A. 0000-0001-9168-5444

[1]Isparta University of Applied Sciences, Faculty of Eğirdir Fisheries, Isparta, Türkiye

[2]Firat University, Fisheries Faculty, Elazığ, Türkiye

Submitted:
09.11.2023

Revision Requested:
02.01.2024

Last Revision Received:
16.01.2024

Accepted:
22.01.2024

Online Published:
05.03.2024

Correspondence:
Mehmet Cilbiz
E-mail:
mehmetcilbiz@isparta.edu.tr

INTRODUCTION

Inland fisheries are defined as "any activity conducted to extract fish and other aquatic organisms from inland waters" by *FAO* (1997), which has vital importance for especially low-income developing countries in the world, as it supports the livelihood of 60 million people and is a source of food for hundreds of millions more (Moutopoulos et al., 2022; Smith et al., 2005; WorldBank, 2012). In addition to nutritional value, inland fisheries are important for poverty alleviation, gender empowerment, cultural services, ecosystem function and biodiversity (Funge-Smith and Bennett, 2019). Inland fisheries provide employment to millions of people in the world (Rabuffetti et al., 2022). The global capture fisheries production was 90.3 million tonnes in 2020, 12.7% of which was obtained from inland fisheries (FAO, 2022). The ratio of inland based capture fisheries production of Türkiye is almost 9.1% of the total production (Anonymous, 2021).

In general, inland fisheries are undervalued and ignored as a national or regional priority, despite their importance, however most sustainably generated source of animal protein of the world may be well-managed inland fisheries (Cooke et al., 2016; Moutopoulos et al., 2022). Marine-based fisheries are always very popular among managers and scientists due to their economic importance; however, inland fisheries are also quite important as marine fisheries in terms of considerable livelihood impacts in some countries (such as Bangladesh, Myanmar, Uganda, Nigeria, Cambodia, etc.)

Lakes, rivers, streams, canals, reservoirs, and other land-locked waters are defined as inland waters (FAO, 2014; Lynch et al., 2016). Commercial inland fishing occurs on mainly lakes and dam lakes. Fisheries cooperatives or private entrepreneur have to take a licence from Ministry of Forestry and Agriculture and should be pay a hire charge to the government. A total of 87 dam lakes, 16 natural lakes and three rivers were licenced by the ministry for fish production to inland fishermen in 2022 (fisheries cooperatives hired 82 fishing areas, while private entrepreneurs hired 24) (Anonymous, 2022a). Although Türkiye has 320 natural lakes (Anonymous, 2022b), the majority of them dry up in the summer, making them unsuitable for fisheries. The most important inland fishing areas of Türkiye are Van Lake (eastern Anatolia, 3, 713 km^2), Beyşehir Lake (southwestern Anatolia 656 km^2), Eğirdir Lake (south-western Anatolia 482 km^2), Atatürk Dam Lake (south-eastern Anatolia, 817 km^2), Keban Dam Lake (eastern Anatolia, 675 km^2) and Ilısu Dam Lake (south-eastern Anatolia, 313 km^2).

Inland fisheries are carried out in a small-scale (*SSFs*) concept in Türkiye. Using the efficient fishing gears, such as purse seine and trawl are banned in inland fisheries by legislation. The Turkish inland fisheries fleet consists of 3181 vessels, 97.36% of them are less than 10 m (Anonymous, 2021). Gillnets (tarek, common carp, gibel carp, pikeperch), trammel nets (common carp, gibel carp, European catfish), fyke nets (crayfish, European ell, northern pike), long lines (northern pike, pikeperch) and beach seines (sand smelt) are used by fishermen for catching the aquatic species. Most (94.0%) of the species caught from Türkiye's inland waters are fish, while others are crayfish, crabs, frogs, and snails (Turkstat, 2022).

Capture based inland fisheries production has decreased in Türkiye year by year; the total production declined 22.75% from 2000 (42,824 tonnes) to 2020 (33,119 tonnes). Increased fishing pressure, damming of rivers, deforestation, water pollution, and inadequate practices for managing fisheries are serious challenges to the sustainability of the inland waters (Barletta et al., 2010; Rabuffetti et al., 2022); however, managing the harvest of freshwater species is important for both food security and reducing biodiversity loss (Shephard et al., 2022; Tickner et al., 2020)

It is very important to use selective fishing gears for the sustainability of the natural fish stock in addition to determining the maximum sustainable yield (*MSY*) or the total allowable catch (TAC). Removing non-target species and under-sized fish that have not yet reproduced from the catch composition can only be achieved using the high size and species-selective fishing gear.

Gill nets (*GNs*) and trammel nets (*TNs*) which are widely used especially in developing countries in small-scale fishery because they are productive and relatively cheap (Acosta and Appeldoorn, 1995) are widely used by fishermen in the inland fishery in Türkiye. Set nets (*GNs* and *TNs*) rigged with monofilament material are typically preferred by fishermen instead of those rigged with multifilament material because they are (i) relatively cheap, (ii) long-lasting, (iii) resistant to contamination, (iv) easy to carry due to their lightness, (v) not gaining weight by taking in water when they are in the water.

In gilling type fishing, small fish may escape from the gill nets as they pass through the mesh and big fish may do the same as their heads do not fit into the mesh (Pope et al., 1975), in this case, gill nests can only catch a range of length where mesh size is larger than the fish head but smaller than fish body (Yüksel and Aydın, 2012). If a suitable mesh size is determined, both fish which are below the initial reproduction length and which have high reproduction potential (super-spawners) will be concurrently protected (Cilbiz et al., 2022). For this reason, set net selectivity parameters are extremely important scientific data for sustainable management of the natural fish stock. Both the FAO and the European Commission encourage the use of more selective fishing practices to reduce or eliminate bycatch and improve sustainability (Pérez Roda et al., 2019; Suárez et al., 2021).

Despite being highly selective, set nets to be used for species having different minimum landing sizes (MLS) (or in other words length at first maturity) are likely to capture bycatch. Therefore, knowing about the interaction between the species fished, minimum landing sizes and mesh sizes will significantly contribute to the maintenance of stocks. In this context, this study compiled and remodelled size selectivity studies conducted on the commercial fish species in the inland fishery in Türkiye and evaluated based on both the initial reproduction size and minimum landing size of species.

MATERIAL AND METHODS

Review of set net fishing technology and selectivity properties

Certain technical characteristics of *GNs* and *TNs*, and data needed for setting forth the model used for determining selectivity and the results achieved were obtained through literature review. To this end, peer-reviewed scientific journals and grey literature (e.g. conference proceedings, scientific research project final reports) that published the results of studies performed in the inland fishery in Türkiye were searched. In this section, (i) manufacturing technique for set net (gill net or trammel net), (ii) mesh size of nets tested (*MS*: stretched mesh size in mm), (iii) estimated model length for respective *MS* (*ML*: in cm) and (iv) methods used for modelling selectivity were investigated.

Review of biological - administrative reference points and commercial fish species

Conformity of estimated model lengths with the biological characteristics of species needs to be set forth in order to reveal the contribution of the results of selectivity studies to the sustainability of stocks. In this context, a literature review was made to (i) determine the commercial species in Türkiye, (ii) for which of the commercial species *GNs* and *TNs* selectivity studies are carried out, (iii) present lengths at first maturity (*LFM*) and (iv) minimum conservation reference sizes (*MCRS*) for those species. In case of presence of multiple studies on the same species, the most recent study was taken into account. Data needed were taken from peer-reviewed scientific journals, governmental institutions' data and legislation review.

Statistical approach

The relationship between MS and ML values obtained in selectivity studies was explained using simple linear regression model in the study. The model is described as follows;

$y = b.x + \varepsilon$

where:

y: denotes the response variable (MS)

x: denotes the predictor variable (ML)

b: the regression coefficient

ε: is the measurement error and any variation unexplained by linear model

For species with only one study, linear regression parameters were taken as basis, however, data were bootstrapped in case of presence of two or more studies. A simple linear regression based bootstrapping resampling method was used for estimating the uncertainties for reported selectivity data. 1000 times bootstraps were applied on *MS~ML* data by using tidymodels (v1.0.0) (Kuhn and Wickham, 2020) in RStudio software.

The ideal MS were computed by using regression model parameters for reference point *LFM* and *MCRS* separately. In case of existence of a legal length limit, *LFM* was primarily taken into account; if there was no available *LFM* value determined for a species, then *MCRS* was taken as the valid biological reference point.

RESULTS

There are almost 20 different fish species caught in Türkiye's inland fisheries (excepting the lagoon areas and other aquatic products such as crustaceans) by the 2021 data, and total production was 30,309 tonnes (Turkstat, 2022). It is determined that selectivity studies were conducted for 14 different fish species *Arabibarbus grypus, Alburnus tarichi, Blicca bjoerkna, Capoeta antalyensis, Cyprinus carpio, Carassius gibelio, Capoeta umbla, Esox lucius, Luciobarbus esocinus, Rutilus rutilus, Oncorhynchus mykiss, Scardinius erythrophtalmus, Sander lucioperca,* and *Vimba vimba*). In this context, it can be said that the set net selectivity is known for 70.0 % of caught fish species.

As a result of the literature review, 34 set net selectivity studies were identified in total. 26 (76.5%) of these studies were carried out with *GNs* and the remaining 8 (23.5%) were carried out with *TNs* (Table 1). 24 of the studies were conducted according to *SELECT* (Share Each Length-class's Catch Total) and 10 of them were conducted according to Holt (1963). As for the number of studies per species; 10 studies were carried out for *C. carpio*, seven for *C. gibelio*, four for *S. lucioperca*, two for *E. lucius*, two for *A. tarichi* and one study for each of all other species. When looked at the methods used for estimating selectivity, it was seen that *SELECT* (Share Each Length-class's Catch Total) method was widely preferred (70.6%) and Holt (1963) method was partly (29.4%) preferred (Table 1). Minimum conservation reference sizes (*MCRS*) determined for species by the Ministry of Agriculture and Forestry were found to vary between a total length range of 18 to 50 cm and no *MCRS* was determined for six species (*B. bjoerkna*, *C. gibelio*, *R. rutilus*, *O. mykiss*, *S. erythrophtalmus* and *V. vimba*) (Anonymous, 2020). Length at first maturity values were known for a major portion (76.9%) of species for which a set net length selectivity study was performed whereas only for three species (*A. grypus*, *B. bjoerkna* and *C. antalyensis*) *LFM* values were not known. *MS* of *GNs* used in selectivity study varied between 16-200 mm while *MS* of *TNs* varied between 34-160 mm (Table 1). Despite being the second mostly fished species in inland fishery in Türkiye, no *MCRS* was determined for *C. gibelio*. In this study, 25 cm of minimum economic length arising under market conditions was considered *MCRS*. For species for which more than one *LFM* reporting was provided for the same species and for species for which separate *LFMs* were reported for female-male individuals in the same study, the lower value was preferred as the reference *LFM* value with a protectionist approach. For example, out of *LFM* values reported by Yüce et al. (2016) as 30.85 for male individuals and as 32.01 for female individuals of *C. carpio*, the lower value proposed for male individuals was taken as reference.

The ideal mesh size for fishing the relevant species was determined in line with the results of the selectivity study (Table 2). In calculations carried out based on *MS~ML* relationship, *MCRS* value was primarily taken as basis as the point of intersection, and *LFM* value was used in the absence of *MCRS*.

As there were adequate number of studies for the species of *C. carpio* (*GNs-TNs*), *C. gibelio* (*GNs-TNs*), *E. lucius* (*GNs*), and *S. lucioperca* (*GNs*), ideal mesh sizes were determined according to bootstrap analysis (Figure 1). Ideal mesh sizes of other species given in Table two were determined according to simple linear regression model. Although there were 2 studies for *A. tarichi*, the fact that only two panels were used in the study carried out by Çetinkaya et al. (1995) made bootstrapping of data impossible. For this reason, the ideal mesh size for the species *A. tarichi* was given according to simple linear regression model. The ideal mesh size could not be modelled for *B. bjoerkna* which has neither an *MCRS* value nor an *LFM* value although a selectivity study was conducted on it. It was seen that *MCRS* values declared for all other species except for *C. umbla* were higher than (*C. carpio, C. gibelio, E. lucius, S. lucioperca, A. tarichi*) or equal to (*A. grypus, L. esocinus*) *LFM* values.

When *MS~ML* relationship graphs given in Figure 1 are reviewed, it is seen that confidence interval for *TNs* is wider compared to that of *GNs* considering the studies carried out on the same species. Mesh sizes of the nets used for *E. lucius* ve *S. lucioperca* selectivity are smaller than those of the nets used for *C. carpio* ve *C. gibelio* (Figure 1).

In order to observe which net mesh size corresponds to which reference point of which species; model lengths of set nets according to species and reference points (*MCRS - LFM*) are given as a whole in Figure 2. A green shaded area remaining on the right of the orange region for the same species means a greater *MCRS* than *LFM* for the species in question (i.e. *MCRS/LFM* is greater than 1). Disappearance of green shaded area may result either from the fact that *LFM* of the species is not known or *MCRS* limit is not available in commercial fishing (e.g. *R. rutilus*, *S. erythrophtalmus*, *V. vimba*). The most notable point in Figure 2 is that modelled mesh size which is in the confident area for a species corresponds to the unconfident area for another. For example, mesh size of 52.9 mm which is considered ideal for *S. lu-*

Table 1. Summary of the selectivity studies and the parameters considered for the analysis.

Gear	Species	*MCRS* (cm)	*LFM* (cm)	*MS* (mm)	*ML* (cm)	*Sel EM*	Reference
GNs	*Arabibarbus grypus*	45	-	40-80	40.12-80.24	Sel	(Anonymous, 2020a; Yuksel et al., 2020)
TNs	*Alburnus tarichi*	18	13.68	34-44	15.7-20.3	Holt	(Anonymous, 2020a; Çetinkaya et al., 1995; Elp, 1996)
GNS	*Alburnus tarichi*	18	13.68	16-48	8.5-25.4	Sel	(Anonymous, 2020a; Demirol and Cilbiz, 2023; Elp, 1996)
GNs	*Blicca bjoerkna*	-	-	40-52	9.8-13.8	Holt	(Balık and Çubuk, 2001)
GNs	*Capoeta antalyensis*	20	-	32-90	14.8-41.7	Sel	(Cilbiz and Yalım, 2017)
GNs	*Cyprinus carpio*	40	30.85	40-140	12.4-43.4	Sel	(Anonymous, 2020a; Aydın et al., 2016; Yüce et al., 2016)
GNs	*Cyprinus carpio*	40	30.85	130-160	53.3-65.6	Sel	(Anonymous, 2020a; Şen, 2016; Yüce et al., 2016)
GNs	*Cyprinus carpio*	40	30.85	140-200	56.9-84.2	Sel	(Anonymous, 2020a; Dereli et al., 2022; Yüce et al., 2016)
GNs	*Cyprinus carpio*	40	30.85	70-140	18.1-42-4	Holt	(Anonymous, 2020a; Balık, 1999a; Yüce et al., 2016)
GNs	*Cyprinus carpio*	40	30.85	56-90	17.6-27.5	Holt	(Anonymous, 2020a; Özyurt and Avşar, 2005; Yüce et al., 2016)
GNs	*Cyprinus carpio*	40	30.85	90-120	30.0-43.4	Holt	(Anonymous, 2020a; Yalçın, 2006; Yüce et al., 2016)
GNs	*Cyprinus carpio*	40	30.85	90-120	30.0-37.1	Holt	(Anonymous, 2020a; Dartay and Atessahin, 2017; Yüce et al., 2016)
TNs	*Cyprinus carpio*	40	30.85	100-140	39.1-54.7	Sel	(Anonymous, 2020a; Cilbiz, Küçükkara, et al., 2015; Yüce et al., 2016)
TNs	*Cyprinus carpio*	40	30.85	40-140	13.0-45.4	Sel	(Anonymous, 2020a; Aydın et al., 2016; Yüce et al., 2016)
TNs	*Cyprinus carpio*	40	30.85	130-160	50.8-62.6	Sel	(Anonymous, 2020a; Dereli et al., 2022; Yüce et al., 2016)
GNs	*Carassius gibelio*	25	9.7	32-90	8.8-24.7	Sel	(Balik et al., 2004; Cilbiz et al., 2014)
GNs	*Carassius gibelio*	25	9.7	32-90	8.7-24.6	Sel	(Balik et al., 2004; Cilbiz et al., 2014)
GNs	*Carassius gibelio*	25	9.7	40-100	12.3-30.7	Sel	(Aydın et al., 2018; Balik et al., 2004)
TNs	*Carassius gibelio*	25	9.7	100-140	24.9-34.9	Sel	(Balik et al., 2004; Cilbiz et al., 2014)
TNs	*Carassius gibelio*	25	9.7	100-140	27.2-38.1	Sel	(Balik et al., 2004; Cilbiz et al., 2014)
TNs	*Carassius gibelio*	25	9.7	100-120	23.8-28.5	Sel	(Balik et al., 2004; Korkmaz and Kuşat, 2014)
TNs	*Carassius gibelio*	25	9.7	40-100	12.0-30.1	Sel	(Aydın et al., 2018; Balik et al., 2004)
GNs	*Capoeta umbla*	20	23.3	56-76	26.0-35.3	Sel	(Anonymous, 2020a; Çoban et al., 2013; Gündüz et al., 2019)
TNs	*Capoeta baliki*	20	-	64-96	26.52-39.78	Sel	(Aydin et al., 2015)
GNs	*Esox lucius*	40	19.7	36-60	20.4-34.3	Sel	(Anonymous, 2020a; Balık, 2008; Balık et al., 2004)
GNs	*Esox lucius*	40	19.7	40-90	21.0-47.2	Sel	(Anonymous, 2020a; Balık et al., 2004; Cilbiz et al., 2017)
GNs	*Luciobarbus esocinus*	50	50	50-70	36.6-51.3	Holt	(Anonymous, 2020a; Eskandary et al., 2001; Yuksel et al., 2014)

Table 1. Continue.

Gear	Species	*MCRS* (cm)	*LFM* (cm)	*MS* (mm)	*ML* (cm)	*Sel EM*	Reference
GNs	*Oncorhynchus mykiss*	-	28.7	32-80	16.06-40.16	Sel	(Cilbiz, Yalım, et al., 2015; Wali et al., 2022)
GNs	*Rutilıs rutilus*	-	16.8	32-80	12.5-30.4	Sel	(Hanol et al., 2015; Tarkan, 2002)
GNs	*Scardinius erythrophtalmus*	-	19.2	40-52	12.6-16.5	Holt	(Balık and Çubuk, 2001; Tarkan, 2002)
GNs	*Sander lucioperca*	26	25.6	40-52	21.2-27.5	Sel	(Anonymous, 2020a; Ozyurt et al., 2011)
GNs	*Sander lucioperca*	26	25.6	32-90	16.3-45.8	Sel	(Anonymous, 2020a; Cilbiz et al., 2022)
GNs	*Sander lucioperca*	26	25.6	34-70	15.9-32.9	Holt	(Anonymous, 2020a; Balık, 1999b; Ozyurt et al., 2011)
GNs	*Sander lucioperca*	26	25.6	40-52	20.6-26.8	Holt	(Anonymous, 2020a; Kiyağa, 2008; Ozyurt et al., 2011)
GNs	*Vimba vimba*	-	12.23	32-90	12.9-36.4	Sel	(Cilbiz, Apaydın Yağcı, et al., 2015; Erol, 2019)

Gear: *GNs* (Gill nets) and *TNs* (Trammel nets; *MCRS*: Minimum Conservation Reference Size as total length; *LFM*: Length at First Maturity; MS: Mesh Size (intended as stretched mesh length, range min-max); *ML*: Modal Length (range min-max); *Sel EM* (Estimation Method): *SEL* (SELECT), Holt (Holt, 1963); Reference for *MCRS*, *LFM*, gear selectivity

Table 2. Some biological reference points and linear regression parameters modelling the relationship between mesh length and modal length for the species targeted by gears.

Species	Net type	*MCRS* (cm)	*LFM* (cm)	*MCRS / LFM* ratio	Ideal mesh size (mm) Mean *(CI 95%)*	Linear regression parameters			
						Intercept *(SE)*	Mesh *(SE)*	*p*	R^2
*C. carpio**	*GNs*	40	30.84	1.29	114.4 (111.0 – 117.8)	35.62 (3.12)	1.95 (0.07)	<0.001	0.94
*C. carpio**	*TNs*	40	30.84	1.29	112.2 (105.7 – 118.7)	20.58 (7.00)	2.25 (0.16)	<0.001	0.93
*C. gibelio**	*GNs*	25	10.67	2.34	86.7 (83.8 – 89.7)	3.52 (2.47)	3.34 (0.13)	<0.001	0.97
*C. gibelio**	*TNs*	25	10.67	2.34	93.5 (88.7 – 99.8)	-4.34 (9.35)	3.96 (0.32)	<0.001	0.90
*E. lucius**	*GNs*	40	24.54	1.62	75.0 (72.8 – 77.1)	-4.26 (2.22)	1.99 (0.07)	<0.001	0.98
*S. lucioperca**	*GNs*	26	22.00	1.18	52.9 (52.0 – 53.8)	1.42 (1.37)	1.95 (0.05)	<0.001	0.98
A. grypus	*GNs*	45	45	1.00	46.00	1.00 (5.00)	1.00 (9.00)	<0.001	1.00
A. tarichi	*GNs*	18	13.68	1.32	34.04	-0.002 (0.005)	1.89 (0.00)	<0.001	1.00
C. antalyensis	*GNs*	20	-	-	43.12	-0.001 (0.007)	2.16 (0.00)	<0.001	1.00
C. umbla	*GNs*	20	23.31	0.86	43.05	-0.006 (0.03)	2.15 (0.00)	<0.001	1.00
L. esocinus	*GNs*	50	50	1.00	68.28	0.018 (0.02)	1.37 (0.00)	<0.001	1.00
O. mykiss	*GNs*	-	28.7	-	57.17	0.006 (0.003)	1.99 (0.00)	<0.001	1.00
R. rutilus	*GNS*	-	16.8	-	42.48	-1.20 (9.10)	2.60 (4.10)	<0.001	1.00
S. erythrophtalmus	*GNs*	-	19.2	-	79.46	0.92 (1.28)	3.09 (0.9)	<0.001	1.00
V. vimba	*GNs*	-	12.23	-	26.66	-3.90 (2.01)	2.53 (7.93)	<0.001	1.00

* Ideal mesh size computed by using bootstrapped model for *MCRS* point; *GNs*: gillnets; *TNs*: trammel nets; *LFM*: length at first maturity: *MCRS*: Minimum conservation reference size.

cioperca is quite below the ideal mesh size for *C. carpio* with which it probably shares the same fishing grounds.

The selectivity indicator diagrams (Figure 3) used in this study which was developed by Lucchetti et al. (2020) for provide an immediate visual representation of whether a net can catch immature individuals. Figure 3 represents four catching scenarios. (*i*) The best scenario -catches mature individuals above the *MCRS*- (Figure 3- green area); (*ii*) discard scenario -catches mature individuals under the *MCRS*- (Figure 3- yellow area); (*iii*) worst case scenario -catches immature individuals under the *MCRS*- (Figure 3- red area); bad case scenario catches immature individuals above the *MCRS*- (Figure 3- pink area).

The selectivity indicator diagrams were applied for only *C. carpio* (*GNs-TNs*), *C. gibelio* (*GNs-TNs*), *E. lucius* (*GNs*) and *S. lucioperca* (*GNs*) for which we have enough studies (Figure 4). A shift towards the upper right quadrant was observed in density graphs for all net types, so it indicates that the nets catch mature individuals above the *MCRS* (Figure 3, Figure 4).

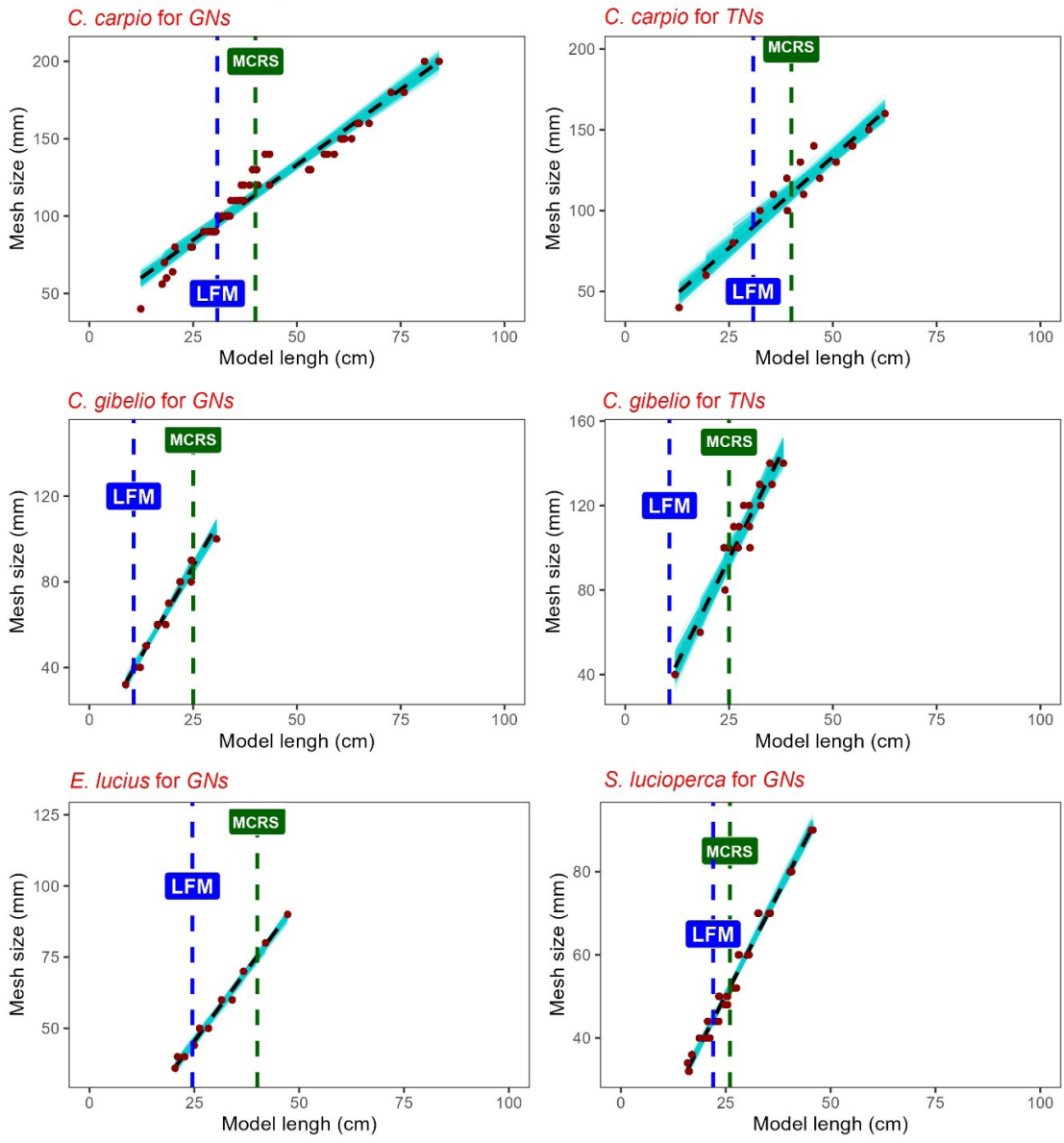

Figure 1. Relationship between mesh size and modal length in gillnets and trammel nets for the species that can be studied. (Shadowed area: 95% confidence interval; solid cyan; black dotted line: linear regression; blue dotted line: *LFM*; green dotted line: *MCRS* obtained from the review).

DISCUSSION

In the present study, in which selectivity features of *GNs* and *TNs* used in fishing 14 fish species in the inland fishery in Türkiye were evaluated, 34 set net selectivity studies were evaluated. The majority of the studies (75.7%) were carried out with *GNs*. Likewise, in a similar study conducted by Lucchetti et al. (2020), selectivity studies were found to be more common on *GNs* (65.0%) compared to *TNs* (35.0%). Specific to Türkiye, this is supposed to be related to cost. The cost to the fishermen of *TNs* made up by combining three different panels instead of *GNs* consisting of a single panel is higher; they prefer this cost only for fishing relatively bigger fish (e.g. *C. carpio*) which they have difficulty in fishing with *GNs*. The big *C. carpio* cause breakage of the net rope because of fluttering while they are caught in the form of gilling on the side of *GNs* and in this way they can break away from the net, however, this is not the case when they are caught with *TNs*. In support of this argument, Balık (1996) reports that *TNs* are more efficient than *GNs* in fishing *C. carpio*.

As for the number of studies per species, 10 studies were carried out for *C. carpio*, seven for *C. gibelio*, four for *S. lucioperca*, two for *E. lucius*, two for *A. tarichi* and one study for each of the other species. This distribution is supposed to be due to the generality across the country of the species in general and their share in total production. According to Turkstat (2022) data, the first four species that are mostly fished in the inland fishery in Türkiye in 2001 and their production amounts are *A. tarichi* (9925), *C.*

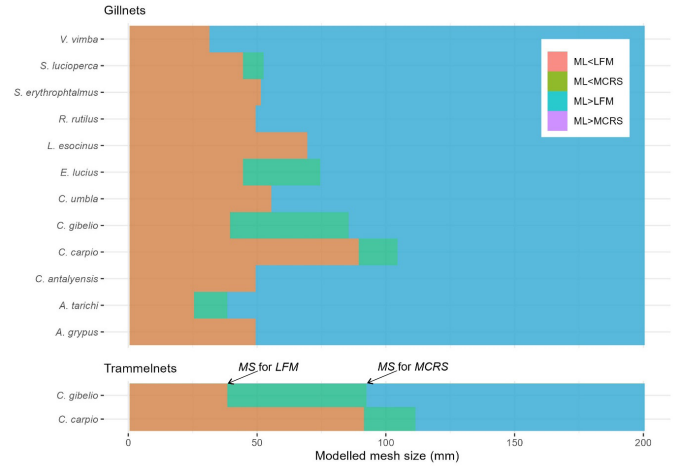

Figure 2. Ideal MS versus reference point comparison for different species (gillnets: upper; trammel nets: lower; MS: mesh size; LFM: length at first maturity; MCRS: Minimum conservation reference size).

Figure 3. Definition of theoretical selectivity indicator diagrams (Lucchetti et al., 2020) (*ML*: Modal length; *LFM*: Length at first maturity; *MCRS*: Minimum conservation reference size).

gibelio (8039 tonnes), *A. boyeri* (6404 tonnes) ve *C. carpio* (3212 tonnes). In fishing *A. boyeri* in Türkiye, the coastal beach seine is used rather than *GNs* and *TNs* (Cilbiz et al., 2020).

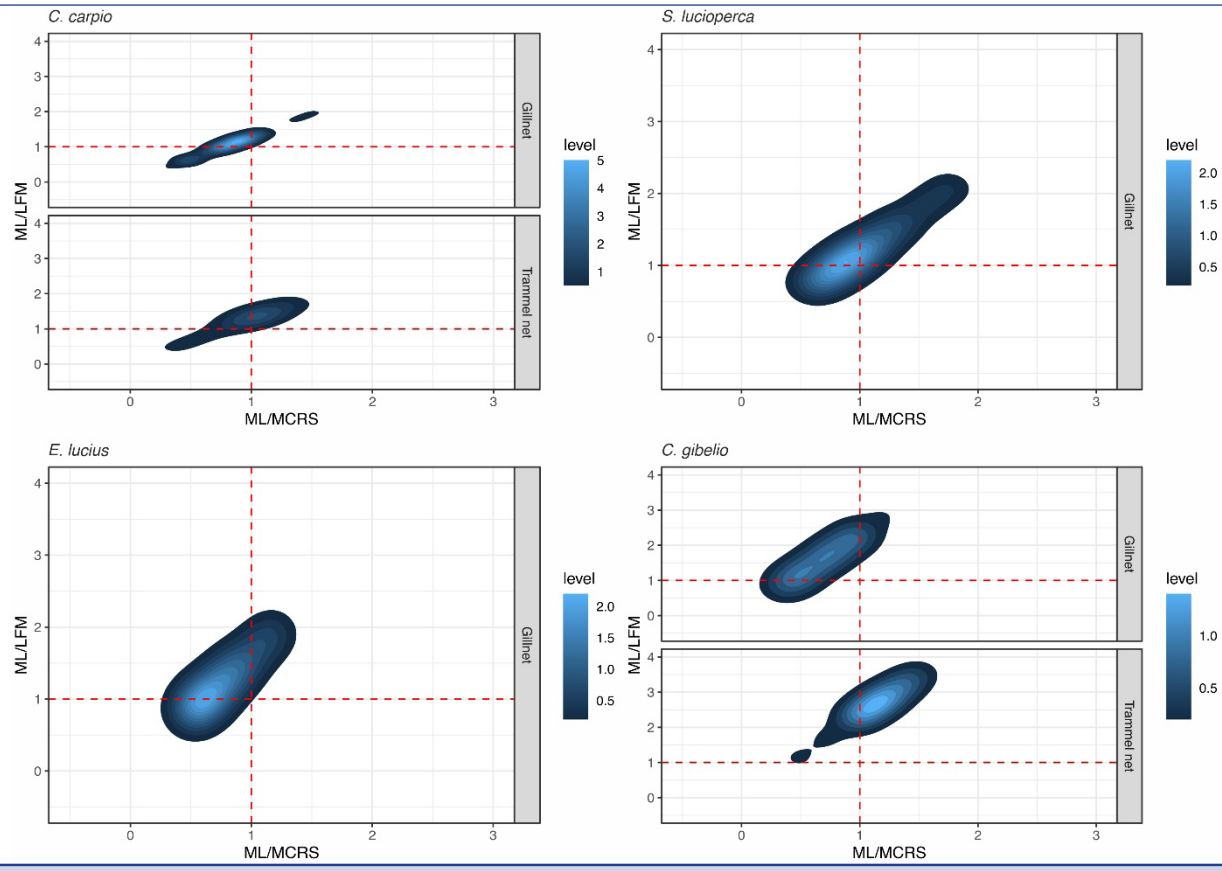

Figure 4. Selectivity indicator diagrams for the different species (*ML*: modal length; *LFM*: length at first maturity; *MCRS*: minimum conservation reference size).

Researchers mostly preferred *SELECT* method (69.7%) and partly Holt (1963) method (30.3%) in their studies for investigating *GNs* and *TNs* size selectivity. In general, it can be said that *SELECT* method was preferred in the more recent studies. This results from the fact that researchers prefer methods with relatively higher reliability within the framework of developing data evaluation technologies. According to Balık (2008), despite being one of the most widely used methods in set net selectivity, Holt (1963) is limiting; instead of it, the use of the *SELECT* method which is a statistical model estimating selectivity curve comparatively among different mesh size fishing and which provides an approach compatible with selectivity analysis has become widespread recently.

In this study, it was found that *LFM* for *A. grypus, B. bjoerkna* and *C. antalyensis* species is still not known and there is need for scientific studies for determining this value which is very important for sustainability of the species. On the other hand, there are species for which *MCRS* is not applied in commercial fishing although their *LFMs* are known (*C. gibelio, O. mykiss, R. rutilus, S. erythrophtalmus, V. vimba, A. boyeri*). There is no length limit in both amateur and commercial fishing for *C. gibelio, O. mykiss, A. boyeri* which are listed among the ecologically harmful (and potentially harmful species) for inland waters in Anonymous (2020b). In particular, although *C. gibelio* and *A. boyeri* that are among the first four species fished mostly in inland waters make significant contributions to the economy of both fishermen and the country, the fact that *MCRS* has not been declared for commercial fishing them may indicate that the ministry is not planning for the sustainability of natural stocks of these species.

The *MCRS* values declared for all species except *C. umbla* was found to be higher (*C. carpio, C. gibelio, E. lucius, S. lucioperca, A. tarichi*) than or equal (*A. grypus, L. esocinus*) to *LFM* value. In this context, the requirement that "the stock should find the chance of reproduction at least once before being caught for ensuring maintainability of the biomass" suggested by Beverton and Holt (1957) for a significant part of species is met in theory. However, whether determined legal regulations have been put into practice, in other words, whether fishermen comply with *MCRS* limits should be strictly inspected. Otherwise, no contribution should be expected from the criteria determined to the sustainability of natural fish stocks.

There is a selectivity study carried out by Balık and Çubuk, in 2001 for *B. bjoerkna*, however, it is a species with a very low economic value in the inland fishery in Türkiye and there is no legal length limit (*MCRS*) in fishing this species as it is often caught as a bycatch of other more important species; in addition, it was not subjected to further evaluation since no studies were found on the reproduction biology of the species.

When the relationship graphs given in Figure 1 are reviewed, it is seen that in general, the confidence interval for *TNs* is wider compared to that of *GNs* considering the studies carried out on the same species. This may be resulting from the higher spread value (*SR*) of *TNs* compared to that of *GNs* (from the fact that they capture a wider length range). In a study by Aydın et al. (2016), selectivity features of *GNs* and *TNs* with the same mesh size in fishing *C. carpio* were investigated and for nets with the mesh sizes of 40, 60, 80 and 100 mm, *SR* values for *GNs* were reported to be 1.10, 1.65, 2.20, 2.75 cm in the same order while *SR* values for *TNs* were reported to be higher being 1.37, 2.06, 2.74 ve 3.43 cm respectively. Similarly, Lucchetti et al. (2020) also state that out of the set nets, especially *GNs* have high selectivity.

Under the legislation regulating commercial fisheries in Türkiye (Anonymous, 2020a), minimum mesh size application in inland waters is in place for removing certain lengths of certain species from the catch composition. Minimum mesh size that can be used by fishers in fishing is determined by local administrative units. At this point, because the legal practice does not follow a strategy that is based on a scientific foundation, different mesh sizes are declared in different fishing places for the same species and this leads to discomfort among the fishers. This study has a lot of promise for overcoming this handicap. When looked at other countries where composition of species is wide, it is observed that a standard ideal mesh size cannot be determined as is the case in Türkiye. For example, in Greece, the mesh size of nets used by professional fishermen in inland fishing is required to be longer than 20 mm (bar length), however, this rule does not apply to *C. carpio* (55 mm), *Alburnus sp.* and *Atherina boyeri* (15 mm) (Petriki et al., 2014).

Consequently, it was found that *MCRS* values determined by the Ministry in the inland fishery in Türkiye are substantially greater than *LFMs* of species. In this context, it can be asserted that the criterion that species should find the opportunity of reproducing at least once before being fished is met, and that sustainability of fishery is significantly guaranteed. On the other hand, fishing gears used for fishing were found to be typically tending to fish mature individuals on *MCRS*. The major problem in the use of set net in the inland fishery in Türkiye is that different ideal mesh sizes are determined for different fish species that are in the same fishing place. A mesh size that is ideal for a species may have the potential of fishing individuals below *LFM* for another species in the same fishing grounds. For resolution of this problem, fishery management authorities are advised to determine vulnerable species (that are exposed to overfishing, economically much more valuable, among endangered species, of high importance in ecological terms) within the frame of fishing place-based on-site management and to determine mesh width based on the objective of protecting them to a minimum extent. This problem which is not very likely to be resolved with conventional fishery management focusing on single or targeted species is likely to be resolved with the Ecosystem Approach to Fisheries Management (*EAFm*) specifically recommended for inland fisheries by FAO (2021).

Authorship: Mehmet Cilbiz and Tuncay Ateşşahin collected the data. Mehmet Cilbiz analysed the data. Mehmet Cilbiz wrote the first draft of the manuscript. Mehmet Cilbiz and Tuncay Ateşşahin contributed to manuscript revision, and read and approved the submitted version.

Funding Information: This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Data Availability Statement: The data that support the findings of this study are available on request from the corresponding au-

thor. The data are not publicly available due to privacy or ethical restrictions.

Conflict of Interest Disclosure: The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this research.

Ethics Approval Statement: Not applicable due to not used any biological material in the research.

Acknowledgements: Some part of this study was represented in 2nd International Limnology and Freshwater Fisheries Research Symposium (*Limnofish 2019*).

REFRERENCES

Acosta AR, Appeldoorn RS (1995). Catching efficiency and selectivity of gillnets and trammel nets in coral reefs from southwestern Puerto Rico. Fish. Res. *22*(3–4):175-196. https://doi.org/http://dx.doi.org/10.1016/0165-7836(94)00328-T

Anonymous (2020a). *5/1 Notification of commercial fish catching regulation in seas and inland water (Notificaiton number 2020/20).* Republic of Türkiye, Minister of Agriculture and Rural Forestry, General Directorate of Fisheries and Aquaculture, Ankara.

Anonymous (2020b). *5/2 Notification of amateur fish catching regulation in seas and inland water (Notification number 2020/21).* Republic of Türkiye, Minister of Agriculture and Rural Forestry, General Directorate of Fisheries and Aquaculture, Ankara.

Anonymous (2021). Fishries Statistics. *Republic of Turkey, Ministry of Agriculture and Forest, General Directorate of Agricultural Research and Policies, Ankara, Turkey*, 21 p.

Anonymous (2022a). Hired Fishing Areas. Republic of Turkey, Minister of Agriculture and Rural Forestry, General Directorate of Fisheries and Aquaculture, Ankara (retrived from: https://www.tarimorman.gov.tr/BSGM/Menu/32/Bilgi-Dokumanlari).

Anonymous (2022b). Soil-water sources. Republic of Turkey, Minister of Agriculture and Rural Forestry, General Directorate of State Hydraulic Works, Ankara (retrived from: https://www.dsi.gov.tr/Sayfa/Detay/754#).

Aydın C, Cilbiz M, İlhan A, Sari HM (2018). Gillnet and trammel net selectivity for Prussian carp (*Carassius gibelio*) in Marmara Lake, (Turkey). Ege J Fish Aqua Sci. 35:79-87. https://doi.org/10.12714/egejfas.2018.35.1.13

Aydın C, Cilbiz M, İlhan A, Sarı HM (2016). Selectivity of multifilament trammel and gillnets for common carp (*Cyprinus carpio* L., 1758) in Lake Marmara. Ege J Fish Aqua *Sci.* 33(3): 183-192. https://doi.org/10.12714/egejfas.2016.33.3.01

Aydin E, Kahraman AE, Göktürk D, Ayaz A (2015). Trammel net selectivity for four barbel scraper *Capoeta baliki* in the Sakarya River, Turkey. Turkish Journal of Fisheries and Aquatic Sciences, 15(3), 9. https://doi.org/10.4194/1303-2712-v15_3_01

Balık İ (1996). *The investigation of selectivity and productivity of multifilament trammel net and multifilament gill net and monofilament gill net that used on fishing for carp fish (Cyprinus carpio L.1758) and pike-perch (Stizastedion lucioperca (L.1758) in Lake Beyşenir [Doctoral dissertation, in Turkish]* Ege Üniversitesi.

Balık İ (1999a). Investigation of the selectivity of monofilament gill nets used in carp fishing (*Cyprinus carpio* L., 1758) in Lake Beyşehir. Turk J Zool. *23*(2): 185-187.

Balık İ (1999b). Investigation of the selectivity of multifilament and monofilament gill nets on pike perch (*Stizostedion lucioperca* (L., 1758)) fishing in Lake Beyşehir. Turk J Zool. 23(2): 179-183.

Balık İ (2008). Gillnet selectivity for pike, *Esox lucius*, in Lake Karamık, Turkey. *Eifac Symposium On Interactions Between Social, Economic And Ecological Objectives Of Inland Commercial And Recreational Fisheries And Aquaculture, Antalya, EIFAC Occasional 44*, 93-99.

Balık İ, Çubuk H (2001). Selectivity of gillnets for catching rudd (*Scardinius erythrophtalmus* L. 1758) and white bream (*Blicca björkna* L., 1758) in Lake Ulubat (Apolyont,) [in Turkish]. XI. Syposium on National Aquaculture, Hatay, 04- 06 September, Hatay, Turkey.

Balık İ, Çubuk H, Özkök R, Uysal R (2004). Reproduction properties of pike (*Esox lucius* L., 1758) population in Lake Karamık (Afyonkarahisar/Turkey). Turk J Zool. 30: 27-30.

Balik I, Özkök R, Çubuk H, Uysal, R (2004). Investigation of some biological characteristics of the silver crucian carp, *Carassius gibelio* (Bloch 1782) population in Lake Eğirdir. Turk J Zool. 28(1):19-28.

Barletta M, Jaureguizar AJ, Baigun C, Fontoura NF, Agostinho AA, Almeida-Val VMF, Val A L, Torres RA, Jimenes-Segura LF, Giarrizzo T, Fabré NN, Batista VS, Lasso C, Taphorn DC, Costa MF, Chaves PT, Vieira JP, Corrêa MFM (2010). Fish and aquatic habitat conservation in South America: a continental overview with emphasis on neotropical systems. *J Fish Biol.* 76(9): 2118-2176. https://doi.org/10.1111/j.1095-8649.2010.02684.x

Beverton R, Holt S (1957). On the dynamics of exploited fish populations. . In *Fish. Invest. Minist. Agric., Fish. Food (G. B.),* Ser. II, 19, 533 p.

Cilbiz M, Apaydın Yağcı M, Uysal R, Yagcı A, Cesur M (2015). Investigation of monofilament gill net selectivity for vimba (*Vimba vimba* Linnaeus, 1758) in Eğirdir Lake, Turkey. Pak J Zool. 47(3): 882-886.

Cilbiz M, Çinar Ş, Cilbiz N, Çapkın K, Ceylan M (2014). Monofilament gill net and trammel net selectivity for the silver crucian carp (*Carassius gibelio* Bloch, 1782) in the Eğirdir Lake, Isparta-Turkey. Iran J Fish Sci. 13(4): 967-978.

Cilbiz M, Hanol Z, Cilbiz N, Çınar Ş, Savaşer S (2014). Multifilament gillnet and trammel Net selectivity for the silver crucian carp (*Carassius gibelio* Bloch, 1782) in Eğirdir Lake, Isparta, Turkey. Turk J Fish Aquat Sci. 14(4):905-913. https://doi.org/10.4194/1303-2712-v14_4_09

Cilbiz M, Küçükkara R, Ceylan M, Savaşer S, Meke T (2015). Trammel net selectivity of common carp (*Cyprinus carpio* L., 1758) in Manyas Lake, Turkey. Limnofish. 1(1):1-7 https://doi.org/10.17216/LimnoFish-5000083989

Cilbiz M, Uysal R, Alp A, Yeğen V, Apaydın Yağcı M, Yağcı A, Küçükkara R (2017). Mesh size recommendation for Turkey pike (*Esox lucius* L., 1758) gillnet fishery. *Turk J Fish Aquat Sci.* 17(4): 815-820. https://doi.org/10.4194/1303-2712-v17_4_17

Cilbiz M, Uysal R, Tosunoğlu Z, Aydın C, Ahmet A, Bilgin F (2020). New approach for codend selectivity: A case study of coastal beach seine for big-scale sand smelt (*Atherina boyeri*) fishery in İznik Lake. Turk J Fish Aquat Sci. 20(9):681-692. https://doi.org/10.4194/1303-2712-v20_9_03

Cilbiz M, Yağcı A, Yener O, Korkmaz B (2022). Gillnet selectivity for pikeperch (*Sander lucioperca* Linnaeus, 1758) in Suğla Lake (Konya-Turkiye) [in Turkish]. Commagene J Bio. 6(1):110-115. https://doi.org/10.31594/commagene.1118152

Cilbiz M, Yalım FB (2017). Gillnet selectiviy of siraz *(Capoeta antalyensis) in Karacaören-I Dam Lake (Burdur-Türkiye)[in Turkish].* 19. Sypmposium on National Aquaculture, 12-15 September 2017, Sinop, Turkey. 162p.

Cilbiz M, Yalım FB, Korkmaz B, Yener O (2015). Determining of gillnet selectivity and efficiency of rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792) in Karacaören-I Dam Lake [in Turkish]. 2. Sypmposium on Fish Introduction and Reservuar Management, 20-22 May 2015 Eğirdir-Isparta,Turkey. 205-206p.

Cooke SJ, Allison EH, Beard TD Jr, Arlinghaus R, Arthington AH, Bartley DM, Cowx IG, Fuentevilla C, Leonard NJ, Lorenzen K, Lynch AJ, Nguyen VM, Youn SJ, Taylor WW, Welcomme RL (2016). On the sustainability of inland fisheries: Finding a future for the forgotten. Ambio, *45*(7): 753-764 https://doi.org/10.1007/s13280-016-0787-4

Çetinkaya O, Sarı M, Arabacı M (1995). A preliminary study on catch composition and selectivity of the trammel net used in fishing of

Chalcalburnus tarichi (Pallas, 1811) in Lake Van (TÜrkiye) [in Turkish]. Ege J Fish Aqua Sc., *12*(1-2):1-13.

Çoban MZ, Gündüz F, Türkgülü I, Örnekçi NG, Yüce S, Demirol F, Alp A (2013). Reproductive properties of *Capoeta umbla* (Heckel, 1843) living in Lake Hazar (Elaziğ , Turkey). Int J Agr Food Res. 2(2): 38-47.

Dartay M, Atessahin T (2017). Selectivity of gillnets with different characteristics for *Cyprinus carpio* Linnaeus 1758 caught in inland waters of Turkey. Fresen Environ Bull. 26(5): 3374-3378.

Demirol F, Cilbiz M (2023). Gillnet selectivity estimating for sustainable stock management of endemik Tarek (*Alburnus tarichi* Güldenstädt, 1814) population in Lake Van (eastern, Turkey). Turk J Fish Aquat Sci. *23*(5). TRJFAS21936 https://doi.org/10.4194/trjfas21936

Dereli H, Kebapçıoğlu T, Şen Y, Ölçek ZS, Dinçtürk E, Ulman A (2022). The effect of gillnet twine thickness on catching efficiency and selectivity for common carp (*Cyprinus carpio* Linnaeus, 1758) fishery in Marmara Lake. Ege J Fish Aqua Sci. 39:88-96. https://doi.org/10.12714/egejfas.39.2.01

Dereli H, Şen Y, Kebapçıoğlu T, Erdoğuş M, Ölçek ZS, Özdemir M, Ulman A (2022). Management recommendations for common carp fisheries in Turkey in light of their reproductivity and gear selectivity. J Fish Environ. 46(1):141-156.

Elp M (1996). *A study on reproductive biology of inci kefali (Chalcalburnus tarichi) [in Turkish]* MSc thesis, Van Yüzüncü Yıl üniversitesi, Van,Turkey, 71p.

Erol HD (2019). *Population structure, growth and reproduction biologyof egrez (Vimba vimba (Linnaeus, 1758)) in the Oymapınar Dam Lake (Antalya) [in Turkish]* MSc thesis, Akdeniz Üniversitesi , Antalya, Turkey, 52p.

Eskandary GR, Dheghan S, Nikpey M, Miahi Y (2001). The biology of *Barbus esocinus* in Dez Dam Lake (Khuzestan Province). *The Scientific Research Results of the Iranian Fisheries Org., Tehran, Iran, 75 pp.*

FAO (1997). Inland fisheries. *FAO Technical Guidelines for Responsible Fisheries. No. 6. Rome, FAO. 1997. 36p.*

FAO (2014). CWP Handbook of Fishery Statistical Standards. Section G: Fishing Areas - General. Rome, Italy.

FAO (2021). Report of the Second Advisory Roundtable on the Assessment of Inland Fisheries, Rome, 25–27 November 2019. FAO Fisheries and Aquaculture Report No. 1314. Rome.

FAO (2022). The State of World Fisheries and Aquaculture 2022. Towards Blue Transformation. Rome, FAO.

Funge-Smith S, Bennett A (2019). A fresh look at inland fisheries and their role in food security and livelihoods. Fish Fish. 20(6):1176-1195. https://doi.org/https://doi.org/10.1111/faf.12403

Gündüz F, Demirol F, Yüce S, Yüksel F, Çoban M Z, Alp A (2019). *Gillnet selectivity for Capoeta umbla (Heckel, 1843) in Uzunçayır Dam Lake*. 2. International Symposium on Limnology and Freshwater Fisheries, Elazığ-Turkey 144p.

Hanol Z, Cilbiz M, Çinar Ş, Korkut SO, Yener O (2015). Investigation the selectivity of gillnet used in roach (*Rutilus rutilus* L., 1758) fishery in Uluabat Lake, Bursa-Turkey. J Surv Fish Sci. 1(2):11-20. https://doi.org/10.17762/sfs.v1i2.56

Kiyağa VB, 2008. *The Investigation of the Selectivity of Monofilament Gill Nets Used in Catch of Pike Perch (Sander lucioperca Bogustkaya and Naseka, 1996) In Seyhan Dam Lake [in Turkish]* Çukuraova University, School of Natural and Applied Sciences]. Adana.

Korkmaz B, Kuşat M (2014). Selectivity of monofilament trammel nets used in Fishing of Prussian carp, *Carassius gibelio* (Bloch, 1782) in Lake Eğirdir. Suleyman Demirel University Journal of Natural and Applied Science, 18(2): 69-74.

Kuhn M, Wickham H (2020). Tidymodels: a collection of packages for modeling and machine learning using tidyverse principles. https://www.tidymodels.org

Lucchetti A, Virgili M, Petetta A, Sartor P (2020). An overview of gill net and trammel net size selectivity in the Mediterranean Sea. Fish Res. 230:105677. https://doi.org/10.1016/j.fishres.2020.105677

Lynch AJ, Cooke SJ, Deines AM, Bower SD, Bunnell DB, Cowx IG, Nguyen VM, Nohner J, Phouthavong K, Riley B, Rogers MW, Taylor WW, Woelmer W, Youn SJ, Beard TD (2016). The social, economic, and environmental importance of inland fish and fisheries. Environ Rev. 24(2):115-121. https://doi.org/10.1139/er-2015-0064

Moutopoulos DK, Koutsikos N, Vardakas L, Perdikaris C (2022). A history of Greek inland fishery development during 1928-2019. *Fisheries Manag Ecol*. *29*(5):681-692. https://doi.org/10.1111/fme.12558

Ozyurt CE, Kiyağa VB, Mavruk S, Akamca E (2011). Spawing, maturity length and size selectivity for pikeperch (*Sander lucioperca*) in Seyhan Dam Lake. J Anim Vet Adv. *10*(4):545-551.

Özyurt CE, Avşar D (2005). Investigation of the selectivity parameters for carp (*Cyprinus carpio* Linnaeus, 1758) in Seyhan Dam Lake. Turk J Ve Anim Sci. *29*(2): 219-223.

Pérez Roda MA, e, Gilman E, Huntington T, Kennelly SJ, Suuronen P, Chaloupka M, Medley P (2019). *A third assessment of global marine fisheries discards*. FAO Fisheries and Aquaculture Technical Paper No. 633. Rome, FAO. 78 pp.

Petriki O, Erzini K, Moutopoulos DK, Bobori DC (2014). Gillnet selectivity for freshwater fish species in three lentic systems of Greece. J App Ichthyol. 30(5):1016-1027.

Pope J, Margetts A, Hamley J, Akyüz E (1975). Manual of Methods For Fish Stock Assessment Part III Selectivity Of Fishing Gear *Fao Fisheries Technical Paper No 41 Revision 1*.

Rabuffetti AP, Espínola LA, Amsler P, Ferreira P, Abrial E, Blettler MCM, Amsler ML (2022). Fishers' knowledge on a large floodplain river in South America. Contributions for sustainable management of inland fisheries. Rev Fish Biol Fisher.1:20. https://doi.org/10.1007/s11160-022-09722-x

RStudio Team (2022). RStudio: Integrated Development 464 for R. RStudio, Inc., Boston, MA. http://www.rstudio.com/

Shephard S, Edwards K, George S, Joseph E, James S, David O, Persaud A, Watson LC, Van Vliet N (2023). Community-based monitoring, assessment and management of data-limited inland fish stocks in North Rupununi, Guyana. Fish Manag Ecol. 30:2. 121-133. https://doi.org/10.1111/fme.12604

Smith LED, Khoa SN, Lorenzen K (2005). Livelihood functions of inland fisheries: policy implications in developing countries. Water policy. 7(4): 359-383. https://doi.org/10.2166/wp.2005.0023

Suárez MD, Sáez MI, Rincon-Cervera MÁ, Hidalgo L, Guil-Guerrero JL (2021). Discarded fish on the Spanish Mediterranean coast: influence of season on fatty acids profiles. Mediterr Mar Sci. *22*(2):232-245. https://doi.org/10.12681/mms.24909

Şen Y (2016). *Determination of the selectivity and catching efficiency of the gill nets used for common carp (cyprinus carpio l., 1758) in Demirkopru Dam Lake[in Turkish]* MSc thesis, İzmir Katip Çelebi Üniversitesi, Fen Bilimleri Enstitüsü, Su Ürünleri Ana Bilim Dalı, izmir, 79.p].

Tarkan AS (2002). *Size at maturity and fecundity of roach (Rutilus rutilus L. 1758) and rudd (Scardinius erythrophthalmus L. 1758) fish populations in Sapanca Lake [in Turkish]* MSc thesis, İstanbul Üniversitesi, 32p.

Tickner D, Opperman JJ, Abell R, Acreman M, Arthington AH, Bunn SE, Cooke SJ, Dalton J, Darwall W, Edwards G, Harrison I, Hughes K, Jones T, Leclère D, Lynch AJ, Leonard P, McClain ME, Muruven D, Olden JD, Ormerod SJ, Robinson J, Tharme RE, Thieme M,Tockner K,Wright M, Young, L (2020). Bending the Curve of Global Freshwater Biodiversity Loss: An Emergency Recovery Plan. *BioScience. 70*(4):330-342. https://doi.org/10.1093/biosci/biaa002

Turkstat (2022) Fishery statistics. http://www.turkstat.gov.tr/PreTablo.do?alt_id=1005 (Access: January 19, 2022).

Wali A, Shah T, Bhat F, Mohd I (2022). studies on gonad histology and length at first maturity of the rainbow trout Walbaum, 1792 *Oncorhynchus mykiss* (Salmoniformes: Salmonidae) from Kashmir. Indian J Ecol. 49(1):178-182. https://doi.org/10.55362/IJE/2022/3499

WorldBank (2012). *Hidden harvest: the global contribution of capture fisheries. The World Bank, Report 66469-GLB, Washington, DC.* http://hdl.handle.net/10986/11873

Yalçın N (2006). *Comparison of the Selectivity Parameters for Common Carp (Cyprinus carpio L, 1758) and Mirror Carp (Cyprinus carpio L., 1758 var. specularis) in Dam Lakes [in Turkish]* I. Syposium on Fish İntroduction and Reservuar Management. Akuademi.net, 281-288 p.

Yuksel F, Demirol F, Gündüz F, Çoban M (2020). Estimation of gillnet selectivity for shabbout (*Arabibarbus grypus* Heckel, 1843). Fresen Environ Bull. 29: 8675-8681.

Yuksel F, Gündüz F, Demirol F (2014). Gillnet selectivity for *Luciobarbus esocinus* (Heckel, 1843) in Keban Dam Lake, Elazig, Turkey. Indian J Fish. 61(2):108-111.

Yüce S, Gündüz F, Demirol F, Çelik B, Alpaslan K, Çoban M, Aydın R, Şen D (2016). some population parameters of mirror carp (*Cyprinus carpio* L., 1758) inhabiting Atatürk Dam Lake. *Limnofish*. 2(1):31-42. https://doi.org/10.17216/LimnoFish-5000149481

Yüksel F, Aydın F (2012). Gillnet selectivity and factors affecting Selectivity. Ecological Life Sciences. 7(2):12-21.

İSTANBUL UNIVERSITY PRESS

EISSN 2602-473X

AQUATIC SCIENCES AND ENGINEERING

Aquat Sci Eng 2024; 39(3): 200-205 • DOI: https://doi.org/10.26650/ASE20241406066

Short Communication

Where Have You Been Hiding? Re-Emergence of *Squatina oculata* in the Sea of Marmara, with a Review of Captures in Turkish Seas

Hakan Kabasakal[1,2], Uğur Uzer[3], F. Saadet Karakulak[3]

Cite this article as: Kabasakal, H., Uzer, U., & Karakulak, F.S. (2024). Where have you been hiding? Re-emergence of *Squatina oculata* in the Sea of Marmara, with a review of captures in Turkish seas. *Aquatic Sciences and Engineering, 39(3),* 200-205. DOI: https://doi.org/10.26650/ASE20241406066

ABSTRACT

On February 27, 2018, a female smoothback angelshark, *Squatina oculata*, was incidentally captured in a gill-net fishery off the Mudanya coast (southeastern Sea of Marmara). An opportunistic photographic record confirmed the emergence of S. oculata in the Sea of Marmara, where its status in the area is considered questionable because of its absence for many years in the region. The current opportunistic records are valuable data sources, which can provide complementary information to fill the knowledge gaps in the life histories of rare, rarely caught, or endangered sharks and rays.

Keywords: *Squatina oculata,* Turkish Sea, emergence, endangered, opportunistic

ORCID IDs of the author:
H.K. 0000-0001-8189-9748;
U.U. 0000-0002-7038-6469;
F.S.K. 0000-0002-6053-5256

[1]İstanbul University, Institute of Science, Fisheries Technologies and Management Program, İstanbul, Türkiye

[2]WWF Türkiye, Asmalı Mescit, İstanbul, Türkiye

[3]Faculty of Aquatic Sciences, Istanbul University, Istanbul, Türkiye

Submitted:
17.12.2023

Revision Requested:
25.03.2024

Last Revision Received:
25.03.2024

Accepted:
28.03.2024

Correspondence:
Hakan Kabasakal
E-mail:
kabasakal.hakan@gmail.com

INTRODUCTION

Angelsharks (Squatiniformes: Squatinidae) are represented by 22 species and *Squatina oculata* Bonaparte, 1840, commonly known as the smoothback angelshark, emerged in the Eastern Atlantic, from Senegal to Ghana coast, and in the Mediterranean Sea (Ebert et al., 2021). Although its present emergence in the Mediterranean Sea has long been well-recognized (Miller, 2016; Serena et al., 2020; Barone et al., 2022), *S. oculata* is considered a rare and one of the least-known species of Mediterranean sharks for which data collection is clearly required owing to knowledge gaps (Zava et al., 2016; Barone et al., 2022; Zava et al., 2022). First accounts of the emergence of smoothback angelshark in Turkish waters were mentioned in the inventories of Slastenenko (1955–1956; cited in Bilecenoğlu et al., 2002, 2014), Akşıray (1987), and Bauchot (1987). Despite being reported in the Sea of Marmara for the first time in the 1950s and then reported from the same region in the early 1990s (Slastenenko, 1955–1956; Meriç, 1994; both were cited in Bilecenoğlu et al., 2002, 2014), the species has not been reported in several bycatch inventories (Bayhan et al., 2006; Bök et al., 2011) or general ichthyological inventories (Karakulak et al., 2017; Daban et al., 2021; ÇŞİDB-TUBİTAK-MAM, 2021) of the Sea of Marmara, which were previously published. Although the emergence of *S. oculata* in the region was also reported by Eryılmaz and Meriç (2005), this review study is based on the ichthyological report by Meriç (1994; cited in Bilecenoğlu et al., 2002). Due to the absence of a recent record of smoothback angelsharks from the region, the emergence status of *S. oculata* was considered questionable (Kabasakal, 2022); thus, it requires confirmation. In the present study, the re-emergence of the smoothback angelshark in the Sea of Marmara after nearly 30 years is reported, and a review of captures of the species in Turkish waters is provided.

MATERIAL AND METHODS

The present record of *S. oculata* was obtained following data mining to extract shark-specific

information (printed or digital articles, social media posts or films, etc.) as part of an ongoing data acquisition survey to regularly update the information on sharks of Turkish seas, which has been continuing since 2000 through monthly screening of published media (2000–2009 records) and Internet media (post2009 records). While scanning and extracting data shared by digital content owners on the Internet or social media, ethical rules proposed by Monkman et al. (2017) were followed using the data in question. Furthermore, care was taken to not violate the rights of content owners and show the faces of people in the visual content to prevent revealing their identities. Information regarding the present smooth angelshark is available on the website of a mainstream newspaper of Turkish media (Türk, 2018).

Species identification follows the descriptions by Miller (2016), Ebert et al. (2021), and Barone et al. (2022). Taxonomic nomenclature follows that reported by Froese and Pauly (2023). Regarding the sampling methodology, the present study is a usual practice of opportunistic sampling, of which opportunistic records are not the direct results of scientific fisheries surveys but are acquired from printed or digital media, social media, logs of naturalists, or recreational fishermen (Grant et al., 2022; Hiddink et al., 2023). Previous captures of *S. oculata* in Turkish waters were extracted from relevant references presented in Table 1.

RESULTS AND DISCUSSION

On February 27, 2018, a female smoothback angelshark (Figure 1) was incidentally captured in a gill-net fishery off the Mudanya coast (southeastern Sea of Marmara; Figure 2). The present specimen was idenified as *S. oculata* based on the following characteristics: a ray-like shark species with a strongly dorsoventrally flattened body, a very wide head, widened pectoral and dorsal fins, and no anal fin; however, the head is separated from the pectoral fins with very deep insertions. The origin of the first dorsal fin is located well behind the free rear tips of the pelvic fins, a primary descriptive characteristic of the three species of angelsharks occurring in the Mediterranean Sea (Figure 1). The area between the eyes is strongly concave. The lower lobe of the caudal fin is larger than the upper lobe. Nasal barbels are quite simple; however, because of the resolution and sight angle of the present image, no details of the nasal barbels, defined as "moderately fringed" in the literature, can be observed in the photo of the present specimen. Large spiracles are observed behind the eyes. The mouth is terminal and wide, and the teeth on the upper and lower jaws are small, pointed, and with one cusp. The above description of the present specimen is in agreement with the published descriptions of *S. oculata* (Miller, 2016; Ebert et al., 2021; Barone et al., 2022).

Captures of *S. oculata* reported from the Sea of Marmara and off the Turkish coasts of the Aegean Sea and the Mediterranean are presented in Table 1. In this table, 22 smoothback angelsharks have been incidentally captured in Turkish waters between pre1956 and 2023. The majority of smoothback angelsharks has been captured in Turkish Mediterranean waters (n = 13; 59.09%), followed by Turkish Aegean waters (n = 6; 27.2%) and Sea of Marmara (n = 3; 13.6%). The size (TL) of the captured smoothback angelsharks varied between approximately 24 and 120 cm, and the

Table 1. Review of captured specimens of Squatina oculata in Turkish waters.

No	Date	Locality	TL (cm)	W (g)	Sex	Depth (m)	Fishing gear	Remarks	Reference
1	Pre1956	SoM	-	-	-	-	-	-	Slastenenko (1955–1956; cited in Bilecenoğlu et al., 2002, 2014)
2	Pre1994	SoM	-	-	-	-	-	-	Meriç (1994; cited in Bilecenoğlu et al., 2002)
3	Pre1996	İskenderun Bay, northeastern MS	75.6	4,000	-	60	BT	Captured over mixed muddy-sandy bottom off Karataş	Başusta ve Erdem (2000)
4	Pre2002	Gökçeada, northern AE	-	-	-	-	-	-	Kabasakal (2002)
5	Pre2002	Fethiye, southern AE	-	-	-	-	-	-	Kabasakal (2002)
6	Pre2002	İskenderun Bay, northeastern MS	-	-	-	-	-	-	Kabasakal (2002)
7	Jul 1997	Gökçeada, northern AE	30	180	♂	-	BT	Captured over sandy bottom mixed with small pebbles and vegetation of *Posidonia oceanica*	Kabasakal & Kabasakal (2004)

Table 1. Continue.

No	Date	Locality	TL (cm)	W (g)	Sex	Depth (m)	Fishing gear	Remarks	Reference
8	Sep 1999	Gökçeada, northern AE	95	6,000	♀	-	BT	Captured over sandy bottom	Kabasakal & Kabasakal (2004)
9	Aug 2009	Bay of Antalya, eastern MS	80	4,500	-	100	BT	-	Özgür Özbek & Kabasakal (2022)
10	Spring 2010	Bay of Antalya, eastern MS	50	850	-	100	BT	-	Özgür Özbek & Kabasakal (2022)
11	Spring 2010	Bay of Antalya, eastern MS	88	5,550	♀	50	BT	A total of seven developing embryos, with a total weight of 500 g, were found in uteri. Photographic documentation of the intra-uterine embryos is available in the relevant reference	Özgür Özbek & Kabasakal (2022)
12	Spring 2010	Bay of Antalya, eastern MS	59	1,600	-	50	BT	-	Özgür Özbek & Kabasakal (2022)
13	Spring 2010	Bay of Antalya, eastern MS	52	1,000	-	100	BT	-	Özgür Özbek & Kabasakal (2022)
14	Winter 2010	Bay of Antalya, eastern MS	67	1,700	-	50	BT	-	Özgür Özbek & Kabasakal (2022)
15	Winter 2010	Bay of Antalya, eastern MS	69	1,800	-	50	BT	-	Özgür Özbek & Kabasakal (2022)
16	Winter 2010	Bay of Antalya, eastern MS	66	2,000	-	50	BT	-	Özgür Özbek & Kabasakal (2022)
17	Winter 2010	Bay of Antalya, eastern MS	88	5,500	-	50	BT	-	Özgür Özbek & Kabasakal (2022)
18	Winter 2010	Bay of Antalya, eastern MS	24	71	-	50	BT	-	Özgür Özbek & Kabasakal (2022)
19	4 Nov 2017	Aydıncık coast, eastern MS	72.6	3,450	♀	65	BT	Captured over sandy bottom	Ergüden et al. (2019)
20	27 Feb 2018	Mudanya coast, southwestern SoM	-	-	-	-	GN	Displayed in the fishmonger	Present study
21	22 Mar 2018	Gökçeada, northern AE	87.5	5,536	♀	110	BT	Symmetrically distributed developing oocytes (n = 6) ranging from 55.22 to 59.55 mm, were found in ovaries	Yığın et al. (2019)
22	8 Aug 2023	Gökçeada, northern AE	ca. 120	-	♀	224–300	TN	Released alive	Kabasakal (2023, in press)

SoM, Sea of Marmara; AE, Aegean Sea; MS, Mediterranean Sea; BT, bottom trawl; GN, gill net; TN, trammel net

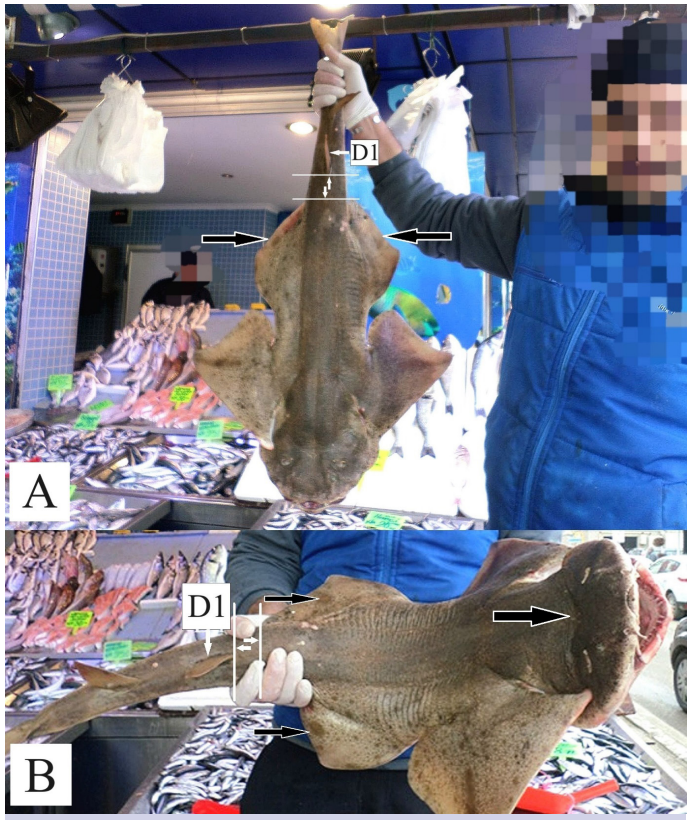

Figure 1. The specimen of *Squatina oculata*, captured in the Sea of Marmara. (Panels a and b) white arrows indicate the origin of the base of the first dorsal fin (D1) located well behind the free rear tips of the pelvic fins; black arrows with white outlines depict the pelvic fins and the characteristic brown spots. (Panel b) The bigger black arrow with a white outline indicates the concave depression on the head.

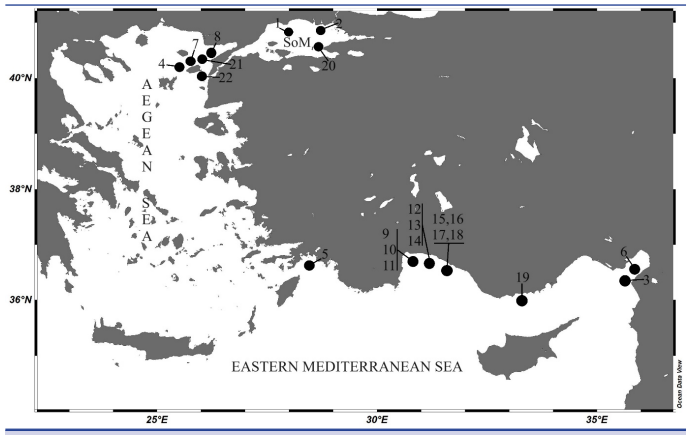

Figure 2. Black dots depict the approximate localities of captures of Squatina oculata in Turkish waters. The numbers on the map correspond to the same individual numbers listed under column "No" in Table 1. SoM, Sea of Marmara.

weight varied between 71 and 6,000 g (Table 1). The collated data by Zava et al. (2022), between September 2005 and March 2021, revealed that 34 smoothback angelsharks have been either captured or sighted from several regions of the Mediterranean Sea. When the study results and data by Zava et al. (2022) are combined, the number of confirmed records of *S. oculata* in the Mediterranean Sea rises to 56, 39.28% of which have been captured in Turkish waters.

During the second phase of the extensive MEDITS survey performed in northern Mediterranean waters between 2012 and 2015, no *S. oculata* was captured (Follesa et al., 2019). Furthermore, during the first phase of the MEDLEM project, which is aimed at collating data on the large elasmobranch species of the Mediterranean Sea between 1666 and 2017, *S. oculata* is considered a "rarely observed" shark species (Mancusi et al., 2020). During the second phase of the MEDLEM, covering 2017–2022, no smoothback angelshark has been recorded in the area (Gallo et al., 2022). Therefore, the smoothback angelshark sighted in the trammel net fishery on August 8, 2023, off the island of Gökçeada (sp No. 22 in Table 1; Kabasakal, 2023), is a recent record of species in the Mediterranean Sea, without ruling out the rarity of *S. oculata*. Pregnant females captured in the Bay of Antalya and off the island of Gökçeada (sp Nos. 11 and 21, respectively, in Table 1) indicate that *S. oculata* reproduction may simultaneously emerge in remote regions of the northern Aegean Sea and Turkish Mediterranean waters. The emergence of pregnant females provides further justification for the International Union for Conservation of Nature Shark Specialists Group's declaration of the Tracean Sea Shelf (northern Aegean Sea) and Bay of Antalya (eastern Mediterranean) important shark and ray areas (Jabado et al., 2023).

Recent records of *S. oculata* in Turkish seas are mostly composed of individuals captured after 2000 (n = 19; 86.36%; Table 1). Similarly, Zava et al. (2022) reported that all 34 smoothback angelsharks were either captured or sighted in 2005 and thereafter. Again, when the results of this study and those of Zava et al. (2022) are combined, the majority of contemporary records of smoothback angelsharks (n = 53; 94.64%) have either been captured or sighted in the last 23 years, which corresponds to 2.3 individuals per year. Although the number of smoothback angelsharks per annum reflects the rarity of the species, the paucity of records may be due to the absence of reporting captures or sightings. Therefore, the available number of records may increase by unraveling the retrospective unpublished smoothback angelshark captures. For example, despite the present capture of a smoothback angelshark on February 27, 2018, the discovery of this unpublished record occurred following an Internet search performed on December 14, 2023, which emphasizes the importance of retrospective data searches with novel investigation methods and digital tools.

In recent years, a notable increase in the use of digital tools, such as digital media, and social media, among others, in searching for rare elasmobranchs and in the number of studies focusing on captures and/or sightings of these species has been reported (Bengil, 2020; Kabasakal & Bilecenoğlu, 2020; Grant et al., 2022; Hiddink et al., 2023; Kabasakal, 2023). A quick review of the contents of such research articles reveals a wide spectrum of topics, such as providing complementary data on the distribution of

well-recognized elasmobranchs (Bengil, 2020) or rare and large sharks (Kabasakal and Bilecenoğlu, 2020) in Turkish waters, filling the knowledge gaps of the distribution range of an endangered elasmobranch species (Grant et al., 2022), or determining spatial and temporal variations of the distribution and abundance of a certain elasmobranch (Hiddink et al., 2023). Compared with traditional systematic scientific sampling, opportunistic methodologies obviously suffer from several drawbacks, such as uncertainties (e.g., fisher's statement-dependent information) and weaknesses (e.g., fisher's data cannot be representative of the true periodicity of fishing days). However, as emphasized by Tsikliras and Dimarchopoulou (2021), the current opportunistic records are considered valuable data sources; these can provide complementary information to fill the knowledge gaps in the life histories of rare, rarely caught, or endangered sharks and rays. Finally, the identifying a species from the photo provided, which is also the case for the present study, is considered a confirmed emergence of a fish species in a given region (Kovačič et al., 2020).

CONCLUSIONS

As a consequence, the contemporary emergence of *S. oculata* in the Sea of Marmara, where the status of the species in the region is considered questionable due to its absence for many years in the area (Kabasakal, 2022), is confirmed based on an opportunistic photographic record (Figure 1). Being a "critically endangered" elasmobranch species of the Mediterranean Sea (Ferretti et al., 2016) and being included in the Annex II of the SPA/BD protocol covered by GFCM/36/2012/3 and GFCM/42/2018/2 recommendations (Barone et al., 2022), *S. oculata* is also a protected species in Turkish seas (Resmi Gazete, 2018). However, even the protected species can be incidentally captured in commercial fisheries, which is also the case for the present smoothback angelshark. Since the data in question can often be hidden due to fear of punishment, social media posts or digital news reports of such hidden captures may be used as an efficient and complementary data-providing tool for monitoring the bycatch of endangered and protected species in commercial fisheries.

Acknowledgments: The authors would like to thank two anonymous reviewers for their comments, which improved the quality and the content of the article.

Conflict of Interest: The author declares that there is no conflict of interest.

Ethics Committee Approval: Since no experimental animal was used in the submitted study, approval of the ethics committee is not required.

Financial Disclosure: This study received no financial support from any funding source.

REFERENCES

Akşıray, F. (1987). *Türkiye Deniz Balıkları ve Tayin Anahtarı, 2nd Edition*. Publications of İstanbul University, İstanbul, no. 3490.

Barone, M., Mazzoldi, C. & Serena, F. (2022). *Sharks, Rays and Chimaeras in Mediterranean and Black Sea – Key to Identification*. FAO, Rome, http://dx.doi.org/10.4060/cc0830en.

Başusta, N. & Erdem, Ü. (2000). A study on the pelagic and demersal fishes of İskenderun Bay. *Turkish Journal of Zoology*, 24(5), 1-19. https://journals.tubitak.gov.tr/zoology/vol24/iss5/1

Bauchot, M. -L. (1987). Requins: In: W. Fischer, M. Schneider & M. -L. Bauchot (Eds.), *Fiches FAO d'identification des espèces pour les besoins de la pêche. (Révision 1). Méditerranée et mer Noire*. Zone de pêche 37. Vol. II. Vertebres (pp. 767-843). Roma, FAO.

Bayhan, Y. K., Çiçek, E., Ünlüer, T. & Akkaya, M. (2006). Catch and by-catch composition of the shrimp fishery by beam trawl in the southeastern Marmara Sea. *E.U. Journal of Fisheries & Aquatic Sciences*, 23(3-4), 277-283.

Bengil, E. G. T. (2020). Can opportunistic methodologies provide information on elasmobranchs? A case study from Seas around Turkey. *Journal of Wildlife and Biodiversity*, 4(Special issue), 68-77.

Bilecenoğlu, M., Taşkavak, E., Mater, S. & Kaya, M. (2002). Checklist of the marine fishes of Turkey. *Zootaxa*, 113(1), 1-194. https://doi.org/10.11646/zootaxa.113.1.1.

Bilecenoğlu, M., Kaya, M., Cihangir, B. & Çiçek, E. (2014). An updated checklist of the marine fihes of Turkey. *Turkish Journal of Zoology*, 38(6), 901-929. doi:10.3906/zoo-1405-60

Bök, T. D., Göktürk, D. & Kahraman, A. E. (2011). Bycatch in 36 and 40 mm PA Turkish twin rigged beam trawl codends. *African Journal of Biotechnology*, 10(37), 7294-7302. http://www.academicjournals.org/AJB.

ÇŞİDB-TUBİTAK-MAM (2021). "*Denizlerde Bütünleşik Kirlilik İzleme Programı 2014-2019 Marmara Denizi Özet Raporu*". Kocaeli, TÜBİTAK-MAM Matbaası.

Daban, İ. B., İşmen, A., Şirin, M., Yığın, C. Ç., Arslan İhsanoğlu, M. (2021). Analysis of Demersal Fish Fauna off the Sea of Marmara, Turkey. *COMU Journal of Marine Sciences and Fisheries*, 4(1), 20-31. DOI: 10.46384/jmsf.912403.

Ebert, D. A., Dando, M. & Fowler, S. (2021). *Sharks of the world, a complete guide*. Princeton, Wild Nature Press.

Ergüden, D., Ayas, D., Gürlek, M., Karan, S. & Turan, C. (2019). First documented smoothback angelshark *Squatina oculata* Bonaparte, 1840 from the North-Eastern Mediterranean Sea, Turkey. *Cahiers de Biologie Marine*, 60(2), 189-194. DOI: 10.21411/CBM.A.23607FF9.

Eryılmaz, L. & Meriç, N. (2005). Review of fish fauna of the Sea of Marmara. *Journal of the Black Sea / Mediterranean Environment*, 11(2), 153-178

Ferretti, F., Morey, G., Serena, F., Mancusi, C., Coelho, R.P., Seisay, M., Litvinov, F. & Buscher, E. (2016). *Squatina oculata* (Mediterranean assessment). The IUCN Red List of Threatened Species 2016: e. T61418A16570000.

Follesa, M. C., Marongiu, M. F., Zupa, W., Bellodi, A., Cau, A., Cannas, R., Colloca, F., Djurovic, M., Isajlovic, I., Jadaud, A., Manfredi, C., Mulas, A., Peristeraki, P., Porcu, C., Ramirez-Amaro, S., Jiménez, F. S., Serena, F., Sion, L., Thasitis, I., Cau A. & Carbonara, P. (2019). Spatial variability of Chondrichthyes in the northern Mediterranean. In: Mediterranean demersal resources and ecosystems: 25 years of MEDITS trawl surveys (M.T. Spedicato, G. Tserpes, B. Mérigot & E. Massutí, eds.) *Scientia Marina*, 83(S1), 81-100. ISSN-L 0214-8358 https://doi.org/10.3989/scimar.04998.23A.

Froese, R. & Pauly. D. (Eds) (2023). FishBase. World Wide Web electronic publication. www.fishbase.org, version (last accession: 16 December 2023).

Gallo, S., Mancusi, C., Mohammed, A., Rigers, B., Barash, A., Barone, M., Bottaro, M., Carbonara, P., Roberto, C., Ćetkovič, I., Clo, S., de Sabata, E., Enajjar, S., Shakman, E., Garibaldi, F., Giglio, G., Giovos, I., Kabasakal, H., Lanteri, L., Lelli, S., Lipej, L., Mazzoldi, C., Micarelli, P., Morey, G., Moro, S., Bradai, M. N., di Sciara, G. N., Romano, C., Saidi, B., Soldo, A., Sperone, E., Tiralongo, F., Zava B. & Serena F. (2022). Sigthings of large elasmobranchs from the Mediterranean: new data from MEDLEM database in the last five years (2017–2022). *2022 IEEE International Workshop on Metrology for the Sea; Learning*

to Measure Sea Health Parameters (MetroSea). pp. 293-297, doi: 10.1109/MetroSea55331.2022.9950984.

Grant, M. I., Bicknell, A. W. J., Htut, T., Maung, A., Maung, T., Myo Myo, K., Rein, T., San, M. K., White, W. T., Ya, K. Z. & Mizrahi, M. (2022). Market surveys and social media provide confirmation of the endangered giant freshwater whipray *Urogymnus polylepis* in Myanmar. *Journal of Fish Biology*, 101(1), 302–307. DOI: 10.1111/jfb.15073.

Hiddink, J. G., Charles, R. & Moore, A. B. M. (2023) Using opportunistic data to study the distribution and abundance of a warm water elasmobranch at the northern edge of its range. *ICES Journal of Marine Science*, https://doi.org/10.1093/icesjms/fsad183.

Jabado, R. W, García-Rodríguez, E., Kyne, P. M., Charles, R., Armstrong, A. H., Bortoluzzi, J., Mouton, T. L., Gonzalez Pestana, A., Battle-Morera, A., Rohner, C. & Notarbartolo di Sciara, G. (2023). *Mediterranean and Black Seas: A regional compendium of Important Shark and Ray Areas*. Dubai, IUCN SSC Shark Specialist Group.

Kabasakal, H. (2002). Elasmobranch species of the seas of Turkey. *ANNALES - Series historia naturalis*, 12(1), 15-22.

Kabasakal, H. (2022). Sharks of the Sea of Marmara: An Overview on the Sharks of A Small Inland Sea. In: (Öztürk, B., Ergül, H. A., Yalçıner, A. C., Öztürk, H. & Salihoğlu, H., Eds.) *8-9 Ocak 2022 Marmara Denizi 2022 Sempozyumu Bildiriler Kitabı*, (pp. 337-343), İstanbul, Türk Deniz Araştırmaları Vakfı, Yayın No: 63.

Kabasakal, H. (2023). A preliminary social media survey of sharks and batoids captured in north Aegean Sea. *ANNALES - Series historia naturalis*, 33(2), 165-186.

Kabasakal, H. & Bilecenoğlu, M. (2020). Shark infested internet: an analysis of internet-based media reports on rare and large sharks of Turkey. *FishTaxa*, 16, 8-18

Kabasakal, H. & Kabasakal, E. (2004). Sharks captured by commercial fishing vessels off the coast of Turkey in the northern Aegean Sea. *ANNALES - Series historia naturalis.*, 14(2), 171-180.

Karakulak, F. S., İşinibilir Okyar, M., Yıldız, T., Uzer, U., Türkeri, E. E., Doğan, O., Peksu, M., Biçer, D., Yemişken, E., Gül, B. & Çanak, Ö. (2017). Doğu Marmara Denizi'nde demersal balıkların stok tesbiti. Proje No: 51922, Sonuç Raporu. T. C. İstanbul Üniversitesi Bilimsel Araştırma Projeleri Koordinasyon Birimi.

Kovačič, M., Lipej, L. & Dulčič, J. (2020). Evidence approach to checklists: critical revision of the checklist of the Adriatic Sea fishes. *Zootaxa*, 4767(1): 001-055. https://doi.org/10.11646/zootaxa.4767.1.1.

Mancusi, C., Baino, R., Fortuna, C., de Sola, L., Morey, G., Bradai, M., Kallianotis, A., Soldo, A., Hemida, F., Saad, A., Dimech, M., Peristeraki, P., Bariche, M., Clo, S., de Sabata, E., Castellano, L., Garibaldi, F., Lanteri, L., Tinti, F., Pais, A., Sperone, E., Micarelli, P., Poisson, F., Sion, L., Carlucci, R., Cebrian-Menchero, D., Séret, B., Ferretti, F., El-Far A., Saygu, I., Shakman, E., Bartoli, A., Guallart, J., Damalas, D., Megalofonou, P., Vacchi, M., Bottaro, M., Notarbartolo di Sciara, Follesa, M., Cannas, R., Kabasakal, H., Zava, B., Cavlan, G., Jung, A., Abudaya, M., Kolitari, J., Barash, A., Joksimovic, A., Marceta, B., Gonzales Vilas, L., Tiralongo, F., Giovos, I., Bargnesi, F., Lelli, S., Barone, M., Moro, S., Mazzoldi, C., Charis, C., Abella, A. & Serena, F. (2020). MEDLEM database, a data collection on large elasmobranchs in the Mediterranean and Black seas. *Mediterranean Marine Science*, 21(2), 276-288.

Meriç, N. (1994). Türkiye denizlerinde az rastlanan bazi baliklar [Some uncommon fishes from the Turkish seas]. *XII. Ulusal Biyoloji Kongresi, Edirne*, (pp. 295-299). [in Turkish].

Miller, M. H. (2016). Status review report of 3 species of angelsharks: *Squatina aculeata*, *S. oculata*, and *S. squatina*. Report to National Marine Fisheries Service, Office of Protected Resources.

Monkman, G. G., Kaiser, M. & Hyder, K. (2017). The Ethics of Using Social Media in Fisheries Research. *Reviews in Fisheries Science & Aquaculture*, http://dx.doi.org/10.1080/23308249.2017.1389854

Özgür Özbek, E. & Kabasakal, H. (2022). Notes on smoothback angel shark, *Squatina oculata* Bonaparte, 1840 (Squatiniformes: Squatinidae), caught in the Gulf of Antalya. *ANNALES - Series historia naturalis*, 32(1), 9-16. DOI 10.19233/ASHN.2022.02.

Resmi Gazete (2018). Resmi Gazete (Official Gazette), 19 April 2018 – No. 30396, page 15, Article 3.

Serena, F., Abella, A. J., Bargnesi, F., Barone, M., Colloca, F., Ferretti, F., Fiorentino, F., Jenrette, J. & Moro, S. (2020). Species diversity, taxonomy and distribution of chondrichthyes in the Mediterranean and Black Sea. *The European Zoological Journal*, 87(1), 497-536. https://doi.org/10.1080/24750263.2020.1805518

Slastenenko, E. (1955-1956). *Karadeniz havzası balıkları* [*Fishes of Black Sea basin*]. Istanbul, Et ve Balik Kurumu Yayınları. [in Turkish].

Tsikliras, A. C. & Dimarchopoulou, D. (2021). Filling in knowledge gaps: Length–weight relations of 46 uncommon sharks and rays (Elasmobranchii) in the Mediterranean Sea. *Acta Ichthyologica et Piscatoria*, 51(3), 249-255. DOI 10.3897/aiep.51.65858.

Türk, Ç. (2018). https://www.turkiyegazetesi.com.tr/yasam/marmara-denizinde-kopekbaligi-yakalandi-546708 (last accession: May 15, 2025).

Yığın, C. Ç., İşmen, A., Daban, B., Cabbar, K. & Önal, U. (2019). Recent findings of rare sharks, Squatina oculata Bonaparte, 1840 and *Squatina squatina* (Linnaeus, 1758) from Gökçeada Island, Northern Aegean Sea, Turkey. *Journal of the Black Sea / Mediterranean Environment*, 25(3), 305-314.

Zava, B., Fiorentino, F. & Serena, F. (2016). Occurrence of juveniles *Squatina oculata* Bonaparte, 1840 (Elasmobranchii: Squatinidae) in the Strait of Sicily (central Mediterranean). *Cybium*, 40(4), 341-343. https:// doi.org/10.26028/cybium/2016-404-011

Zava, B., Insacco, G., Deidun, A., Said, A., Ben Suissi, J., Nour, O. M., Kondylatos, G., Scannella, D. & Corsini-Foka, M. (2022). Records of the critically endangered *Squatina aculeata* and *Squatina oculata* (Elasmobranchii: Squatiniformes: Squatinidae) from the Mediterranean Sea. *Acta Ichthyologica et Piscatoria*, 52(4), 285-297. DOI 10.3897/aiep.52.94694.

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Tables should be included in the main document, presented after the reference list, and they should be numbered consecutively in the order they are referred to within the main text. A descriptive title must be placed above the tables. Abbreviations used in the tables should be defined below the tables by footnotes (even if they are defined within the main text). Tables should be created using the "insert table" command of the word processing software and they should be arranged clearly to provide easy reading. Data presented in the tables should not be a repetition of the data presented within the main text but should be supporting the main text.

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Type of manuscript	Page	Abstract word limit	Reference limit
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Short Communication	≤5	250	20

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All references, tables, and figures should be referred to within the main text, and they should be numbered consecutively in the order they are referred to within the main text.

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- American Psychological Association. (2010). Publication manual of the American Psychological Association (6th ed.). Washington, DC: APA.
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- **An eBook:** Millbower, L. (2003). *Show biz training: Fun and effective business training techniques from the worlds of stage, screen, and song.* Retrieved from http://www.amacombooks.org/ (accessed 10.10.15)
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- **An article with DOI:** Gaudio, J. L. & Snowdon, C. T. (2008). Spatial cues more salient than color cues in cotton-top tamarins (saguinus oedipus) reversal learning. *Journal of Comparative Psychology,* https://doi.org/10.1037/0735-7036.122.4.441
- **Websites - professional or personal sites:** The World Famous Hot Dog Site. (1999, July 7). Retrieved January 5, 2008, from http://www.xroads.com/~tcs/hotdog/hotdog.html (accessed 10.10.15)
- **Websites - online government publications:** U.S. Department of Justice. (2006, September 10). Trends in violent victimization by age, 1973-2005. Retrieved from http://www.ojp.usdoj.gov/bjs/glance/vage.htm (accessed 10.10.15)
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- **Artwork - from library database:** Clark, L. (c.a. 1960's). *Man with Baby.* [photograph]. George Eastman House, Rochester, NY. Retrieved from ARTstor
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