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Research Articles

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Research Article

Evaluation of Food Wastes in *Chlorella vulgaris* Cultivation for Remazol Brilliant Blue R Biosorption

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ABSTRACT

The current study demonstrates the biosorption efficiency of *Chlorella vulgaris* for the removal of Remazol Brilliant Blue R (RBBR), which is often used in the textile industry. For this, optimization of microalgal growth was investigated under photoautotrophic conditions including only BG-11 medium and photoheterotrophic conditions containing 0.5 g/L of pumpkin waste, apple pomace, or glucose. Some critical parameters for RBBR biosorption onto dry C. vulgaris biomass, such as pH (2-10), initial concentration of RBBR (100-800 mg/L), biosorbent concentration (1-3 g/L), and biosorption time (0-120 min) were optimized. As a result of the study, the best growth of microalgae was determined as 0.502 g/L under photoheterotrophic cultivation condition, including 0.5 g/L of pumpkin waste sugar. The highest dye removal was calculated as 99.49% in the presence of 3 g/L microalgal biosorbent and 103.38 mg/L RBBR concentration at pH 4. These results indicate that *C. vulgaris* has a promising biosorbent for waste management and dye removal.

Keywords: *Chlorella vulgaris*, agro-industrial waste, dye removal, biosorption, photoheterotrophic growth

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INTRODUCTION

The growing textile industry and the use of synthetic dyes are the primary causes of environmental pollution. Moreover, water pollution caused by dyeing processes in the textile industry is approximately 17-20% (Premaratne et al., 2021). Synthetic dyes, defined as highly polluting, have toxic (cytotoxic, genotoxic, and mutagenic) effects (Verma, 2021). Up to 50% of dyes are used in different areas, such as skin and clothing, do not bind to the cloth's fibers, and are mixed into the aquatic environment as pollutants (Benkhaya, M'rabet & El Harfi, 2020). Therefore, the dyes used in the textile industry threaten the living ecosystem due to them mixing with natural water. Low amounts of dye in water can even significantly affect the photosynthetic activity of plants and toxicity for animals (Verma, 2021). Azo dyes constitute the biggest class (up to 60%) of textile industry dyes. Of these dyes, 15-50% of them are mixed with effluent because they can not remain fixed into the product (Al-Tohamy et al., 2022). For example, Remazol Brilliant Blue R (RBBR), an anionic-azo dye utilized in polymeric material production and the textile industry, is quite dangerous for aquatic organisms due to its low biological disjunction and high toxicity (Aracagök, 2022).

For many years, chemical (electrochemical etc.), physical (coagulation etc.), and biological (enzyme or microorganisms) methods have been utilized to remove these dangerous dyes from natural life (Shabir et al., 2022). Among the methods used, biosorption from biological methods is a cheap, effective, and successful mechanism. Biosorption is a passive process, without the need for energy, in which xenobiotic chemicals from contaminated sources are removed by microbial biomass such as bacteria, yeast, and algae (Gadd, 2009). Furthermore, the microbial biomass used in biosorption can be active (live) or inactive (dead) character. However, the use of dead microbial biomass is more effective than live biomass because of its features, such as reusability, high surface-to-volume ratio, storage convenience, metabolic independence, economic efficiency, and environmental friendliness (Goud et al., 2020). The biosorption mechanism occurs as a result of the interaction of azo dyes and different functional groups (such as carboxyl, hydroxyl, and amino) on the microbial cell surface. Among microorganisms used, microalgae can live adaptively in an environment contaminated with textile dyes since they are aquatic organisms. Phosphorus and nitrogen compounds in dyes can be used as nutrient sources for microalgae growth (Zohoorian et al., 2020). It is also indicated in the literature that it is a good biosorbent with its high binding affinity and high surface-to-volume ratio for dyes because of its functional groups (carboxyl, sulfhydryl, etc.) on the cell wall surface (Chu & Phang, 2019).

Furthermore, biosorption with microalgal biomass has a low carbon footprint profile due to its photosynthetic properties (Mustafa et al., 2021). Thus, it can be said that using microalgae in industrial dye removal is quite advantageous. *Chlorella vulgaris* is among the microorganisms frequently used in the literature because it has a high affinity for removing heavy metals (Joo, Lee & Choi, 2021) and dyes (Aksu & Tezer, 2005). Even though they typically grow photoautotrophically, microalgae can be cultivated in heterotrophic, mixotrophic, or photoheterotrophic conditions containing a carbon source (Saratale et al., 2022). In addition, in the literature, it is indicated that microalgae growth and yield significantly were supported in photoheterotrophic conditions using organic carbon and a light source. For microalgal growth, industrial and agricultural wastes with rich sugar content are often utilized in photoheterotrophic conditions as a carbon source (Isleten-Hosoglu et al., 2013). Apple pomace (AP) and pumpkin waste (PW) are important by-products of the food industry and are abundant in carbohydrates, protein, lipids, minerals, vitamins, and polyphenols. While pumpkin production is about 23 million tons worldwide, 93,144,358.17 tons of apples were generated in 2021 worldwide (FAOSTAT, 2021; Kidoń & Uwineza, 2022). Therefore, AP and PW are promising carbon sources for microalgae production.

Moreover, the contents and surface structures of microbial cells may be associated with the growth conditions (Joo et al., 2021). In addition, it is emphasized that *C. vulgaris* cells change the efficiency of their contents (macro (protein, carbohydrates, lipids etc.) and micro (phosphorus etc.) compounds) under different growth conditions (Joo et al., 2021). In this context, as a result of the growth of *C. vulgaris* under photoheterotrophic conditions, it can be expected to obtain high biomass and have a more effective cell surface for biosorption.

This work aimed to evaluate the biosorption of anionic RBBR dye onto dead *C. vulgaris* biomass produced in photoheterotrophic conditions in the presence of AP or PW. For this aim, carbon source and its concentrations were optimized for *C. vulgaris* growth. Using microalgal biosorbent cultivated at optimized photoheterotrophic conditions, important parameters such as pH, initial dye concentration, biosorbent concentration, and time were optimized for RBBR biosorption. Thus, the present study aims to suggest an effective, eco-friendly, and cost-efficient biosorbent cultivated with AP or PW sugar in photoheterotrophic conditions for anionic dye biosorption to the literature for the first time.

MATERIAL AND METHODS

Microorganism and its cultivation conditions

C. vulgaris microalgae was supplied from the Ankara University culture collection. Microalgae stock culture was cultivated at 30°C with 2400 lx light in sterile BG-11 medium that contain of 1.5 g $NaNO_3$, 6 mg ferric ammonium citrate, 40 mg $K_2HPO_4{\cdot}3H_2O$, 6 mg citric acid H_2O, 1 mg $Na_2EDTA{\cdot}2H_2O$, 75 mg $MgSO_4{\cdot}7H_2O$, 2.86 mg H_3BO_3, 20 mg Na_2CO_3, 1.81 mg $MnCl_2{\cdot}4H_2O$, 0.22 mg $ZnSO_4{\cdot}7H_2O$, 0.39 mg $Na_2MoO_4{\cdot}2H_2O$, 0.049 mg $Co(NO_3)_2{\cdot}6H_2O$, 0.08 mg $CuSO_4{\cdot}5H_2O$, and 36 mg $CaCl_2{\cdot}2H_2O$ per liter (Rippka, 1988; Park et al., 2014). The experiments were carried out in photoautotrophic and photoheterotrophic conditions for 10 days. In photoautotrophic conditions, *C. vulgaris* was cultivated in the standard BG-11 medium under constant illumination. For photoheterotrophic condition, microalgae cultivation was carried out in BG-11 medium adding 0.5 g/L glucose, 0.5 g/L AP sugar, or 0.5 g/L PW sugar with constant illumination in the first part of the study. Furthermore, in further experiments, photoheterotrophic cultivation was carried out at different sugar concentrations of PW, the waste from which the highest microalgal biomass was obtained.

Pretreatment of agro-industrial wastes

PW and AP as carbon sources for photoheterotrophic conditions were provided by a local company in Sakarya and Aroma Bursa Fruit Juices and Food Industry, Turkey, respectively. Agro-industrial wastes were dried at 80°C in an oven, ground in a laboratory-type mill (Miprolab, Turkey), and stored at room temperature.

Before experiments, dried and ground wastes were pretreated with 1% H_2SO_4 by dilute acid pretreatment method The solution was autoclaved for 15 min at 121°C to obtain monomeric sugars (Germec & Turhan, 2018). Hydrolyzates were filtered with Whatman No. 1 filter paper, and the solutions were stored at +4°C.

The effect of initial PW sugar concentration on microalgal growth

Different concentrations of PW sugar (0.25-1 g/L) were investigated for the growth of *C. vulgaris* under photoheterotrophic condition. For this, 10% (v/v) of microalgae culture was added into 250 mL flasks including 100 mL of BG-11 media containing desired PW sugar concentrations and cultivated at 2400 lx and 30°C for 10 days.

Preparation of microalgal biosorbent and stock dye solutions for biosorption

To obtain biosorbent, microalgae culture photoheterotrophically cultivated in the presence of PW sugar was harvested at 5000 rpm for 10 min by centrifugation and dried overnight at 70 °C using an oven. For stock biosorbent solution, 10-30 g of dried microalgal biomass was homogenized in 1L of distilled water with a homogenizer (IKA T18 digital Ultra Turrax) for 45 seconds at 13400 rpm.

To prepare the RBBR stock solution, 20g of dye was dissolved in 1L of distilled water and the solution was diluted to arrive desired RBBR (100-800 mg/L) concentration in experiments.

Biosorption studies

The experiments for biosorption were actualized in 150 mL flasks including 50 mL of RBBR dye solution. The flasks containing desired concentrations of dye and biosorbent were shaken at 100 rpm for 120 min. The samples taken at definite minutes were centrifuged for 4 min at 5000 rpm. After centrifugation, the supernatants were spectrophotometrically measured to determine the dye removal efficiency.

The effect of pH on RBBR biosorption

The pH effect was analyzed in the range of 2 to 10 in the dye solution containing about 100 mg/L of RBBR with 1 g/L microalgal biosorbent. The pH of dye solutions was brought to the determined value with 0.1 M NaOH or 0.1 M H_2SO_4 before mixing the microalgal biosorbent.

The effect of initial RBBR concentration on biosorption

To determine the effect of increasing dye concentrations, biosorption experiments were performed approximately from 100 to 800 mg/L (from 103.38 mg/L to 818.30 mg/L) RBBR at optimum pH value. The flasks containing dye solutions were shaken at 100 rpm for 120 min.

The effect of biosorbent concentration and time on RBBR biosorption

1, 2 and 3 g/L of biosorbent were tested for all dye concentrations at optimum pH value. In addition, during all the biosorption experiments, the samples were taken at 0, 5, 15, 30, and 120 minutes and spectrophotometrically analyzed.

Analytical methods

Reducing sugar concentration in agro-industrial wastes was analyzed according to Dinitrosalicylic acid Method (Miller, 1959) and spectrophotometrically measured at 540 nm using a Shimadzu UV-1201 model UV-VIS spectrophotometer.

Microalgal growth in different conditions was spectrophotometrically analyzed at 600 nm using a Shimadzu UV-1201 model UV-VIS spectrophotometer. Absorbance measurements for the concentration of RBBR in the dye solutions were spectrophotometrically carried out at 590 nm.

The biosorption percentage (%) and removal capacity (q_m) of RBBR were calculated using the equations below (Eq. 1 and Eq. 2) (Gül, 2022):

$$Biosorption(\%) = \frac{Co-Cf}{Co} x100 \quad \text{(Eq.1)}$$

$$q_m(mg/g) = \frac{Co-Cf}{X_m} \quad \text{(Eq.2)}$$

Where C_o shows the initial RBBR concentration in solution (mg/L), C_f definites the final concentration of RBBR at any time (mg/L), and X_m shows the microalgal biosorbent concentration (g/L).

RESULT AND DISCUSSION

Microalgal growth in different cultivation conditions

Macro and microelements such as carbon, protein, mineral, and vitamins are important for microalgae production because they affect microbial metabolic activities. In this sense, agro-industrial wastes are unique sources containing substances required by microalgae. Therefore, the current study determined the effect of different cultivation conditions for the growth of microalgae with BG-11 media adding 0.5 g/L of AP, PW, or glucose. Figure 1 shows the growth of *C. vulgaris* in photoautotrophic (BG-11) and photoheterotrophic (agro-industrial wastes or glucose) condition. As shown in Figure 1, the highest microalgal biomass was 0.502 g/L in medium including 0.5 g/L sugar of PW after 10-days. According to this, the supplementation of PW sugar increased microalgal growth 2.3 times compared with photoautotrophic cultivation. The BG-11 medium containing PW sugar caused higher biomass production than the BG-11 medium containing synthetic glucose. This increase may be due to the fact that the pumpkin waste has compounds that support microbial growth, such as minerals, vitamins, protein and lipid, in addition to its sugar content (Valdez-Arjona & Ramírez-Mella, 2019). While proteins are important sources of nitrogen in cell growth (Wang et al., 2016), vitamins and minerals can participate as coenzymes or cofactors for enzymes involved in microalgal metabolism pathways such as carbon and lipid metabolism (Golub and Voyevoda, 2013). Thus, despite the same sugar concentration, microalgal growth was significantly increased thanks to the rich nutritional content of the pumpkin waste.

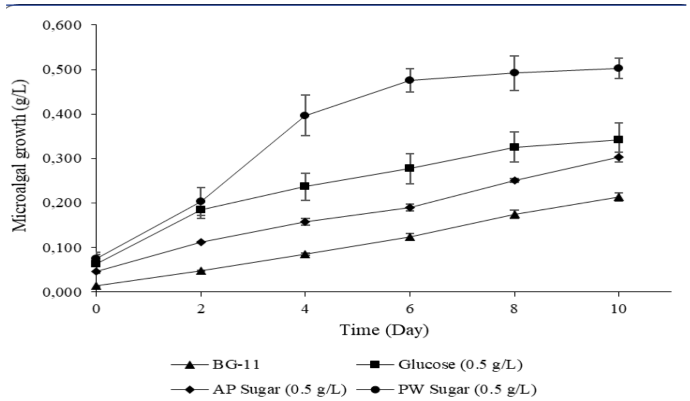

Figure 1. The effects of photoautotrophic and photoheterotrophic conditions on *C. vulgaris* growth (BG-11 media; 2400 lx; 30 °C; pH 7; 10 days).

Moreover, AP sugar supported microalgae production but was less effective than glucose. This situation may be due to the sugar and composition of apple pomace. Furthermore, 0.25 - 1 g/L of PW sugar was investigated in BG-11 Medium to determine the optimum PW sugar concentration and the results were demonstrated in Figure 2. According to the obtained results, when sugar concentration increased from 0.25 to 0.5 g/L, biomass production significantly enhanced and reached from 0.357 g/L to 0.502 g/L. Moreover, 1 g/L PW sugar caused a slight decrease in microalgal growth. This decline can be explained by the fact that high organic matter content causes a decrease in biomass productivity (Manzoor et al., 2020).

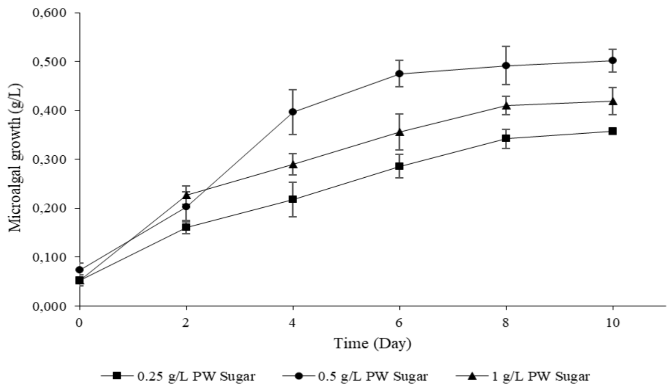

Figure 2. The effects of different PW sugar concentrations on *C. vulgaris* growth (BG-11 medium; 2400 lx; 30 °C; pH 7; 10 days).

In previous studies, the addition of various carbon sources has been shown to increase microalgal biomass production. For instance, Kassim et al. (2022) determined that the highest biomass of *Tetraselmis suecica* and *Halochlorella rubescens* was 0.669 ± 0.01 g/L and 0.653 ± 0.009 g/L, respectively, when molasses were used in photoheterotrophic conditions. Mohammad Mirzaie et al. (2016) observed that under the mixotrophic conditions using cane molasses and corn steep liquor for carbon and nitrogen sources, the dry weight of *C. vulgaris* was 4 and 2.5 times higher than the heterotrophic and autotrophic conditions, respectively. Moreover, adding a carbon source improves microalgae growth and its valuable content, such as carbohydrates, lipids, and protein, for fields such as biofuel production and food supplementation. For example, supplementation of carbon sources in growth of different *Chlorella* species has been stated to increase lipid accumulation for biodiesel production compared to the photoautotrophic condition (Sharma et al., 2016).

Thus, according to the results obtained, for use as a biosorbent in biosorption experiments, *C. vulgaris* biomass was produced under the photoheterotrophic cultivation conditions containing 0.5 g/L PW sugar.

The effect of pH on RBBR biosorption

The pH of the solution is a critical parameter in the biosorption activity of the biosorbent, depending on whether the dyes are anionic or cationic. Therefore, in the current study, the effect of pH on RBBR biosorption was tested in the range of 2-10 in the presence of 1 g/L microalgal biosorbent and about 100 mg/L RBBR. Figure 3 shows that the pH change significantly affected the biosorption of RBBR. For this dye, which has an anionic character, the increase at pH value caused a decrease on biosorption onto *C. vulgaris* biomass, and the highest biosorption percentage was obtained at pH 4 as 93.06% at the end of 120 min (Figure 3). In addition, there was not a significant difference between pH 2 and pH 4. Higher biosorption activity at low pH values for

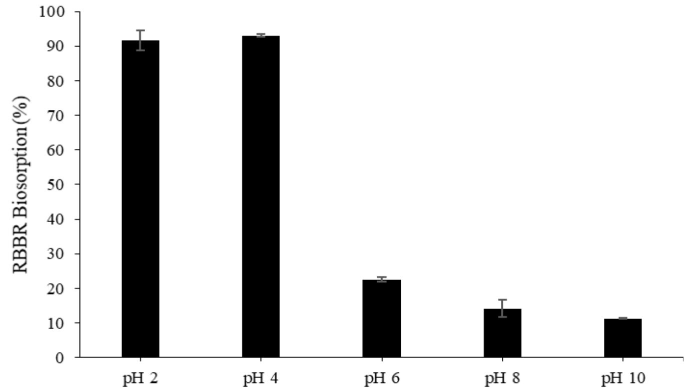

Figure 3. The effect of pH on RBBR biosorption (1 g/L biosorbent, 100 mg/L RBBR, 30 °C; 100 rpm, 120 min).

an anionic dye is due to the ionized dye molecules producing electrostatic charges. When the pH of the solution is low, the solution positively charges and the biosorbent surface also becomes protonated, and the adsorption of negative-charged dye increases (Salleh et al., 2011). In addition, the decrease in biosorption at high pH can be explained by decreasing attraction force between negative-charged cell surfaces and anionic dye molecules with the increase of negative charges in the environment (Yu et al., 2018).

Furthermore, in the studies where *C. vulgaris* was used as a biosorbent, Aksu & Tezer (2005) determined the highest biosorption capacities for Remazol Red RR, Remazol Black B and Remazol Golden Yellow at pH 2 while Kumar, Ahluwalia & Charaya (2019) obtained the maximum biosorption value at pH 5 in dye solution containing 5 ppm Orange G (an anionic dye) and 50 mg biosorbent. Similar effects of pH were shown in other studies using microalgal biosorbent. For example, Khataee, Vafaei & Jannatkhah (2013) used *Spirogyra* sp. biomass as a biosorbent for the removal of acid orange 7. In the study, the highest percentage of dye removal at pH values of 2, 4, 6, 8 and 10 was determined as 42% at pH 4, and a significant decrease in dye removal was observed after pH 4. Gunasundari et al. (2020) investigated the adsorption of Naphthol green-B using *Spirulina platensis* biomass. pH 3 was determined as the best solution pH for biosorption. The dye removal was noticeably decreased when the pH increased from 3 to 7. In another study, the removal of anionic Methyl orange dye using *Chlorella* biomass decreased from 96.3% to 18.7% when pH increased from 2.5 to 11, and the decrease was evident after pH 4 (El Amri, Elkacmi & Boudouch, 2023).

Moreover, in the current study, the biosorption percentage for RBBR sharply decreased when the pH value was increased from 4 to 10. This trend of RBBR biosorption was demonstrated in the study of Ergene et al. (2009) performed with *Scenedesmus quadricauda* biomass, and a significant decrease was determined after pH 4.

The effect of initial RBBR concentration on biosorption

In the present study, the effect of initial dye concentration was investigated approximately from 100 to 800 mg/L (103.38 mg/L -

818.30 mg/L) RBBR concentrations at pH 4 in the presence of 1 g/L biosorbent, and the results were demonstrated in Figure 4. It is clearly seen in Figure 4 that increasing RBBR concentration from 100 to 800 mg/L decreased the biosorption from 93.06% to 16.11% at the end of 120 min. RBBR biosorption efficiency was better for low initial dye concentrations due to the availability of active binding sites on the adsorbent (Aracagök, 2022). Similarly, Hernández-Zamora et al. (2015) observed that biosorption of the anionic azo dye Congo red onto inactive *C. vulgaris* biomass decreased when the initial concentration of Congo red increased from 5 mg/L to 25 mg/L. The authors stated that this is due to the fact that low concentrations of dye molecules in solution interact more easily with the binding sites of biosorbent.

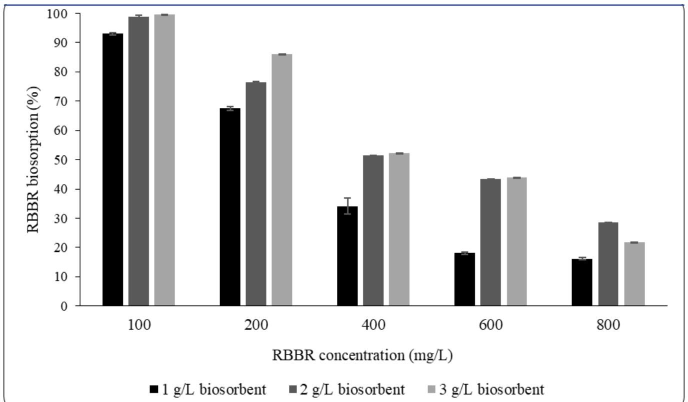

Figure 4. The highest biosorption values obtained with different biosorbent concentrations in increasing initial dye concentrations (pH 4; 30 °C; 100 rpm; biosorption time: the 30th min for 100 and 200 mg/L RBBR, the 120th min for 400, 600 and 800 mg/L RBBR in the presence of 3 g/L biosorbent; the 15th min for 100 mg/L RBBR, 30th for 400 and 800 mg/L RBBR, the 120th min for 200 and 600 mg/L RBBR in the presence of 2 g/L biosorbent; the 0th min for 400 mg/L RBBR, the 30th min 200 mg/L RBBR, the 120th min for 100, 600 and 800 mg/L RBBR in the presence of 1 g/L biosorbent).

In the current study, 600 and 800 mg/L RBBR removals were concluded with similar biosorption values at the end of the process. This situation can be explained by the fact that the amount of fixed dye molecules decreases the attractiveness of biosorbent surface's functional groups (Lai, 2021).

According to results obtained from the current study and literature, it has been seen that effective results can be obtained using *C. vulgaris* as a biosorbent for the biosorption of azo dyes.

The effect of initial biosorbent concentration and time on biosorption

Time and biosorbent concentration parameters for the removal of RBBR were found to be correlated with each other in the current study. The effect of biosorbent concentration (1, 2, and 3 g/L) on the RBBR biosorption is also shown in Figure 4. RBBR removal accelerated when biosorbent concentration in dye solution was risen from 1 to 3 g/L. According to this, the highest RBBR biosorption was 98.91% at 15th min in 100 mg/L dye solution in the presence of 2 g/L biosorbent. Furthermore, for 3 g/L biosorbent concentration, the highest dye removal was calculated as 99.49% at the 30th min while it was similarly examined as 99.18% at the 15th min, and no significant change was observed between these values. Correspondingly with the current study, Radwan et al. (2020) showed that the biosorption rate of the 10 mg/L reactive yellow 145 dye was accelerated when the concentration of *C. vulgaris* biosorbent modified with citric acid increased from 0.1 to 0.5 g/L. For example, while equilibrium was reached at 60 min with 0.5 g/L modified- *C. vulgaris*, the dye uptake process continued for 0.1 g/L modified- *C. vulgaris*. The authors also stated that this increase is due to the increase in active binding sites on surface with increasing biosorbent concentration.

In addition, for 200 mg/L initial RBBR concentration, an increase from 1 to 3 g/L in biosorbent concentration reduced biosorption time up to the 15th minute and the biosorption percentage ranged from 67.45% to 85.88%. However, at dye concentrations after 200 mg/L, removal percentages were slightly decreased at the end of 30 min when biosorbent concentration increased from 2 to 3 g/L. This decline can be clarified by the crowding of the biosorbent and by releasing some dye molecules from the biosorbent surface (Mohd Khori et al., 2018).

Behl et al. (2019) examined the range of 0.25-1.5 g/L of *C. pyrenoidosa* biomass in Direct Red 31 removal. As a result of the study, no increase in dye removal efficiency was observed at concentrations after 1 g/L biosorbent. Furthermore, Revathi et al. (2017) demonstrated that moderate *C. vulgaris* concentration is more effective than higher cell concentrations.

According to the results of the present study, a significant increase in RBBR biosorption was observed at biosorbent concentrations above 1 g/L. At low RBBR concentrations (about 100 and 200 mg/L), the most effective biosorbent concentration was detected as 3 g/L *C. vulgaris* biomass. In addition, although there is no significant difference between 2 and 3 g/L biosorbent concentrations for approximately 400 and 600 mg/L RBBR concentrations, the best biosorption of 800 mg/L RBBR was observed in the presence of 2 g/L biosorbent. The decrease for 800 mg/L RBBR biosorption percentage in the solution containing 3 g/L biosorbent concentration may be due to the crowding of the biosorbent particles, which declined the number of active binding sites on the surface for adsorption, and the adsorption sites overlapped. This causes a decrease in the dye biosorption percentage (Mohd Khori et al., 2018).

Biosorption capacity of microalgal biosorbent on biosorption

Table 1 shows q_m values at the 15th min on RBBR biosorption in the presence of increasing biosorbent concentrations and increasing RBBR dye concentrations. As seen in Table 1, maximum q_m values at the 15th min were obtained in 200 mg/L RBBR for 1 g/L microalgal biosorbent and in 600 mg/L RBBR for 2 and 3 g/L microalgal biosorbent. When the q_m values for the RBBR concentrations of about 100, 200, and 400 mg/L were evaluated, a de-

Table 1. q_m values at 15th min on RBBR biosorption in the presence of increasing biosorbent concentrations and increasing RBBR dye concentrations (30 °C; 100 rpm; pH 4)

100		Initial RBBR dye concentration (mg/L)				
		100	200	400	600	800
Biosorbent concentration (g/L)	1	90.14±2.72	135.92±0.11	132.92±0.01	81.34±4.37	79.21±5.67
	2	52.42±0.27	68.23±0.01	100.28±0.43	112.47±0.08	102.77±0.01
	3	34.18±0.04	55.97±0.04	65.31±0.04	78.72±1.61	56.31±0.15

crease in the q_m was observed as the concentration of biosorbent increased. The highest q_m values for 100, 200 and 400 mg/L dye concentrations were observed at 1 g/L biosorbent concentration. Furthermore, at 600 and 800 mg/L initial RBBR dye concentrations, the highest q_m value was observed at 2 g/L biosorbent concentration. The lowest q_m value in all dye concentrations was at 3 g/L biosorbent concentration.

Similarly, studies in the literature show that the biosorption capacities of microalgal biosorbents for the removal of dyes decreased by increasing biosorbent concentration. da Rosa et al. (2018) investigated the *C. pyrenoidosa* biosorbent amount effect on the biosorption of rhodamine B at pH 4.5 in the presence of 100 mg/L dye. The biosorption capacities decreased from about 20 mg/g to 3.6 mg/g due to increasing biosorbent concentration from 0.1 to 2.0 g. Seth et al. (2022) determined that the increasing biosorbent concentrations (1-5 g/L) caused the decrease on sorption (mg/g) of 100 mg/L anionic dye Indigo Carmine. The authors also indicated that a decrease in sorption with the increase in biomass concentration is associated with a decrease in the availability of dye molecules per unit of biomass. In addition, the aggregation of biomass particles as another factor can decrease sorption (mg/g) due to the slowing of the intraparticle diffusion of dye molecules. Another study also showed that biosorption capacity decreased from 34.89 mg/g to 9.61 mg/g with increasing concentrations (1-5 g/L) of wet-torrefied *Chlorella* biochar on Congo Red biosorption (Yu et al. 2021). Thus, the results from the current study are consistent with the mentioned studies.

CONCLUSION

In this study, the maximum *C. vulgaris* biomass for RBBR biosorption was obtained as 0.502 g/L under photoheterotrophic cultivation conditions containing 0.5 g/L PW sugar. After optimization of microalgal growth, the highest RBBR biosorption percentage was determined as 99.49% at pH 4 in the presence of 3 g/L biosorbent and 103.38 mg/L initial RBBR concentration. Thus, the current study shows that PW for microalgae production and *C. vulgaris* for RBBR removal are potent, eco-friendly, and cost-effectively materials.

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Research Article

Investigation of Growth Performance, Proximate and Fatty Acid Composition of Freshwater (*Euglena gracilis, Chlorella vulgaris*) and Marine (*Pavlova lutheri, Diacronema vlkanium*) Microalgae

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ABSTRACT

This work is focused on investigating the nutrient compositions, growth, and fatty acid composition of *Chlorella vulgaris*, *Euglena gracilis, Pavlova lutheri*, and *Diacronema vlkanium*, which are natural diets of bivalve, crustaceans, live prey such as rotifer, copepods, daphnia and feed ingredients in aquaculture nutrition. Microalgae culture was performed in a live feed laboratory under controlled physical and chemical conditions. The initial concentration of microalgae species was adjusted as 2×10^6 cells/mL and growth performance was calculated by Neubauer Hemocytometer daily. The maximum growth performance was detected in *Diacronema vlkanium* culture with 1.78×10^7 cells/mL. In the case of proximate composition, the highest dry matter content was found in *Pavlova lutheri* (6.21%). Freshwater microalgae species *Chlorella vulgaris* (50.5%) and *Euglena gracilis* (42.5%) had high crude protein compared to *Pavlova lutheri* and *Diacronema vlkanium*. Fatty acid compositions of microalgae were also determined. The highest EPA (C20:5n-3) content was found in *Pavlova lutheri* (6.85%) whereas arachidonic acid (C20:4n-6) and docosahexaenoic acid (C22:6n-3) contents were only found with a level of (3.32%) and (1.79%) in *Euglena gracilis*, respectively. Microalgal culture should have high biomass in a short time of culture and in this study, *E.gracilis* and *P.lutheri* showed high growth and essential nutrients gain in laboratory scale production and this result could be applied in larger volume photobioreactor.

Keywords: Microalgae, growth, fatty acids, proximate, biomass

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INTRODUCTION

Microalgae contribute greatly to both the marine and freshwater food-web and they are able to synthesize inorganic matter into organic compounds such as lipids, polysaccharides and pigments (Chiu et al., 2011). They are used for live prey enrichment and feeding (Eryalçın, 2018; Eryalçın, 2019; Turcihan et al., 2021; Turcihan et al., 2022), wastewater treatment (Wollmann et al., 2019), biodiesel production (Goh et al., 2019), fish diet ingredients (Eryalçın et al., 2013; Eryalçın and Yıldız, 2015; Eryalçın et al., 2015; Camacho-Rodríguez et al., 2018; Soto-Sánchez et al., 2023) and bivalve culture (Shah et al., 2018). Microalgae must be nutritionally riched in essential biochemical compounds such as polyunsaturated fatty acids (PUFAs), highly unsaturated fatty acids (HUFAs), essential amino acids (EAA), and pigments (Raja et al., 2004; Patil et al., 2005; Patil et al., 2007; Hemaiswarya et al., 2011; Singh et al., 2015; Peltomaa et al., 2017). Moreover, they have antigonistic effects on bacterial communities in culture tanks (Spolaore et al., 2006; Neori, 2011). The first priority of microalgae culture is to get fast high biomass gain in a short time. The fast growth performance of microalgae is based on several parameters. The rapid proliferation of microalgae contributes to the high biomass in wet weight and this leads to the possible production of nutrients such as lipid, protein, carbohydrate, and pigment. For example, dinoflagellate *Crypthecodinium cohnii* can contain DHA up to 40% in dry weight that is nesessary for both growth and stress resistance at fish larval cultivation (Eryalçın et al., 2013). Nutrient contents of microalgae such as protein, lipids, and pigments can be species-specific which means each alga can contain specific nutrients (Das et al., 2012; Eryalçın, 2019; Gharajeh et al., 2020). For instance, *Nannochloropsis oculata* contains a high amount of EPA whereas dinoflagellate *Crypthecodinium cohnii* is famous for DHA. In comparison, freshwater microalgae are rich in essential 18C chain fatty acids such as linoleic (C18:2n-6), and α-linolenic acid (C18:3n-3) which are also important for freshwater fish.

Moreover, microalgae are the main energy source and substantial for enhancing the survival and growth of bivalve larvae (Parrish et al., 1998; Pazos et al., 1997; Budge et al., 2001). The nutritional value of microalgae is changed during their culture time. There are two main phases of the culture period called the exponential and stationary phases where algae should be harvested (da Silva Ferreira and Anna, 2017). Not only the culture phase but also the culture medium affects the nutritional value of microalgae biomass. These nutrient profiles consist of macronutrients (nitrogen, phosphorus, and sulphur) and micronutrients (iron, manganese, sodium molybdenum oxide, zinc, copper, and selenium) (Aslam et al., 2021; Shaaban et al., 2010). The nitrogen source of microalgae increases the growth performance and nutritional content by synthesizing large nutrient molecules like minerals and proteins (Procházková et al., 2014; Kumaran et al., 2023).

The growth performance and nutritional composition of microalgae also depend on physical and chemical parameters such as light, temperature, salinity, pH, and cultivation methods such as heterotrophic, autotrophic, and mixotrophic culture (Bashir et al., 2019; Zhao et al., 2011). Salinity and light conditions are very important in the cultivation of microalgae. For instance; Nannochloropsis sp. shows high growth performance at high salinity levels but it shows slow growth performance in heterotrophic culture (Bashir et al., 2019). In particular, autotrophic microalgae species directly affect the synthesis of biochemical substances and growth performance due to the intensity of the light (Sandnes et al., 2005). The reason is because these microalgae species use light as an energy source. As a result, biomass, proximate and fatty acid composition change depending on the light intensity. Another agent affecting microalgal growth performance, and fatty acid composition, microalgae cell metabolism, and the initial enzyme used for photosynthesis is culture temperature (Chaisutyakorn et al., 2018; Chiu et al., 2011). The increasing temperature in the culture adversely affects the fatty acid composition of microalgae. Most importantly, higher biomass gain and growth performance as well as protein and lipid contents in most microalgae are linearly related to light intensity and photoperiod such as *Chlorella vulgaris, Ankistrodesmus falcatus*, *Monoraphidium* sp., *Botryococcus braunii* (He et al., 2015; Metsoviti et al., 2019).

The other reason that affects biomass gain of microalgae depends on the growth potential of the species. As the growth performance increases, the biomass recovery rate also increases (Lau et al., 2022). The size of microalgae cells also affects their growth performance. Small-sized microalgae show higher growth performance than larger cell microalgae due to doubling time. For example, Arkronrat et al. (2016) have stated that *Nannochloropsis sp.* are smaller microalgae, and its growth performance is faster than *Tetraselmis sp.* due to its size. The unicellular freshwater eukaryote *Euglena gracilis* obtains flagellates and instead of a cell wall it has a pellicle based protein layer by a substructure of microtubules (Zhang et al., 2023). *Euglena gracilis* is rich in Paramylon which is a linear β-1,3-glucan polysaccharide polymer, antioxidants such as β-carotene, α-tocopherol and L-ascorbic acid, and PUFAs (Kottuparambil et al., 2019). The other freshwater microalgae *Chlorella vulgaris* belongs to Chlorophyceae with the thick cell walls and contains a high level of protein, minerals, vitamins, and pigments (Spínola et al., 2023). *Pavlova lutheri* is unicellular motile marine prymnesiophyte algae containing flagellate and it is known for high sterols, EPA and, DHA contents (Ahmed et al., 2015). *Diacronema vlkanium* is another marine green microalgae that belongs to the Haptophyceae family and is also rich in high levels of EPA and DHA (Fradique et al., 2013).

In this study, growth performance, proximate, and fatty acid compositions two unicellular both freshwater (*Euglena gracilis* and *Chlorella vulgaris*) and two marine microalgae species (*Pavlova lutheri*, *Diacronema vlkianum*) were investigated under laboratory conditions for biomass utilization.

MATERIAL AND METHODS

Microalgae strains and stock culture

Culture mediums f/2 and 3N-BBM-V were sterilized at 121 °C for 15 min before they were used (Guillard, 1975). Stock culture of microalgae was cultured in 50 mL test tubes to 250 mL, followed by 1-L, and 5-L erlenmayers. Microalgae were counted in each experimental flask during the experiment. The microalgae growth trial was conducted in the Phytoplankton and Zooplankton Laboratory of the Faculty of Aquatic Sciences of Istanbul University, for 32 days. Four microalgae species were obtained from CCAP (Culture collection of algae and Protozoa, Scotland) which are *Pavlova lutheri* (Strain number: CCAP940/2), *Diacronema vlkianum* (Strain number: CCAP914/1), *Euglena gracilis* (Strain number: CCAP1224/38), *Chlorella vulgaris* (Strain number: CCAP211/110).

Experimental design and growth performance

In this study, the microalgae species were cultured from the initial to 15 days under 250 mL volume. Each experimental group was studied in three replicates. The initial cell density of the microalgae species was adjusted at $2x10^6$ cells/mL for the second part of the culture experiment after the 15^{th} day culture, all volumes were inoculated into 1000 mL erlenmayers with gentle aeration till the 32nd day. This method was chosen by the same culture procedure at commercial hathcheries where first culture occured in small-scale flasks and then continuously up-scaled in larger volumes. At the end of the culture, all biomass was harvested and stored at -80 °C in the refrigerator. During culture, the growth performance of microalgae species was calculated daily with a Neubauer Hemocytometer.

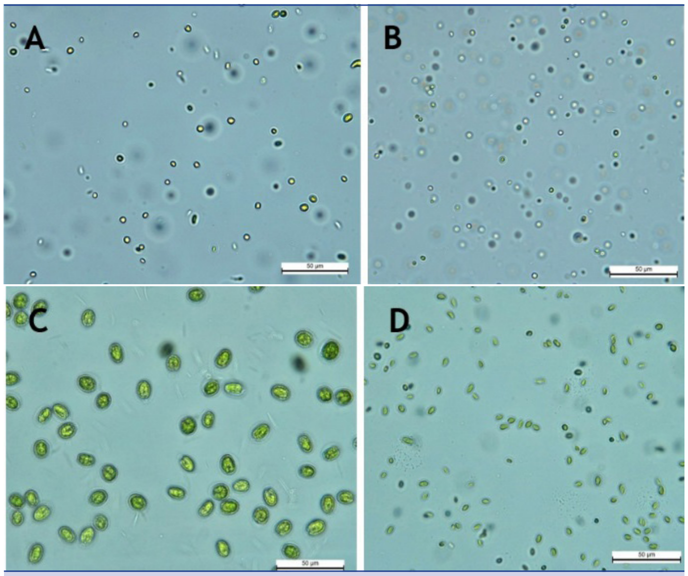

Figure 1. *Chlorella vulgaris* (A), *Diacronema vlkanium* (B), *Pavlova lutheri* (C) and *Euglena gracilis* (D).

Proximate analysis

Dry matter analysis of microalgae, the samples were first filtered using a vacuum filtration system. Vacuum filter papers (Schleicher&-Schuell GF-52, 47 mm, nominal pore opening 0.7 µm.) were dried in an oven at 105°C for 3 hours. When the papers were cooled at room temperature, their empty weights were weighed. Microalgae (100 mL) were filtered by a vacuum filtration system. The filter papers obtained after the filtration process were taken back to the oven at 105 °C and the drying process was carried out. After drying methods, the papers were taken into a desiccator. The dry matter (%) was calculated by measuring the papers that were cooled in the desiccator (AOAC, 1995). Kjeldahl method was preferred for crude protein analysis of microalgae. Microalgae samples were weighed 0.5 g–0.8 g and placed in Kjeldahl tubes (AOAC, 1995). Two pieces of Kjeldahl tablets and 20 mL sulphuric acid (H_2SO_4) were added to the microalgae samples placed in the tubes. The samples burned to 450°C for 120 minutes. The tubes were placed in the Kjeldahl device (Gerhardt VAPODEST®), and distillation and titration were performed. The amount of crude protein in the samples was calculated by determining the amount of 0.1N HCl consumed in the titration. Microalgae samples to be analyzed for crude lipid were weighed around 1 g and placed in the lipid extraction device. The glass VELPs (VELP® Scientifica), were previously dried in an oven at 105°C and kept in the desiccator. The samples were completed in the Soxhelet device for 60 minutes. After extraction, the glass VELPs were placed in an oven at 105 °C. The weights of the glass VELPs were weighed, and the percentage of crude lipid was calculated (Folch et al., 1957). Microalgae samples were placed in ceramic and burned in a muffle furnace at 550°C for 5-6 hours. The samples were taken into a desiccator to come to room temperature. The samples at room temperature were weighed. After weighing, the amount of ash was calculated (AOAC, 1995).

Fatty acid analysis

Fatty acid methyl esters were analyzed by GC (GC-2030; Shimadzu, Tokyo, Japan) in a Supercolvax-10 fused silica capillary column (constant pressure with 100KPa, length: 100 m; internal diameter: 0.25 mm; 0.20 i.d (Ref.: 24080-U) Supelco, Bellefonte, PA, USA) using H_2 as a carrier gas. Fatty acid methyl esters in algae biomass were gained by the transmethylation method with 1% sulfuric acid in methanol (Christie, 1982). The column temperature was 180 °C for the first 10 min, increasing to 260 °C at a rate of 2 °C min^{-1} and then held at 260 °C for 15 min. Then they were quantified by FID following the conditions described by Izquierdo et al. (1990) and identified by comparison with external standards well-characterized fish oils (EPA 28, Nippai, Ltd Tokyo, Japan).

Statistical analysis

Each sampling was conducted in triplicate and all data were treated with one-way analysis of variance (ANOVA) and the averages in the study were compared with the Duncan test ($p<0.05$) method in the SPPS program (SPSS for Windows 11.5; SPSS Inc., Chicago, IL, USA) and significance was adjusted at $p<0.05$.

RESULTS AND DISCUSSION

Growth performance of microalgae

In this study, the growth performance of *Chlorella vulgaris*, *Pavlova lutheri*, *Euglena gracilis* and *Diacronema vlkanium* microalgae species were investigated. Growth performance was measured in two different volumes and time-lapse. The first measurement was between the initial and 15th days of culture and had a 250 mL culture volume while the second culture process was upscaled to 1000 mL culture volume with stable aeration between the 15th and 32nd days. The highest growth was determined in DV (*Diacronema vlkanium*) culture with 1.68×10^{7} and 1.59×10^{7} cells/mL density at 13th days and 30th days, respectively. Freshwater microalgae *Chlorella vulgaris* (CV) showed the highest growth rate on the 15th day of culture in 250 mL and cell density continuously increased until the end of the experiment with gentle aeration in 1000 mL erlenmayer. In terms of *Euglena gracilis* (EG) rapid growth was obtained between the 18th and 28th days in the presence of 1000 mL volume and regular aeration. The cell density is higher from the 20th and 28th days (3.50×10^{6}) compared to the culture of between the 2nd and 17th days (9.50×10^{6}). *Pavlova lutheri* (PL) maximum cell density was recorded at (7.77×10^{6}) at 15 days in and (8.50×10^{6}) at 32th days of culture, respectively. Microalgae growth performances are shown the Figure 2.

Proximate composition of microalgal biomass

In this study, the nutritional compositions were examined and it was reported that the highest crude protein content was found in *Chlorella vulgaris* (50.05±0.01%) and *Euglena gracilis* (42.15±0.52%) had the second highest level ($p<0.05$). However, the lowest crude protein (38.4±0.55%) was detected in marine microalgae *Diacronema vlkanium* ($p<0.05$). In terms of crude lipid content, the highest crude lipid (19.96±0.97 %) was found in marine microalgae *Pavlova lutheri* species whereas the lowest value was found in freshwater microalgae *Chlorella vulgaris* (11.2±0.02 %) ($p<0.05$). *Pavlova lutheri* had the highest dry matter (6.21±0.33%) content among groups ($p<0.05$). The table below shows the nutritional content of microalgae (Table 1).

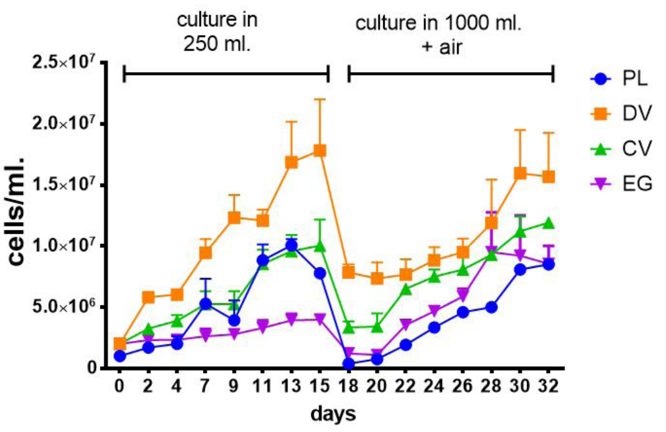

Figure 2. Growth performance of microalgae species; PL (*Pavlova lutheri*), DV (*Diacronema vlkanium*), CV (*Chlorella vulgaris*), and EG (*Euglena gracilis*).

feeding and formulated diets in marine fish. The nutritional value and growth performance of microalgae are also essential for biomass production. In microalgae culture, growth (doubling time), fatty acid content, and nutritional values are directly affected by the cultivation method. Moreover, the ingredients of the culture medium and stress conditions also affect the growth and proximate composition of the microalgae. For instance, *Scenedesmus sp*. can accumulate high levels of lipids under stress conditions (Khatoon et al., 2019). The other halophilic microalgae *Dunaliella salina* can contain a high amount of pigments under high salinity conditions (de Souza Celente et al., 2022), and freshwater microalgae *Chlorella vulgaris* may have high protein under low salinity conditions (Liu et al., 2008). In this study, the growth performance, proximate, and fatty acid composition of four microalgae cultured under constant laboratory conditions were investigated for potential aquaculture purposes such as live prey feeding or microalgae biomass.

Jeong et al. (2016) reported that the highest growth performance in *E. gracilis* was obtained by mixotrophic cultivation compared to phototrophic and heterotrophic cultivation methods with values of 2.48×10^6, 0.61×10^6 and 0.49×10^6 (cells/mL), respectively. A similar study was conducted by Gu et al. (2022) that calculated the growth performance of *E.gracilis* at autotrophic and mixotrophic culture methods and they reported autotrophic culture had a higher growth result (0.6×10^6 cell/mL) at 12 days of culture. In our study, *E.gracilis* was cultivated in a phototrophic way and found higher growth than other studies was obtained at a larger volume (1000 mL) with 9.2×10^6 (cells/mL), and lower growth was detected 3.97×10^6 (cells/mL) cell density in smaller culture (250 mL). In terms of fatty acid results, the highest levels of ALA, and EPA (14.92±0.10% and 4.79±0.08%) were obtained in phototrophic cultivation whereas the highest ARA, and DHA levels (3.39±0.03%, 1.74±0.02%) were found in mixotrophic cultivation method (Jeong et al., 2016). In our study, the highest levels of ALA, ARA, and DHA (14.98±0.10%, 3.32±0.05% and 1.79±0.02%) were found in *E.gracilis* biomass among microalgae whereas the EPA level (4.56±0.03%) was found the lowest in other microalgae species. This result could be related to different cultivation methods of the *Euglena gracilis*. Similar to Jeong et al. (2016) results, our study also showed that the phototrophic cultivation method enhanced fatty acid contents of *E. gracilis*. *Chlorella vulgaris* has a high potential for biomass production in both indoor and outdoor culture systems. It has been evaluated as feed ingredients in aquaculture (Ahmad et al., 2020). At laboratory scale production, we obtained the highest cell densitiy at 1.05×10^7 (cells/mL)

Table 1. Proximate composition of microalgal biomass.

Proximate Analysis (%)	*Chlorella vulgaris*	*Euglena gracilis*	*Pavlova lutheri*	*Diacronema vlkanium*
Crude Protein	50.05±0.01^a	42.15±0.52^b	39.02±0.32^c	38.4±0.55^c
Crude Lipid	11.2±0.02^c	15.35±0.31^b	19.96±0.97^a	18.01±0.99^a
Crude Ash	7.2±0.00^c	5.01±0.22^d	10.01±0.88^b	18.45±0.83^a
Dry Matter	3.00±0.01^c	2.44±0.00^d	6.21±0.33^a	5.89±0.96^b

Dissimilar lettering show significant differences among groups (*p<0.05; Duncan's multiple range test).

Fatty acid composition of microalgae biomass

Microalgae is an important source of essential fatty acids such as EPA (C20:5n-3), DHA (C22:6n-3), and ARA (C20:4n-6) for aquaculture. *Euglena gracilis* had the highest EPA level (0.32±0.01%) among groups ($p<0.05$). The highest DHA level (1.79±0.02%) was found in *Euglena gracilis* biomass ($p<0.05$). *Euglena gracilis* and *Pavlova lutheri* had the highest ARA levels (3.32±0.05% and 3.16±0.00%) ($p<0.05$). Oleic acid (C18:1n-9) content highest values had *Chlorella vulgaris* and *Diacronema vlkanium* and the lowest value was *Euglena gracilis.* Freshwater microalgae had the highest (9.24±0.14% and 8.27±0.06%) linoleic acid (C18:2n-6) content ($p<0.05$). ALA (α-linolenic acid) (C18:3n-3) was found in *Euglena gracilis, Pavlova lutheri* and *Diacronema vlkanium* microalgae species with a level of 14.98±0.10%, 6.62±0.14% and 0.21±0.00%, respectively. γ-linolenic acid (C18:3n-6) was only found in *Pavlova lutheri.* The highest ∑ n-3 (24.87±0.03), ∑ n-6 (17.28±0.56) fatty acid contents were found in *Euglena gracilis.* Additionally, the highest ∑ n-3 HUFA (8.47±0.15%) and ∑ PUFA contents (39.58±1.33%) were found in *Euglena gracilis.* The lowest value (0.30±0.02%) was found in *Chlorella vulgaris* ($p<0.05$).

Microalgae are rich in lipids (Fields et al., 2014), carbohydrates (Chen et al., 2013), proteins (Becker, 2007), pigments (Begum et al., 2016), and fatty acids such as; PUFA, EPA and ARA (Eryalçın et al., 2013; Eryalçın et al., 2015) and therefore, they are very important future food supply not only for aquaculture purpose but also direct utilization of their biomass for human and animal diets. From this respect, recent studies are focused on the utilization of microalgae in both live prey

Table 2. Fatty acid composition of microalgae species.

Fatty Acid Compositions (% total fatty acid)	*Euglena gracilis*	*Pavlova lutheri*	*Diacronema vlkanium*	*Chlorella vulgaris*
C8:0	-	-	0.19±0.01	-
C10:0	-	0.06±0.00[b]	0.11±0.01[a]	0.03±0.00[c]
C11:0	-	0.62±0.02	-	-
C12:0	-	0.86±0.00[a]	0.44±0.03[b]	0.16±0.00[c]
C14:0	12.30±0.15[b]	1.01±0.01[c]	3.69±0.07[a]	0.60±0.02[c]
C14:1	-	-	-	0.11±0.00
C15:0	2.24±0.04[a]	0.14±0.01[c]	0.39±0.03[b]	0.26±0.01[b]
Iso16:0	5.81±0.03	-	-	-
C16:0	27.25±0.23[b]	24.50±0.09[b]	33.59±0.27[a]	18.80±0.21[c]
C16:1n-5	3.08±0.01	-	-	-
C16:2n-4	0.46±0.02	-	-	-
C16:1	-	0.57±0.03[b]	26.33±0.08[a]	28.76±0.18[a]
C17:0	0.90±0.01[a]	n.d.	0.35±0.03[b]	0.23±0.02[b]
C16:3n-4	1.26±0.02	-	-	-
C18:0	2.42±0.01[b]	1.14±0.01[b]	1.86±0.02[b]	14.38±0.05[a]
C18:1n-9	1.32±0.01[d]	15.94±0.02[c]	22.24±0.15[b]	28.21±0.13[a]
C18:2n-6	8.27±0.06[b]	9.24±0.14[a]	3.18±0.03[d]	7.46±0.01[b]
C18:2n-4	0.28±0.00	-	-	-
C18:3n-3	14.98±0.10[a]	6.62±0.14[b]	0.21±0.00[c]	n.d
C18:3n-6	-	1.05±0.01	-	-
C20:0	0.16±0.02[a]	-	-	0.14±0.01[a]
C20:1	-	0.70±0.01[a]	-	0.09±0.01[b]
C20:2	-	1.12±0.01	-	-
C20:2n-6	2.57±0.13	-	-	-
C20:3n-3	1.42±0.05[a]	-	-	0.24±0.00[b]
C20:3n-6	0.65±0.02	-	-	-
C20:4n-6	3.32±0.05[a]	3.16±0.00[a]	0.08±0.00[b]	-
C20:4n-3	1.72±0.02	-	-	-
C20:5n-3	4.56±0.03[b]	6.84±0.03[a]	2.20±0.03[c]	0.06±0.01[d]
C22:0	-	-	-	0.06±0.01
C22:4n-6	0.28±0.05	-	-	-
C22:5n-6	2.19±0.01	-	--	-
C22:4n-3	0.08±0.01	-	-	-
C22:5n-3	0.32±0.01	-	-	-
C22:6n-3	1.79±0.02[a]	-	-	0.09±0.02[b]
C24:0	-	0.08±0.00[b]	-	0.16±0.01[a]
Σ Monounsaturated	4.40±0.05[d]	17.21±0.04[c]	22.24±0.15[b]	29.0±0.16[a]
Σ Saturated	51.08±0.23[a]	28.61±0.13[d]	40.60±0.25[b]	34.8±0.18[c]
Σ n-3	24.87±0.03[a]	6.84±0.03[b]	2.40±0.03[c]	0.3±0.02[d]
Σ n-6	17.28±0.56[a]	13.45±0.13[b]	0.08±0.00[d]	7.5±0.01[c]
Σ n-9	1.32±0.22[d]	15.94±0.02[c]	22.24±0.15[b]	28.2±0.13[a]
Σ n-3 HUFAs	8.47±0.15[a]	6.84±0.03[b]	2.40±0.03[c]	0.30±0.02[d]
EPA/ARA	1.37±0.02	-	-	-
DHA/EPA	0.39±0.01	-	-	-
DHA/ARA	0.54±0.04	-	-	-
n-3/n-6	1.44±0.02[b]	0.51±0.01[b]	30.00±0.38[a]	0.04±0.02[c]
Σ PUFA	39.58±1.33[a]	26.90±0.03[b]	27.90±0.21[b]	7.61±0.04[c]

Dissimilar lettering shows significant differences among groups (*p<0.05; Duncan's multiple range test).

in 1000 mL volume at phototrophic culture. Taş and Dalkıran (2022) reported that the *C. vulgaris* initial cell densitiy was 1.1×10^6 (cells/mL) and the highest cell density obtained was 2.4×10^7 (cells/mL) on the 3rd day of mixotrophic culture. This higher algal productivity might be related to the nutrient composition of cultures medium by affecting the metabolism of microalgae cells (Fields et al., 2014). Light, temperature, and cultivation methods are important factors in microalgal growth and proximate composition. In our study, all parameters were constant therefore we assumed that growth and nutrient compositions were positively affected by culture mediums even when we used phototrophic culture methods.

Total lipid and protein accumulation should be higher in microalgae cells in order to be evaluated as feed ingredients. *E. gracilis* has distinctive features in the phototrophic cultivation method such as high protein content and high digestibility (Nwoye et al., 2017). In our study, the crude protein content of *Euglena gracilis* was higher than the marine microalgae species. On the other hand, the highest crude lipids were found in marine microalgae both *Diacronema vlkanium* and *Pavlova lutheri*. Yeh et al. (2010) found the crude protein and lipid contents of *C.vulgaris* as 25-30% and 30-40%, respectively. In our study, crude protein (50.05%) was found to be higher comparied to Yeh et al. (2010), moreover, the crude lipid (11.2%) value was lower. This result could be related to cultured microalgae in photobioreactor culture. Moreover, salinity highly affects of fatty acid contents of microalgae. Teh et al. (2021) investigated the fatty acid content of *C. vulgaris* at different salinity levels and oleic acid (C18:1n-9), linoleic acid (C18:2n-6), and α-linolenic acid (C18:3n-3) levels were found as 24.6%, 15% and 4.7%, respectively. In our study, we had higher oleic acid (C18:1n-9) (28.21%) and lower linoleic acid (C18:2n-6) (7.46%) levels compared to Teh et al. (2021). Marine haptophyte species *Pavlova lutheri* is known rich in protein and lipid content due to their large cell and ability to accumulate nutrients from culture water. *Pavlova lutheri* is known as rich in protein content among microalgae species (Shah et al., 2014). In our study, the crude protein content (39.02±0.32%) was detected highest value among four microalgae species. The other nutrients also showed good levels of crude lipid, crude ash, and dry matter contents at a level of 19.96±0.9%, 10.01±0.88%, and 6.21±0.33%, respectively.

Fradique et al. (2013) reported crude protein (38.4±0.2%), crude lipid (17.9±0.5%), crude ash (18.04±0.8%), and dry matter content (91.03±0.01%) determined in ‰25 salinity culture conditions of *Diacronema vlkanium*. In our study, salinity was adjusted at ‰30 – ‰32 salinity, the contents of crude protein, lipid, ash, and dry matter were found as 38.4±0.55%, 18.01±0.99%, 18.45±0.83%, 5.89±0.96%, respectively. In another study, Cañavate and Fernández-Díaz (2022) showed lipid and fatty acid composition of *D.vlkanium* at different salinity levels. According to this study, EPA and DHA levels were found 7.6% and 6.6% of total fatty acids between ‰20 - ‰35 different salinity ranges in *D. vlkanium* production. In our study, essential fatty acids showed moderate levels of EPA and DHA (0.06±0.01% and 0.09±0.02%) at similar salinity levels. This result could be related to the fatty acid elongation of microalgae as long as salinity increases (Cañavate and Fernández-Díaz., 2022). We assume that EPA and DHA levels were linearly correlated with a high salinity in *D.vlkanium* species. In terms of marine haptophyte *Pavlova lutheri* has essential fatty acids such as EPA and ARA of total fatty acids. We obtained high accumulation of EPA (6.84%) and ARA (3.16%) levels in this haptophyte algae.

As a result, *D. vlkanium* can be cultured with the highest growth performance under phototrophic cultivation when compared to other microalgae species that were examined. However, all microalgae enhanced cell density after the 18th day of the experiment due to gentle aeration and flow current of culture water. The aeration positively effects the microalgal cell density and growth performance due to increases in the amount of CO_2 and nutrient content in the photrophic culture conditions (Mohsenpour and Willoughby, 2016). *Euglena gracilis* and *Chlorella vulgaris* are productive species together with high protein and biomass contents. Dry matter is important when powder product is concerned with microalgae. The highest dry matter content was found in *Pavlova lutheri*. From this point, *P.lutheri* is a suitable species for the production of biomass and turn into dry material which features of highest dry matter content. *Euglena gracilis* have high content of ALA, ARA, EPA, and DHA which are important for fish feed raw materials (Wang et al., 2018). Microalgae fatty acid contents depend on the aeration, amount of CO_2, light intensity, temperature, and culture medium (Schwarzhans et al., 2015, Guedes et al., 2010, Go et al.,2012). In our study, the highest ALA, ARA, and DHA fatty acids contents were found in *E. gracilis*. This result could be related to, the cultivation of microalgae by phototrophic methods. However, the highest EPA content was found in *Pavlova lutheri*. EPA is highly important for fish feeding and larval development. EPA and DHA fatty acids are very difficult to synthesize from fish (Guedes et al., 2010). That's the reason why the aquaculture industry has to use rich EPA and DHA contents from microalgae species.

CONCLUSION

In conclusion, within this study, we applied the same microalgae culture procedure at commercial hatcheries in our laboratory where microalgae culture start with small vessels and then continuously inoculated a large volume with gentle aeration. The purpose of this work was to investigate both the growth of algae during 32 days of culture (250 mL and 1000 mL glass flasks) and nutritional value and fatty acid composition at the end of the 32nd day of culture just before they were inoculated in 30 L plastic bags. The data obtained from our laboratory is valuable for both commercial hatcheries where those microalgae are utilized. To sum up, the success of microalgal up-scale culture in both freshwater and marine microalgae species are strongly related to inoculation time and volume. As a result, all microalgae have a high potential for biomass gain in a very short time with good enough nutrients. Moreover, *Pavlova lutheri* and *Euglena gracilis* can supply promising levels of highly unsaturated essential fatty acids such as ARA, EPA, and DHA. Most importantly, we suggest based on obtained data these two microalgae have high potential for dry biomass production due to their high dry matter content. Therefore, those biomass have high potential to use feed ingredients in aquaculture and this can lead to a positive effect on sustainable production. Additionally, we conclude that laboratory-scale production of those four microalgae should be inoculated from a 250 mL culture flask to 1000 mL flasks at two weeks. Microalgae biomass production and its nutrient compositions are affected by culture systems like photobioreactors, volumes, and culture types such as phototrophic, myxotrophic, and heterotrophic culture. Further studies are needed for larger photobioreactor production and biomass investigations.

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Research Article

Growth Performance, Survival Rate, and Water Quality in an Aquaculture System Using Different Feeding Strategies for Juveniles of Nile Tilapia (*Oreochromis niloticus*)

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ABSTRACT

Aquaculture is a rapidly growing industry worldwide, with Nile tilapia (*Oreochromis niloticus*) being one of the most intensively farmed fish species. This study aimed to evaluate the growth performance and water quality parameters in different culture systems for Nile tilapia. Six treatments were tested, including variations in feed type (commercial or microalgae), aeration, and their combinations. The results showed that the presence of commercial feed and aeration (T2) resulted in the highest weight gain and specific growth rates, while treatments without commercial feed showed lower growth performance. The addition of microalgae supplementation did not significantly improve growth compared to commercial feed alone. Water quality parameters, particularly nitrite levels and dissolved oxygen, played crucial roles in the production of tilapia. It was observed that high nitrite levels were associated with decreased growth and survival rates. Proper monitoring and management of water quality, including nitrite levels and dissolved oxygen, are essential to ensure the survival and growth of tilapia in aquaculture systems. These findings highlight the importance of implementing sustainable practices and appropriate feeding strategies to optimize the growth and well-being of farmed tilapia while minimizing environmental impacts.

Keywords: Fish nutrition, fish production, fish farming, water quality, microalgae, *Oreochromis niloticus*

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INTRODUCTION

Aquaculture is a thriving industry worldwide, characterized by diversity and expansion (Sarker et al., 2016; Verdegem et al., 2023). In developing countries, this sector has experienced significant growth due to ongoing efforts in regulation, professionalization, and modernization. Farmers have become increasingly knowledgeable about management practices and essential inputs (Scorvo-Filho et al., 2010; Moreira et al., 2012).

Fish farming is expanding at a faster rate compared to other livestock commodities (FAO, 2022). However, despite notable progress, the adoption of technology and technical capabilities still restricts overall achievements and expectations. Addressing the reduction of production costs and environmental impact is crucial. These are critical obstacles that need to be overcome for a more optimistic and sustainable outlook (Leonardo et al., 2009).

In Brazil, Nile tilapia (*Oreochromis niloticus*) is the most intensively farmed fish species. This is primarily due to its rapid growth, efficient feed conversion, desirable meat quality, and market acceptability both locally and internationally (Schwarz et al., 2010). In fact, tilapia is the second most cultivated fish worldwide, following carp (Fonseca et al., 2013).

Successful fish farming in ponds relies on effective management of nutrition and water quality. In most cases, the use of complete diets is necessary to meet the nutritional requirements of fish, ensuring growth, high productivity, and profitability (Furuya et al., 2001; Carvalho et al., 2012). This becomes particularly crucial when fish are bred or fattened under confined conditions, such as cages, ponds, and raceways, where high biomass per unit area is present.

In an attempt to increase income, farmers often increase stocking rates (density), but this may negatively impact growth and result in poor water quality for the fish (Lima et al., 2016). Such conditions affect survival rates since suitable water quality is essential for the physiological functions of fish, including breathing, reproduction, feeding, and defecation. Water quality in fishponds may also depend on the availability and nutritional quality of food (Bhatnagar and Devi, 2013).

At the farm level, intensification typically leads to deteriorating water quality in fish ponds, posing hazards and increased risks to surrounding ecosystems. Effluents from aquaculture activities can contain substantial amounts of feces, leftover feed, and bacterial biomass (Gorlach-Lira et al., 2013). Therefore, ensuring constant monitoring is crucial to maintain the sustainability of fish farming, preventing downstream contamination and environmental degradation. In ponds with limited water renewal, the effects of intensification become even more problematic. Aeration techniques offer alternatives to increase the carrying capacity of a pond for fish (Boyd et al., 2018). Nonetheless, water renewal remains important to dilute the concentration of toxic metabolites.

The concentration of microalgae in fishponds also influences fish performance and water quality. Microalgae are responsible for recycling nutrients excreted by fish (Gorlach-Lira et al., 2013). They also contribute to the dissolved oxygen content in the water through photosynthesis and can adsorb significant amounts of heavy metals from contaminated water sources (Coêlho et al., 2014).

Moreover, certain microalgae serve as direct or indirect sources of food for fish and their ecosystem (Sarker et al., 2016). This is particularly interesting considering that production costs, especially the high cost of feed, pose major constraints in the global fish farming scenario (Taelman et al., 2013). Consequently, research efforts have focused on identifying alternative feeding strategies (Leonardo et al., 2009; Carvalho et al., 2012). In general, these alternatives aim to promote growth, minimize stress, and enhance the efficiency of the fish immune system (Ungsethaphand et al., 2010; Sarker et al., 2016; Zeinab et al., 2015).

The aim of this study was to evaluate the growth performance and the survival rates of Nile tilapia (*Oreochromis niloticus*) and the water quality in an aquaculture system using different feeding strategies. For that, we examined the ability of fish to thrive in ponds without water renewal under various experimental conditions and monitored water quality to assess variations based on treatments involving feed, aeration, and microalgae supplementation.

MATERIALS AND METHODS

An aquaculture system consisting of circular open ponds without water renewal was utilized for raising Nile tilapia (*Oreochromis niloticus*). The ponds were made of polyethylene plastic, with a capacity of 1000 L, filled with 500 L of water. Juvenile Nile tilapia (53.2 ± 5.4 g; 8.0 ± 1.0 cm) were bred at an initial stocking density of 30 fish per pond (n=30), resulting in an average density of 3,192 g m^{-3}. The system was built on a fish farm located in Dourados, MS, Brazil.

Six treatments were designed to evaluate the impact of different factors on the growth and development of juvenile Nile tilapia. These treatments included: commercial feed (T1), commercial feed with aeration (T2), microalgae supplementation (T3), microalgae supplementation with aeration (T4), commercial feed with microalgae supplementation (T5), and commercial feed with microalgae supplementation and aeration (T6) (Table 1).

The commercial feed contained 40% crude protein. The microalgae used in the study was *Chlorella sorokiniana* CTT 7727 (3.9 x 10^6 cells mL^{-1}; 0.39 g L^{-1}), obtained from the André Tosselo Foundation (FAT). The strain was cultivated in a separate open pond using the Bold Basal medium (Bischoff and Bold, 1963). The temperature of the ponds was maintained at 28 ± 2°C using thermostats, while the photoperiod followed a 12 h light/12 h dark cycle.

The experiment was conducted for two weeks. In the first week, the amount of commercial feed provided was fixed at 4% of the initial live weight (g) for treatments T1 and T2. For treatments involving multiple food sources (T5 and T6), the amount of feed was reduced to 50% of the other treatments as per dilution criteria (Table 1). In the second week, the feed amount was halved for all treatments. Feed was administered daily during four feeding periods throughout the entire experiment.

Table 1. Treatments offered to juvenile tilapia bred in open ponds with no water renewal during the early stages of development.

	Treatment	Day 0 – 7	Day 8 – 14
T1	Commercial feed	64 g	32 g
T2	Commercial feed and aeration	64 g	32 g
T3	Microalgae	2,000 mL	1,000 mL
T4	Microalgae and aeration	2,000 mL	1,000 mL
T5	Commercial feed and microalgae	32 g + 1,000 mL	16 g + 500 mL
T6	Commercial feed, microalgae, and aeration	32 g + 1,000 mL	16 g + 500 mL

Water parameters such as pH, hardness, dissolved oxygen, toxic ammonia, and nitrite were measured daily using a commercial colorimetric disk kit. At the end of the experiment (day 14), water samples were collected for microbiological analysis, including total thermo-tolerant coliforms, *Escherichia coli*, and mesophilic bacteria, using the classical multiple-tube technique (APHA, 1998).

To evaluate the growth performance, fish were length measured and weighed before being immediately released into the ponds without inflicting any harm. The other parameters monitored were the specific growth rate (SGR) (Eq. 1), the daily weight gain (DWG) (Eq. 2) (Haque et al., 2023), and the condition factor (K) (Eq. 3) (Pauly, 1983):

$$SGR\ (\%\ day^{-1}) = [(W_2 - W_1) / W_1] \times (100 / T) \quad 1$$

$$DWG\ (\%\ day^{-1}) = [(W_2 - W_1) / W_1] \times 100 \quad 2$$

$$K\ (g\ cm^{-3}) = 100\ W / L^3 \quad 3$$

Where: W = fish weight (g); W_1 = initial fish weight (g); W_2 = final fish weight (g); L = fish length (cm); T = the experimental period in days.

The survival rate (SR) was evaluated at the end of the experiment and expressed as follows (Eq. 4) (Haque et al., 2023):

$$SR\ (\%) = (FH / FS) \times 100 \quad 4$$

Where: FH = number of fish harvested; FS = number of fish stocked.

Variance tests (one-way ANOVA) and comparison of means (Tukey test, 5%) were conducted separately according to data of weight (g) and total length (cm) during the experimental trial. All analyzes were performed in triplicate.

RESULTS AND DISCUSSION

Development of *Nile tilapia*

The weight of the fish varied among treatments on both the 7th and 14th days of the experiment (Fig. 1). During the first week (day 7), treatments T2 and T5 exhibited better results. Subsequently, in the second week, T2 and T6 showed superior outcomes. Conversely, the absence of commercial feed (T3, T4) was associated with lower weight gain in both experimental periods (days 7 and 14) (Fig. 1). Similarly, fish body length was highest in T2 and T6, while it was lowest in T3 and T4 during the final evaluation trial (Fig. 2).

These results showed that the growth performance of Nile tilapia was influenced by various factors, primarily associated with the type of food provided (commercial or natural). However, it was expected that due to the efficient capacity of Nile tilapia to harvest microalgae from the water through their gill rakers (Turker et al., 2003), T6 and T5 would present, respectively, the best results for weight by supplying an extra source of nutrients to the fish diet (T5) in addition to an increment in the oxygen availability (T6), which is critical for fish to achieve their full growth and activity potential (Obirikorang et al., 2020).

The best treatment in terms of total weight gain was obtained by combining feed and aeration (T2), which resulted in an increase

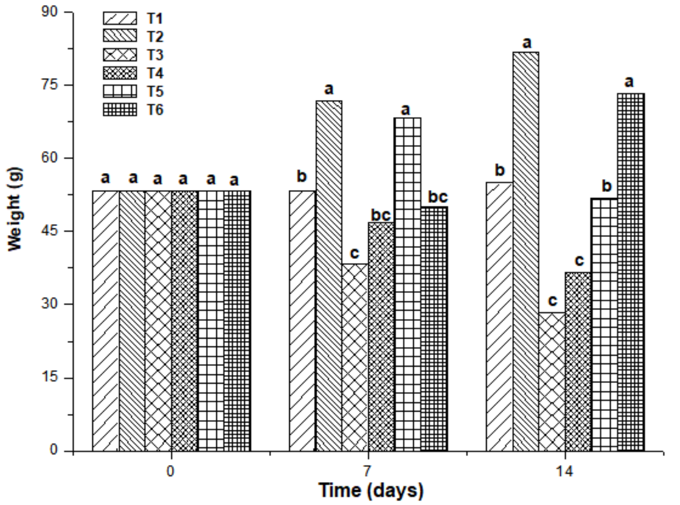

Figure 1. Analysis of variance of the increased weight (g) of Nile tilapia in relation to six treatments and time (Table 1). The results presented are the average of the weighted juveniles (N=5). *Means followed by the same letter do not differ by the Tukey test at the 5% probability level.

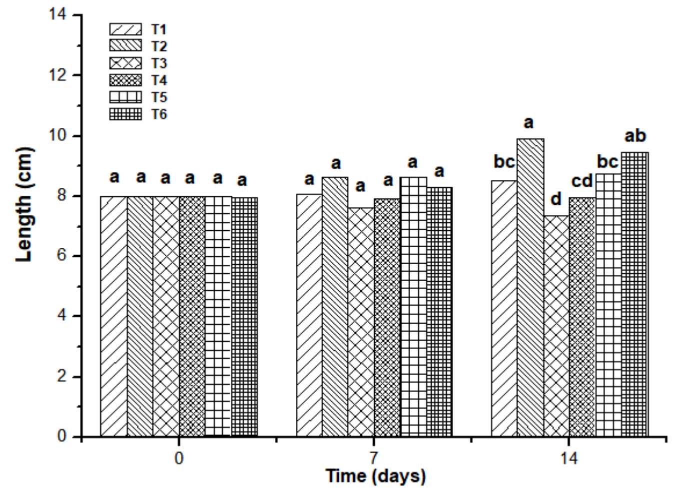

Figure 2. Analysis of variance of the increased total body length (cm) of juvenile Nile tilapia in relation to six treatments and time (Table 1). The results presented are the average of the measured juveniles (N=5). *Means followed by the same letter do not differ by the Tukey test at the 5% probability level.

of 53.33%. The treatment involving feed, microalgae, and aeration (T6) demonstrated the second-highest weight gain rate of 37.77%. However, this treatment also had the lowest survival rate of 53.3%. Solely relying on commercial feed (T1) was associated with poor performance in daily weight gain. In fact, treatments T3, T4, and T5 exhibited negative daily weight gain throughout the experiment. Thus, weight loss was not correlated with lower survival rates ($S_{\%}$) (Table 2). Weight loss can occur when fish are transitioning to a different feed or adjusting to new dietary conditions. Moreover, environmental stressors, such as water quality issues, handling stress, or competition, can indeed lead to weight loss in fish without negatively impacting survival rates. These stressors can affect fish health and behavior, potentially causing reduced feeding, increased energy expenditure, or physiological changes that result in weight loss. The impact on survival rates will depend on the severity and duration of these stressors (Martins et al., 2012; Toni et al., 2017; Yavuzcan et al., 2017; Hvas et al., 2020; Canosa and Bertucci, 2023).

On the other hand, the specific growth rates of tilapia cultivated in T2 (3.80% day^{-1}) and T6 (2.69% day^{-1}) (Table 2) were higher or comparable to those reported in the literature for juveniles (Table 3).

Finally, the condition factor seems to better explain the growth behavior by combining information from weight and length (Table 2). The results underline that T3 and T4 were the worst treatments for the Nile tilapia development, *i.e.*, microalga is not a good enough source of nutrients to sustain growth, at least at the utilized concentration. However, the other treatments (T1, T2, T5, and T6) did not show statistical differences (P>0.05) between them for this parameter.

Various studies have reported success in the partial replacement of commercial fish feed by microalgae biomass, but the inclusion of *Chlorella* sp. into feed in most cases evolves biomass processing, pre-treatment, and extrusion into pellets (Teuling et al., 2017; Tibbetts et al., 2017; Batista et al., 2018; de Cruz et al., 2018). However, a study conducted to evaluate the use of *Chlorella vulgaris* and *Scenedesmus obliquus* as an autotrophic bio floc technology during Nile tilapia cultivation with commercial feed showed a positive effect on water purification and to prevent fish mortality. Nevertheless, their system utilized water exchange every 10 days, which included the microalgae supply of 0.01 g L^{-1} (equivalent to 2 g per pond per day). Here it was utilized the maximum concentration of 0.78 g L^{-1} (equivalent to 0.78 g per pond per day) (2000 mL of microalgae solution per pond per day; Table 1).

Evaluation of water quality

Table 4 presents the profile of the physical and chemical parameters of the water during two stages of the experimental trial. In the initial stage, the water in all ponds met the appropriate standards outlined in Resolution 357 (Brazil, 2005). This condition persisted until the end of the experiment, with only one exception.

By the end of the experiment, water in T1 was associated with low levels of dissolved oxygen, falling below the recommended thresholds. This is likely due to T1 ponds receiving twice the amount of commercial feed compared to treatments involving commercial feed (T2, T5, and T6). The concentration of dissolved oxygen (DO_2 (mg L^{-1})) may gradually decrease with increased feed input. In terms of nitrite (NO_2^-), treatments exhibited critical levels in the final stage of the experiment, except for T1.

Microbiological indicators of water quality revealed the presence of thermotolerant coliforms in all treatments at the end of the experiment. Regarding fecal coliforms (*E. coli*) and mesophilic bacteria, treatments involving commercial feed (T1, T2, T5, and T6) exhibited the highest values, ranging from 10^4 to 10^5 CFU 100 mL^{-1} (Table 5).

Table 2. Specific growth rate, total weight gain, survival rate, and condition factor of Nile tilapia juveniles during 14 days of cultivation.

Treatment	Specific growth rate (% day^{-1})	Weight gain (% day^{-1})	Survival rate (%)	Condition factor (g cm^{-3})
T1	0.34 (± 0.29)^{b}	4.80 (± 3.1)^{b}	86.7^{a}	8.96^{a}
T2	3.80 (± 0.37)^{a}	53.33 (± 5.25)^{a}	96.7^{a}	8.42^{ab}
T3	-3.26 (± 0.60)^{c}	-45.70 (± 8.41)^{c}	93.3^{a}	7.11^{c}
T4	-2.18 (± 0.52)^{c}	-30.55 (± 7.40)^{c}	93.3^{a}	7.58^{c}
T5	-0.19 (± 0.60)^{b}	-2.72 (± 8.48)^{b}	83.3^{ab}	7.67^{bc}
T6	2.69 (± 0,58)^{a}	37.77 (± 8.14)^{a}	53.3^{b}	8.64^{ab}

Different letters in the same column differ (Tukey test, 5% significance level). Treatments are according to Table 1.

Table 3. Specific growth rates (% day^{-1}) of juvenile Nile tilapia depending on treatment (feed), time period (days), and culture system reported elsewhere.

Feed	Time (days)	System	Specific growth rates (% day^{-1})		Reference
			Min.	Max.	
Microalgae supplementation	28	pond/constant aeration	0.21	0.35	Costa et al. (2011)
Organic wheat	15	pond/constant aeration	0.3	0.3	Lui et al. (2012)
Alternative protein	180	pond/constant aeration	1.0	1.1	Assano et al. (2011)
Commercial fed (CF)	29	pond/constant aeration	1.34	1.82	Coêlho et al. (2014)
Agroindustrial byproducts	60	cages/water recirculation	2.03	2.27	Carvalho et al. (2012)
CF and enzyme complex	62	pond/water recirculation	2.28	2.37	Signor et al. (2010)
Agroindustrial byproducts	70	pond/constant aeration	3.43	3.96	Workagegn et al. (2014)

Table 4. Physical and chemical parameters of water depending on feed and the presence of microalgae and aeration in Nile tilapia culture systems.

Treatment	pH		DO_2 (mg L^{-1})		NH_3 (mg L^{-1})		NO_2^- (mg L^{-1})	
	Day 1	Day 14	Day 1	Day 14	Day 1	Day 14	Day 1	Day 14
T1	7.2	6.6	7.9	8.3	< LD	0.02	< LD	0.2
T2	7.2	6.2	7.9	7.9	< LD	0.001	< LD	2.8
T3	7.2	6.2	7.9	8.0	< LD	0.02	< LD	2.8
T4	7.2	6.2	7.9	7.9	< LD	0.02	< LD	2.8
T5	7.2	6.6	7.9	7.9	< LD	0.003	< LD	2.8
T6	7.2	6.4	7.9	7.9	< LD	0.02	< LD	2.8
Rv	6.0-9.0		≥ 5 mg L^{-1}		≤ 2.0 mg L^{-1}		≤ 1.0 mg L^{-1}	

< LD = Values below the limit of detection. RV = reference values (guidelines of CONAMA 357/05); pH = hydrogen potential; DO_2 = dissolved oxygen; NH_3 = toxic ammonia; NO_2^- = nitrite. Treatments are according to Table 1.

Table 5. Counts of mesophilic total bacteria present in the water from ponds used to raise tilapia fish under different experimental conditions.

Treat-ment	TTC		*E. coli*	Mesophilic bacteria
	24 h	48 h	(CFU 100 mL^{-1})	(CFU 100 mL^{-1})
T1	+	+	$6.06 \times 10^{4\,a}$	$8.67 \times 10^{4\,c}$
T2	-	+	$7.00 \times 10^{4\,a}$	$4.70 \times 10^{5\,a}$
T3	+	+	$1.45 \times 10^{4\,b}$	$5.20 \times 10^{4\,d}$
T4	-	+	$1.04 \times 10^{4\,b}$	$6.50 \times 10^{4\,d}$
T5	+	+	$1.80 \times 10^{4\,b}$	$2.28 \times 10^{5\,b}$
T6	+	+	$1.30 \times 10^{4\,b}$	$2.23 \times 10^{5\,b}$

TTC: total thermo-tolerant coliforms after 24 and 48 h (+ presence or - absence); CFU: Colony-Forming Unit. *Means followed by the same letter do not differ by the Tukey test at the 5% probability level. Treatments are according to Table 1.

The level of dissolved oxygen (DO_2) in ponds with low water renewal is likely the major constraint in tilapia production. Thus, monitoring the feed ratio and regularly testing for dissolved oxygen levels is advisable. Tilapia can survive at low levels of dissolved oxygen as long as temperature, pH, and stocking density remain favorable (Caldini et al., 2013; Abdel-Tawwab et al., 2015).

High levels of nitrite can challenge the immune system of fish, leading to diseases and sudden death (Kroupova et al., 2005). Surprisingly, the nitrite levels in this study were at least twice as high as the reference values (RV) (Table 2). However, the reported levels of nitrite in the literature vary significantly and are frequently higher than the recommended values. Pereira and Lapolli (2009), Gorlach-Lira et al. (2013), Coêlho et al. (2014), and Lima et al. (2016) found lower values ranging from 0.1-2.0, 0.004-0.005, 0.0-0.27, and 0.26-0.46 mg L^{-1}, respectively. Interestingly, Santos et al. (2013) reported higher values ranging from 1.03-3.61 mg L^{-1} in cultivation systems using water recirculation.

Overall, similar to the results observed in this study, the data reported in the literature show a discreet association between high nitrite values and mortality or weight trends in tilapia (Pereira and Lapolli, 2009; Gorlach-Lira et al., 2013; Santos et al., 2013; Coêlho et al., 2014; Lima et al., 2016). However, this association may not be straightforward, limiting our understanding and comparisons between the cultivation strategies.

Recirculation systems are often more effective in controlling nitrite levels in fishponds. However, these systems require larger amounts of water, which can increase environmental footprints. Exploring alternatives to remove nitrite from the water may enhance production while also limiting the adoption of technology for family farmers. On the other hand, biotransformation techniques such as denitrification may reduce the investment required to mitigate the toxicity of effluents derived from tilapia production.

As per the reference values established in Resolution 357 (Brazil, 2005), confined animals should not be exposed to thermotolerant coliforms beyond 1000 per 100 milliliters (1.0×10^3 CFU 100 mL^{-1}); *Escherichia coli* may be used as a substitute for thermotolerant coliforms based on the limits set by the competent environmental agency. The contamination of aquaculture systems and fish by human pathogens such as *E. coli* is usually attributed to infected handlers or storage (Rocha et al., 2014; Dewi et al., 2022).

The relatively high bacterial contamination observed in the experiment reflects the nature of fish farming conducted in ponds, which involves a considerable volume of dietary inputs in a relatively small area (Gorlach-Lira et al., 2013). Bacterial contamination was higher than the values recorded by Ahmed and Naim (2003) in tilapia culture ponds and by Gorlach-Lira et al. (2013) in tilapia culture floating net cages, ranging from 10^3-10^4 and 10^4-10^5 CFU 100 mL^{-1}, respectively. The results were similar to the values reported by Ntengwe and Edema (2008) in tilapia culture ponds (10^6 CFU 100 mL^{-1}).

However, bacterial contamination is comparable to the critical values observed for dissolved oxygen and nitrite. The low levels of dissolved oxygen may be attributed to the decomposition of leftover feed (excess of commercial feed). The levels of total mesophilic aerobic bacteria support the evaluation of water in terms of decomposition activity (Gorlach-Lira et al., 2013). Microbiological decomposition requires dissolved oxygen from the water, which can lead to critical levels of DO_2 that threaten the survival of fish. High nitrite values may result from this type of decomposition. Lima et al. (2016) observed that higher loads of organic matter are associated with greater amounts of total ammonia nitrogen, which serves as a substrate for *Nitrossomonas* to release nitrite into the water.

CONCLUSION

In conclusion, this study examined the growth performance and water quality parameters of Nile tilapia (*Oreochromis niloticus*) in different culture systems. The results demonstrated the significant impact of various factors, particularly the type of food provided, on the growth and development of the fish. The treatments combining commercial feed with aeration (T2) and commercial feed with microalgae supplementation and aeration (T6) showed the highest weight gain rates, while treatments without commercial feed exhibited lower weight gains. The specific growth rates of tilapia in T2 and T6 were comparable to those reported in the literature for juveniles. Proper monitoring and management of water quality, including nitrite levels and dissolved oxygen, played a crucial role in the survival and growth of Nile tilapia.

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Research Article

The Relationship of Bristle Worm, *Protodorvillea kefersteini* (McIntosh, 1869) (Eunicida, Dorvilleidae) Abundance with Environmental Variables in Çardak Lagoon (Turkish Straits) Exposed to domestic Discharge

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ABSTRACT

This study presents the correlations between opportunistic polychatea, *Protodorvillea kefersteini* (McIntosh, 1869) abundance, and environmental variables in Çardak Lagoon. Samplings were carried out on the bottoms using a 30x30 cm quadrate seasonally between 1 and 1.8 m depths of seven sampling points in October 2018, February, April, and June 2019. A total of 1094 specimens belonging to *P. kefersteini* were collected. Environmental variables such as gravel content in sediment, pH, and salinity levels in the water had the highest correlations with the abundance through the sampling periods. Considering the sampling points, the highest correlation value was between water salinity and the abundance. Sediment gravel content, pH, salinity, temperature, anionic surfactant levels, and NO_2+NO_3 were major environmental variables affecting *P. kefersteini* abundance in the study area spatially and temporally.

Keywords: *Protodorvillea kefersteini*, abundance, environmental variables, Çardak Lagoon, Turkish Straits

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INTRODUCTION

Anthropogenic environmental variables have negative effects on marine biodiversity, especially on some benthic communities dominant in polluted environments that are sensitive to disturbances (Santos *et al*. 2021). Sudden daily and seasonal fluctuations in the environmental variables in marine systems, mainly in lagoon areas, may change the abundance of benthic organisms (Koutsoubas *et al*. 2000). Water movement, dissolved oxygen concentration, salinity, sediment grain size, and organic matter content effectively affect the polychaeta abundance and distribution in marine environments (Guerra-Garcia & Garcia-Gomez 2004). Among these, organic matter accumulation in sediment with anthropogenic origin controls the macrofaunal communities (Magni *et al*. 2004). Sewage discharges are one of the most common anthropogenic disturbances on marine benthos. There is important information in the relevant literature on the temporal and spatial status of benthic assemblages, especially on conspicuous disturbances caused by organic enrichment in sediments (Del-Pilar-Ruso *et al*. 2009). Opportunistic macrozoobenthic species adapt easily to a new marine environment and can form dense populations in a short time (Carlton 1985).

Polychaetas with high sensitivity to changes on soft bottoms (Del-Pilar-Ruso *et al*. 2009) may be preferred for monitoring studies (Muxika *et al*. 2005). Some polychaetas are also highly tolerant to pollution and low oxygen stress caused by excessive accumulation of organic matter. Besides, they are important in detecting the effect of pollutants between water and sediment (Elias *et al.* 2003). Additionally, they can tolerate high organic matter content in marine soft bottoms (Fernández-Romero *et al.* 2019) and play an important role in the bioturbation of marine sediments (Hutchings 1998).

Effects of pollutants on polychaetes were previously studied by many researchers (Karakassis *et al.* 2000; Warwick 2001; Ergen *et al.* 2004; Como *et al.* 2007; Afli *et al.* 2008; Dauvin 2008; Zaaiba *et al.* 2009; Terlizzi *et al.* 2010; Zaâbi *et al.* 2010; Martins *et al.* 2013; Zaâbi-Sendi 2013; Cabral-Olivera *et al.* 2014; Hamdy *et al.* 2023). Those dorvilleid polychaetes are known as opportunistic communities in marine sediments enriched with organic matter (Alalykina & Polyakova 2022). Dorvilleid polychatea, *Protodorvillea kefersteini* which is an important biotope descriptive and pollution indicator (MES 2010), is one of the 14 known species of the genus, *Protodorvillea* (Worms 2023). *P. kefersteini* is a species that is 1-3 cm long, lives on mud, gravel, or sandy bottoms at depths of 10-30 m, and mostly uses empty tubes of serpulids under stones. *P. kefersteini* is distributed in the North Atlantic to the North Sea and English Channel, Mediterranean, and Black Sea (Tillin 2016). *P. kefersteini* is known from the Mediterranean (Núñez *et al.* 2013; Çınar *et al.* 2015; Mikac 2015) and the Black Sea (Kurt Şahin *et al.* 2017; Kopiy 2018). This study presents the relationships between *P. kefersteini* abundance and environmental variables measured in Çardak Lagoon affected by domestic discharges.

MATERIALS AND METHODS

The sampling area included the depths of 1 to 1.8 m of 7 different sampling points (GPS Coordinates: 40 ° 23'14 "K, 26 ° 43'30" D) chosen in Çardak Lagoon located in the northeast of Çanakkale Strait (Figure 1).

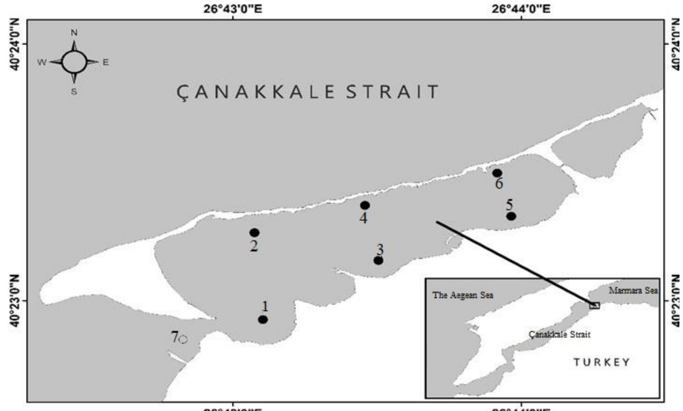

Figure 1. Map of the study area showing sampling points.

Benthos material including *Protodorvillea kefersteini* specimens was collected by a SCUBA diver with a 30x30 cm metal framed quadrat system in October 2018, February, April, and June 2019. Samples were fixed in 4% neutralized formaldehyde in 5 L plastic bottles. In the laboratory, the bottom samples were washed with the help of pressurized water and passed through a triple sieve system with apertures of 0.5, 1, and 2 mm. Faunal species remaining on the sieves were extracted at macro and micro levels and fixed in 70% ethanol in 50 cc glass tubes on a group basis. *P. kefersteini* specimens were identified on the trinocular stereomicroscope according to definitions based on previous studies, and all were counted. The definitions of Fauchald (1977) and Fauchald and Rouse (1997) were used for diagnosis.

The correlations between values of environmental variables and *P. kefersteini* abundance recorded for 7 different sampling stations in 4 seasons were calculated according to Pearson coefficient correlation (r) in the PAST program.

Water quality variables of lagoon water were measured in *situ* using a YSI 650 MDS. The number of nutrients (NO_2, NO_3, NH_4, PO_4-P, SiO2) and total suspended solids (TSS) in the lagoon water were measured based on the analysis method proposed by Strickland and Parsons (1972) at different wavelengths in the spectrophotometer. Analyses were made using the Jasco Brand UV spectrophotometer in the Laboratory of the Faculty of Marine Sciences and Technology. Analyses of % organic matter and % particle content in the sediment were performed in Çanakkale Onsekiz Mart University Central Laboratory. Particle size analyses in the sediment were done according to Allen (1997).

RESULTS

A total of 1094 specimens belonging to *P. kefersteini* were sampled in the study area (Figure 2).

Figure 2. General view of *Protodorvillea kefersteini* (Photographed by E. Dağlı).

Although it is known that *P. kefersteini* is tolerant to pollution, reference station (Stn. 7) with low sediment organic matter content and water chl.-*a* amount (OM%= 1.73% and chl.-*a*= 1.49 µg L^{-1}, respectively) was the most abundant in terms of specimen number (1001 out of a total of 1094 specimens) in the study area. Except for October 2018, *P. kefersteini* showed a regular distribution in terms of specimen number in other sampling periods (the lowest 34 individuals, di%= 3.10) (Table 1).

Considering the relationships between environmental variables and *P. kefersteini* abundance, the highest positive correlation (r= 0.93; p <0.05) was found between *P. kefersteini* abundance and

Table 1. Mean water quality, nutrient, chl-a, total suspended solids (TSS), chemical oxygen demand (COD), anionic surfactant (AS), and sediment variables values measured at sampling periods and points.

Sampling period	A	OM (%)	WOM (mg L^{-1})	T (°C)	S (‰)	O_2 (mg L^{-1})	ORP	pH	Chl.-a (µg L^{-1})	SiO_2
Autum 2018	34	6.42	11.02	14.76	20.77	8.07	80.41	8.14	3.79	0.34
Winter 2019	338	5.98	12.2	7.99	23.46	7.45	-83.37	8.15	1.19	0.39
Spring 2019	382	6.61	10.11	15.5	22.53	6.48	-90.98	8.31	1.83	0.17
Summer 2019	340	6.76	11.48	25.66	21.04	7.83	-100.52	8.29	5.31	0.83
Mean		6.44±0.15	11.2±0.38	15.98±3.15	21.95±0.55	7.46±0.30	-48.62±37.3	8.22±0.04	3.03±0.81	0.43±0.12
Sampling point										
Station 1	58	10.65	11.1	12.53	22.45	8.19	-27.63	7.89	3.63	0.36
Station 2	7	3.03	11.65	13.12	22.35	7.95	-31.6	8.32	2.64	0.56
Station 3	7	**15.52**	11.62	12.4	22.13	6.9	-31.23	8.14	3.38	0.29
Station 4	20	3.49	10.85	12.68	22.35	6.93	-30.16	8.27	2.96	0.57
Station 5	1	7.91	11.2	12.41	22.36	6.74	-35.23	8.22	2.59	0.55
Station 6	-	2.75	11.17	12.93	21.87	6.93	-35.1	8.27	4.47	0.18
Station 7 (ref.)	1001	1.73	10.85	13.1	22.66	7.7	-31.16	8.29	1.49	0.48
Mean		6.44±1.79	11.2±0.11	12.73±0.10	22.31±0.08	7.33±0.20	-31.73±0.94	8.2±0.05	3.02±0.32	0.42±0.05
Total number	**1094**									

Table 1. Continue.

Sampling period	TP	NO_2+NO_3 (mg L^{-1})	TN	TSS (mg L^{-1})	COD (mg L^{-1})	AD (mg L^{-1})	PO_4 (mg L^{-1})	Sand (%)	Gravel (%)	Mud (%)
Autum 2018	0.02	0.13	0.61	9.66	197.83	0.027	0.01	75.36	16.56	8.11
Winter 2019	0.02	0.09	0.23	35.28	178.71	0.029	0.01	69.29	19.9	10.74
Spring 2019	0.04	0.06	0.41	13.57	80.75	0.051	0.01	68.87	23.14	7.96
Summer 2019	0.09	0.03	0.21	6.64	74	0.032	0.02	72.91	21.1	5.95
Mean	0.04±0.01	0.08±0.02	0.37±0.08	16.29±5.62	132.82±27.95	0.03±0.0	0.01±0.0	71.61±1.34	20.18±1.19	8.19±0.85
Sampling point										
Station 1	0.048	0.083	0.48	11	127.87	0.045	0.03	61.8	24.1	14.16
Station 2	0.029	0.071	0.23	8.07	152.12	0.027	0.02	78.6	18.1	3.17
Station 3	0.026	0.071	0.42	22.15	126.66	0.032	0.02	55.2	31.6	13.24
Station 4	0.035	0.036	0.16	9.35	103.33	0.04	0.02	86.2	8.49	5.27
Station 5	0.051	0.089	0.49	19.07	201	0.034	0.01	57.6	25.6	**16.71**
Station 6	0.065	0.089	0.49	28.41	148.25	0.035	0.03	**92.8**	3.98	3.29
Station 7 (ref.)	0.053	0.094	0.26	15.98	163	0.032	0.01	69.1	**29.4**	1.49
Mean	0.04±0.004	0.07±0.006	0.36±0.04	16.29±2.60	146.03±10.95	0.035±0.002	0.02±0.002	71.61±5.10	20.18±3.68	8.19±2.19
Total number										

average % gravel content in sediment for the sampling periods. For sampling points, the highest correlation (r=0.64; p <0.05) was between abundance and salinity. There were positive correlations between *P. kefersteini* abundance and seawater pH, salinity, and anionic detergent amount (r= 0.67, 0.62, 0.58; p <0.05, respectively) in the sampling points. The weakest positive relationships were between the % of mud content in sediment and the amount of organic matter in water and sediment for the sampling points (r= 0.01, 0.02, 0.09; p <0.05, respectively) (Table 2).

Table 2. Correlations between total abundance and mean environmental variables for all sampling periods and sampling points (Pearson coefficient correlation, $p < 0.05$).

	Sampling periods	Sampling points
EV	**A (ind. 0.09 m^{-2})**	**A (ind. 0.09 m^{-2})**
OM (%)	0.09	-0.39
WOM (mg L^{-1})	0.02	-0.50
Temperature (°C)	0.10	**0.50**
Salinity (‰)	**0.62**	**0.64**
O_2 (mg L^{-1})	-0.68	0.31
ORP	-0.99	0.14
pH	**0.67**	0.22
Chl.-α (μg L^{-1})	-0.31	-0.72
SiO_2	0.13	0.15
TP	0.43	0.28
NO_2+NO_3 (mg L^{-1})	-0.82	**0.39**
TN	-0.81	-0.31
TSS (mg L^{-1})	0.30	-0.05
COD (mg L^{-1})	-0.71	0.21
AD (mg L^{-1})	**0.58**	-0.18
PO_4 (mg L^{-1})	0.28	-0.52
Sand (%)	-0.84	-0.09
Gravel (%)	**0.93**	**0.39**
Mud (%)	0.01	-0.46

A: Abundance, EV: Environmental variable, OM: Organic matter in sediment, WOM: organic matter in water, ORP: Oxygen reduction potential, TP: total phosphate, TN: Total nitrogen, TSS: Total suspended solids, COD: Chemical oxygen demand, AD: Anionic detergent.

DISCUSSION

Effects of environmental variables such as temperature and salinity were previously studied at the population level in opportunistic polychaetas such as *Capitella* sp., *Ophryotrocha diadema*, and *Streblospio benedicti* in polluted areas (Simonini & Prevedelli, 2003). Based on the previous studies regarding *P. kefersteini* which is another opportunistic polychaeta, *P. kefersteini* is a recognizable species in areas affected by hypoxia (Leonhard 2006). Similarly, Warwick *et al.* (1986) stated that *P. kefersteini* is abundant in organic matter-rich habitats with sewage discharge. Hiscock *et al.* (2004) also indicated that *P. kefersteini* is an increasingly abundant species on sea bottoms where slight organic enrichment is observed. *P. kefersteini* abundance on the organic matter-rich bottoms of the Mediterranean and its relationship with other environmental variables were previously studied (Karakassis *et al.* 2000; Ergen *et al.* 2004; Como *et al.* 2007; Afli *et al.* 2008; Zaaiba *et al.* 2009; Terlizzi *et al.* 2010; Zaâbi *et al.* 2010; Martins *et al.* 2013; Zaâbi-Sendi 2013; Hamdy *et al.* 2023). Among these studies, Karakassis *et al.* (2000) found that *Protodorvillea kefersteini* and *Cirrophorus lyra* were dominant (more than 20% of the total abundance) on the bottoms of fish farms established at the Sounion coast (eastern Greece) at a depth of 13-20 m, with 80% silt and high carbon content.

Similarly, Ergen *et al.* (2004) found dense populations of *P. kefersteini* (3060 ind. m^{-2}) on the polluted or semi-polluted bottoms of cage farms in the eastern Aegean Sea. In addition, regarding organic matter content in sediment, Como *et al.* (2007) found that *P. kefersteini* dominated the inlet area of Oristano Bay (Sardinia, Italy) with high organic matter content (mean value of 25% of the total sediment dry weight). Terlizzi *et al.* (2010) also observed *P. kefersteini* in abundance on mud-character bottoms rich with organic matter where fish cages are located, approximately 100 to 500 m offshore of Corsica Island (France, Mediterranean). On the contrary, Hamdy *et al.* (2023) recorded *P. kefersteini* only on the bottoms with low organic matter content (max. 1.25%) off the coast of Alexandria (Egypt, Mediterranean). Considering the sediment type depending on the sediment grain size, Zaaiba *et al.* (2009) found *P. kefersteini* to be the most dominant (1900 ind. m^{-2}) at Cap Bon Peninsula coasts (northern Tunisia) in the bottoms where the sediment-gravel ratio varies between 0.2 and 60.6%. *P. kefersteini* is known as of the few polychaeta species that preferred the bottoms with a coarser particle in the Bizerte Lagoon (southern Tunisia) (Afli *et al.* 2008). The Bizerte Lagoon example presented for *P. kefersteini* specimens was observed in this study. We found the highest number of individuals on the bottoms where the average sand+gravel content was the highest and mud content was the lowest. Similarly, Zaâbi-Sendi (2013) stated that *P. kefersteini* was well represented in coarse sand bottoms where the coarse fraction between 500 and 630 μm dominates the Tunisia coasts. Further, *P. kefersteini*, characteristic of the fine and coarse sandy bottoms of Cap Bon Peninsula coasts, was abundant in all seasons with an average abundance of 66 ± 32.73 (ind. m^{-2}) (Zaâbi *et al.* 2010).

Considering the northeastern Atlantic specimens of *P. kefersteini*, the species was defined as a characteristic species for Portugal coast bottoms with a mean depth of 50 m, 68.8% sand, 27.2% gravel, and 1% mean organic matter content (Martins *et al.* 2013). Lourido *et al.* (2008) also found *P. kefersteini* in abundance on the bottoms of the Ria de Aldan coasts (northwest Spain) with an average content of 1.46% organic matter, 19.57% gravel, and 78.06% sand. On the other hand, although *P. kefersteini* prefers shallow waters as its habitat, it was also recorded at depths between 984 and 1113 m in Capbreton Canyon (Biscay Bay, NE Atlantic) (Aguirrezabalaga & Ceberio 2003). Furthermore, *P. kefersteini* was dominant in surface waters with an average redox potential (ORP) value of +61 ± 177 mV on the western Scottish coast (Pearson & Stanley 1979). In this study, no significant relationship was found between ORP and *P. kefersteini* abundance.

Considering only *P. kefersteini* abundance, Kopiy (2018) observed *P. kefersteini* on Sevastopol coasts (Crimea, Black Sea) throughout the year. Besides, there are many studies regarding polychaeta assemblages including *P. kefersteini* in Turkish Seas. Among these, Çınar *et al.* (2011) found *P. kefersteini* was most abundant (2900 ind. m^{-2}) at 17 m depth of Erdek coasts (the southern Marmara Sea). Then, Kurt Şahin *et al.* (2017) recorded *P. kefersteini* (8675 ind. m^{-2}) during spring in fine particle sand bottoms at 3 m depths of the Sinop Peninsula (southern Black Sea) being the most abundant species.

Apart from all these studies, a study examining polychaeta communities and their relationship with environmental variables was conducted by Can Yılmaz (2009) in Homa Lagoon (Turkish Aegean Sea). Can Yılmaz (2009) has found negative correlations between polychaeta abundance and sediment surface temperature in winter, and pH and abundance in spring (p= -0.730; p= -0.782; p<0.05, respectively) in Homa Lagoon. Can Yılmaz (2009) has also stated that the environmental variable that shows a positive correlation with polychaeta abundance (p= 0.697; p<0.05) in Homa Lagoon was % sand content in the sediment for the summer period. In this study, on the contrary, while % sand content of bottoms in the study area was negatively correlated with abundance temporally and spatially, seawater salinity moderately affected *P. kefersteini* abundance both temporally and spatially. In addition, very weak positive and negative correlations were recorded between *P. kefersteini* abundance and % sediment organic matter content in this study. This may be because the abundance of *P. kefersteini* was greatest at the reference point outside the lagoon area with very low organic matter bottoms.

Our study did not agree with the results regarding the correlation between *P. kefersteini* abundance and % sediment organic matter content presented by Warwick *et al*. (1986), Ergen *et al*. (2004), and Como *et al*. (2007). On the contrary, findings by Hiscock *et al*. (2004), Lourido *et al*. (2008), Martins *et al*. (2013), and Hamdy *et al*. (2023) were supported in the present study. Moreover, based on our results, we may point out that excessive accumulation of organic matter in the sediment may not be a major environmental variable that positively affects *P. kefersteini* abundance since the abundance was the greatest in the sediment with the lowest % organic matter content in the study area.

CONCLUSION

Sediment gravel content is one of the most important environmental variables that positively correlated with *P. kefersteini* abundance temporally. According to the results of this study and other studies performed on the Mediterranean and eastern Atlantic coasts, we may state that salinity, temperature, sediment particle size, and % sediment organic matter content had the most effect on *P. kefersteini* abundance. Although *P. kefersteini* is known as a pollution indicator species, we think that it cannot be concluded that the sediment of an area is polluted based only on *P.kefersteini* abundance.

Conflict of Interests: The authors declare that they have no financial interests or personal relationships that could affect this work, hence no conflict of interest.

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Research Article

Validity of Visible Ectoparasite Intensity As a Non-invasive Biomarker for Fish welfare: Parasitic Copepod, *Lernantropus kroyeri* in Sea Bass As an Example

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ABSTRACT

Ensuring fish welfare is essential from the ethical, legal, environmental, economic, and social perspectives. It plays a vital role in maintaining the health and sustainability of aquaculture practices while respecting the intrinsic value and welfare of the fish themselves. The presence of reliable welfare assessment schemes is of utmost importance to appraise the well-being of animals in aquaculture and uphold stringent welfare standards. In determining fish welfare, conducting welfare assessments with non-invasive biomarkers is crucial thus the primary objective of this study is to explore the potential usability of visible parasites as welfare biomarkers in fish without causing any harm to the fish. In this research, certain secondary stress indicators (hematocrit, plasma glucose and lactate) were employed as biomarkers for assessing the well-being of European sea bass (*Dicentrarchus labrax*). The study aimed to investigate whether there is a possible correlation between the presence of visible ectoparasites on the gills (specifically, the Copepod parasite, *Lernantropus kroyeri*) and the aforementioned stress parameters. Thus, in this study, the examination was conducted to establish the validity of ectoparasites as non-invasive biomarkers for evaluating the welfare of fish. The results showed that there was a statistically significant relationship between the intensity of ectoparasites and the stress parameters used as indicators of welfare. The observable presence and intensity of ectoparasites on the gills of the fish can be proposed as a non-invasive biomarker for evaluating fish welfare in aquaculture.

Keywords: Fish welfare, non-invasive biomarker, ectoparasite intensity, validity

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INTRODUCTION

The importance of animal welfare in the aquaculture industry, which is responsible for producing 52% of the fish consumed by humans (FAO, 2022) has started to be better understood. Poor welfare conditions in aquaculture can lead to negative outcomes for the environment, animal well-being, and global food security. By promoting fish well-being, the aquaculture industry can contribute to improving sustainability and fish food safety, which is beneficial for the environment and human health. Therefore, it is crucial to ensure sustainable growth in aquaculture while prioritizing fish welfare.

Non-invasive biomarkers for assessing fish welfare have gained significant attention in recent years. These biomarkers allow for the evaluation of fish well-being without causing unnecessary stress or harm to the animals. The need for non-invasive fish welfare biomarkers arises from the increasing concern and recognition of the ethical and practical importance of fish welfare in various industries, including aquaculture, fisheries, and research. Fish are sentient beings capable of experiencing pain, stress, and suffering, and as such, there is a growing responsibility to ensure their well-being and minimize any potential harm they may experience (Garrath & McCulloch, 2022). Assessing fish welfare has been challenging due to their environment and the fact that they are not as expressive as some land animals. Many conventional welfare assessment methods involve capturing, handling, or even sacrificing fish for analysis, which can cause additional stress and harm to the animals (Browning, 2023). Non-invasive biomarkers offer a more humane and sustainable approach to monitoring and assessing fish welfare. These biomarkers can be indicators of physiological, biochemical, or molecular changes in fish, providing valuable insights into their well-being without causing harm or stress. For example, cortisol concentration in fish skin mucus has been proposed as a non-invasive technique to assess fish welfare and stress (Guardiala et al., 2016; Carbajal et al., 2019). Similarly, the cutaneous stress response system (CSRS) in fish skin has been reported as a new source of information on the welfare status of farmed fish (Kulcykowska, 2019). Although not strictly biomarkers, observing fish behavior can provide valuable insights into their welfare. Non-invasive methods such as video recording or automated image analysis can be used to monitor behaviors such as feeding patterns, swimming activity, aggression, or abnormal behaviors, which can indicate stress or welfare issues (Martins et al., 2012). The establishment of standardized indicators and assessment of welfare needs in aquaculture has been an ongoing discussion (Segner et al., 2019; Magalhães et al., 2020; Barreto et al., 2021; Yavuzcan Yildiz et al., 2021). The use of welfare indicators provides crucial insights into fish welfare, reflecting the extent to which their needs are met. These indicators should possess characteristics such as usability, reliability, scalability, recognizability, minimal damage, and feasibility within a reasonable timeframe. An ideal assessment framework should encompass both operational and laboratory-based welfare markers (Segner et al., 2019; Browning, 2023).

Fish health assessments are one component of on-farm welfare appraisals, particularly in intensive aquaculture systems. Segner et al. (2012) explained that good welfare is reflected in the ability of the fish to cope with various stressors, thereby maintaining homeostasis associated with good health, while stressful aquaculture conditions will cause the loss of coping capacity and impaired health. The welfare status of fish, as measured by the indices of stress can be unveiled. The biochemical and physiological changes result from the effects of the factors released during the primary stress response (Ellis et al., 2012; Schreck & Tort, 2016). The secondary stress response refers to the physiological and behavioral adaptations that occur in response to stressful conditions. These adaptations involve the activation of various metabolic pathways, leading to significant changes in blood chemistry, hematology, respiration, acid-base balance, and ion losses in the gills (Iwama, 2007). Hematocrit, blood lactate, and glucose are considered secondary stress biomarkers in fish. These biomarkers are used to assess the physiological responses of fish to stress and provide valuable information about their overall well-being and health (Seibel et al., 2021).

It is generally recognized that parasitic diseases in fish are one of the most important indicators in relation to fish health (Segner et al., 2012; Stien et al., 2013; Bui et al., 2019). The visible parasites are considered in operational indicators and assessment of fish welfare (Bui et al., 2019), and visible ectoparasites can provide insights into fish well-being without necessarily causing the death of the fish. Thus, the study by Overli (2014) has contributed to our understanding of the relationship between parasites, stress, and welfare. This study revealed that infestation by sea lice led to increased brain stem levels of the 5-Hydroxytryptamine(5-HT) catabolite 5-Hydroxyindolaceticacid (5-HIAA) in Atlantic salmon, indicating a general stress response in infested fish. It was also found that infected fish showed depressed feeding and reduced locomotion, suggesting a negative impact on animal welfare.

The European sea bass (*D. labrax*), one of the most commonly farmed fish in the Mediterranean, faces challenges from the ectoparasite Lernanthropus (Yavuzcan Yildiz & Korkmaz, 2021). *Lernanthropus kroyeri*, belonging to the genus of parasitic copepods, is visible in the gills of sea bass and causes several pathologies such as erosion and necrosis on gill filaments (Henry et al., 2009). The parasite *L. kroyeri* in sea bass has been examined in various studies (Tokşen, 2007; Er&Şevki, 2015; Özak et al., 2016; Yavuzcan Yıldız & Korkmaz, 2021), however, this parasite has not been studied as a welfare biomarker till now. Yet, the easy visibility of Lernantropus to the naked eye may provide the basis for its potential use as a biomarker for European sea bass.

The development and implementation of non-invasive fish welfare biomarkers are essential for ensuring the ethical treatment of fish, promoting sustainable practices in the aquaculture industry, and enhancing our understanding of fish welfare in various settings. Despite the extensive attempts made towards the authentication of welfare indicators that are specific to each species, the identification of suitable combinations of these measures still proves to be limited for the majority of farmed species as stated by Magalhães et al. (2020).

In this study, the aim was to validate the potential use of visible gill parasites (*L. kroyeri*) as the non-invasive biomarker of fish welfare by correlating them with key stress parameters such as hematocrit, blood glucose, and lactate.

MATERIAL AND METHODS

The fish examination was done immediately after harvesting on a commercial farm in the Aegean Sea. Fish samples ($N = 37$) were randomly selected among the harvested batch from one cage in the autumn. The weight of European sea bass (*D.labrax*) was around 350 g. During the harvesting period, the water temperature was measured as 23 °C and the dissolved oxygen level as 6.5 mg/L.

Blood samples were collected promptly upon the taking of the fish out. Prior to blood sampling, the fish underwent a brief anesthetic procedure involving a clove oil solution (5 mg/L) for a duration of 5 minutes. Heparinized syringes were utilized to collect blood via the cardiac puncture. Hematocrit measurements were conducted immediately following blood sampling. Plasma was obtained by centrifugation of heparinized blood at 3000*g* for 10 min and pooled where necessary. The plasma was then preserved at a temperature of -18°C until further analysis. Commercial kits (Cayman Chemicals, USA) and a Shimadzu UV-1210V spectrophotometer were used to measure plasma glucose and lactate, in accordance with the manufacturer›s instructions.

The copepod parasite *L. kroyeri* was carefully plucked from the gills by using forceps in the newly harvested fish (Figure 1). Parasites on both sides of the gills were counted on the gills with the naked eye (Figure 2). The sex of the parasite was not considered.

Statistical analyses: The variation in stress parameters was examined using an ANOVA test based on parasite counts. Regression analyses were conducted to test whether there is a significant relationship between parasite counts on the gills and secondary stress parameters (hematocrit, plasma glucose and lactate).

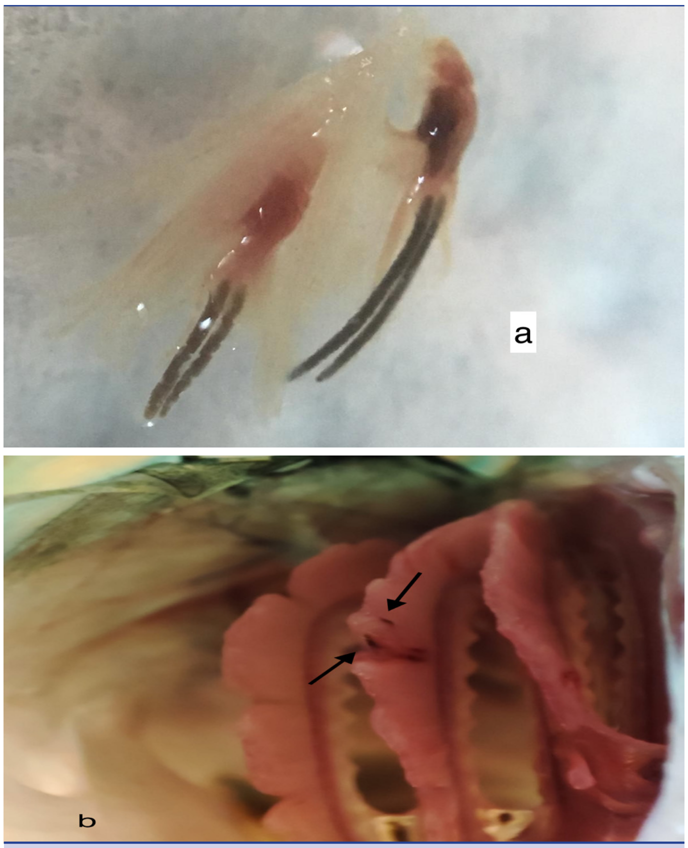

Figure 1. a) Copepod parasite, *Lernantropus kroyeri* b) *L. kroyeri* on the gills of European sea bass.

RESULTS AND DISCUSSION

In this study, the validation of visible ectoparasites (crustacean parasite, *L. kroyeri*) on the fish gills as a non-invasive fish welfare biomarker has been evaluated. The welfare status of the fish was assessed using the stress indicators as the concept of stress is closely connected to that of fish well-being (Galhardo & Oliveira, 2009). When an organism's coping mechanisms fail to adapt to its environment adequately, it leads to poor welfare (Broom, 2008). Stress can negatively impact fish health, growth, reproduction, and overall well-being of fish.

The prevalence of *L. kroyeri* on sea bass gills was 78.37%. Stress indicators (hematocrit, plasma glucose, and lactate) showed significant differences by the parasite *L. kroyeri* number in the gills of the fish ($p < 0.05$). The variation in the stress indicators was parasite number-dependent (Table 1).

Wells & Pankhurst (1999) noted that the persistence of altered states of glucose and lactate as well as hematological changes following stress are clearly-defined indicators of the stress response although the variations in these indicators may be considered high to observe chronic stress (Magalhães et al., 2020). The increased hematocrit is an indication of the increase in the oxygen requirement of the fish body. Under stressful circumstances, there exists a possibility for hematocrit values to exhibit an elevation, which is a compensatory mechanism aimed at augmenting the supply of oxygen to the organs as a response to the escalated metabolic demand (Fazio et al., 2015). Increased hematocrit levels have been reported for the hypoxic conditions in sea bream (Bermejo-Nogales et al 2014). Thus, here, the higher levels of hematocrit in sea bass with higher numbers of *L. kroyeri* can be explained by the disrupted oxygen balance in the gills. The increase in glucose and lactic acid levels in fish during stress reflects their physiological response to challenging conditions (Levy de Carvalho Gomes, 2007). These metabolic changes help the fish adapt to the stressor, providing the necessary energy for survival, but prolonged or severe stress can have negative effects on fish health and welfare (Wells & Pankhurst, 1999). Blood glucose serves as the primary energy fuel in stressful conditions (Wendelaar-Bonga, 1997) and blood lactate is produced as a metabolic byproduct during certain physiological conditions, including stress (Schreck & Tort, 2016). However, high blood lactate can limit oxygen transport to the tissues in fish (Olsen et al., 1992). Plasma glucose and lactate increased by the parasite numbers on the gills in a manner that is parasite number-dependent in this study. Here, the increase in parasite numbers being associated with an increase in hematocrit, plasma glucose, and lactate levels is an important finding of this study.

Table 1. The changes of stress indicators in sea bass by the *L. kroyeri* intensity.

L. kroyeri intensity in the gills	Frequency of parasitized fish (N_{total}=37)	Hematocrit (%)	Plasma glucose (mg/dL)	Plasma lactate (mg/dL)
0 (uninfested fish)	8	30.62*	67*	10.25*
1-3	9	33	83.88	15.33
4-10	11	35	91.72	17.18
>11	9	36.11	129.11	17.44

*refers the difference for the values in the column

The regression analysis resulted in a significant overall effect of the independent variables (parasite intensity on the gills) on the dependent variables; stress indicators including hematocrit, plasma glucose, and plasma lactate (Table 2).

The regression results for hematocrit provides a moderate-to-good explanation of the hematocrit variability (R^2=0.56 and F=44.75), however, the individual regression coefficient is statistically significant (p<0.01), suggesting that parasite number has some predictive power for hematocrit values of sea bass (Figure 2).

For plasma glucose, the regression model appeared to be highly significant (R^2=0.85 and F=200.45), indicating that the ectoparasite numbers have a substantial impact on the plasma glucose of sea bass (Figure 3).

The regression model was statistically significant (F = 36.41) and explains a moderate amount of the variability in the plasma lactate (R^2=0.50), indicating that the model has identified significance between parasite intensity and plasma lactate (Figure 4).

Regarding the potential use of visible ectoparasite presence as a non-invasive biomarker, regression analyses have shown a strong relationship between changes in stress indicators and parasite numbers in the present study. The regression results provide strong evidence that the parasite numbers on the gills have a significant impact on the secondary stress parameters of hematocrit, plasma glucose, and plasma lactate. Based on these regression results, the presence and intensity of parasites can be utilized as a non-invasive biomarker for fish welfare assessment. Furthermore, to strengthen the argument for the usability of parasites as biomarkers, it should be emphasized that parasites serve as a biomarker indicating both fish welfare and environmental quality in aquaculture. The rise in parasite numbers in fish can serve as a warning sign of an unsuitable environment. Thus, a comprehensive approach has been implemented for addressing sea lice in salmon farming in Norway. Sea lice are regarded as indicators of regional environmental sustainability, and certain limitations on biomass production are imposed based on the recorded parasite levels on farms within a specific area. Farms that consistently maintain low parasite abundance on fish are permitted to produce larger biomasses (Bui et al., 2019). It is also essential to highlight their consistent presence and relevance in different ecological contexts. Copepod parasites such as *L. kroyeri* and their copepodites are available throughout the year, resulting in constant infection pressure in the sea cages (Yavuzcan Yildiz & Korkmaz 2021). The crustacean parasite's continuous presence provides suitability for using these parasites as non-invasive biomarkers.

Table 2. The key parametric in regression analysis for stress indicators.

Dependent variable*	R-squared	*F* value	*P* value
Hematocrit	0.56	44.75	<0.01
Plasma glucose	0.85	200.45	<0.01
Plasma lactate	0.50	36.41	<0.01

* The independent variable was the parasite number on the gills

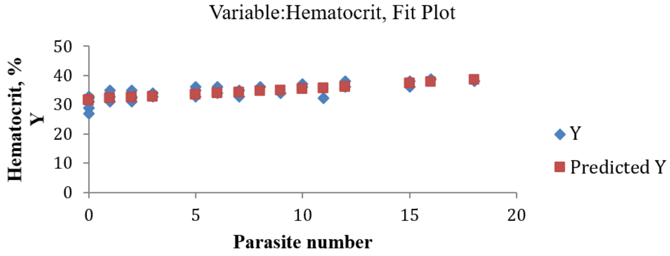

Figure 2. Hematocrit.

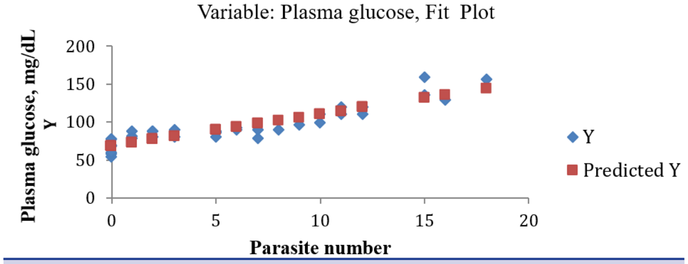

Figure 3. Plasma glucose.

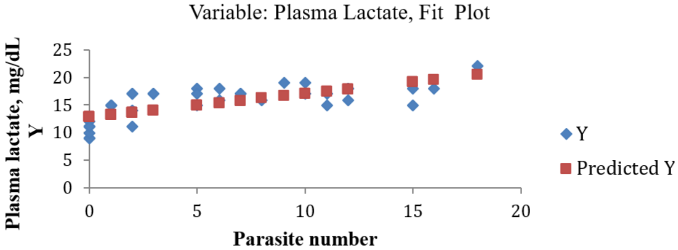

Figure 4. Plasma lactate.

CONCLUSION

While varying between moderate and strong, a statistically significant relationship exists between the numbers of copepod parasites (*L. kroyeri*) on the gills and secondary stress indicators (hematocrit, blood glucose, and lactate) in sea-caged European sea bass. In the regression analysis, R-squared values (approximately 50%) for the hematocrit and plasma lactate indicate that the independent variables in the model explain half of the variance in the dependent variable, necessitating cautious interpretation. An R-squared value of 0.85 is relatively high and suggests a strong positive linear relationship between the plasma glucose and the parasite number on the gills in the regression. Nevertheless, with R-squared values of each biomarker consistently above 50%, it is considered that the results of this study can support the utilization of parasite count as a non-invasive biomarker for fish welfare assessment. Therefore, the visible ectoparasite presence on the surface of the fish and their intensity can be suggested as a non-invasive biomarker for assessing fish welfare in aquaculture and an effective tool in explaining the welfare status of the fish. The validation process typically included defining requirements, establishing testing protocols, conducting tests, analyzing results, and documenting the findings. The goal was to provide confidence that the presence of visible parasites is fit to welfare assessment as a non-invasive biomarker, however, both validation and verification would be essential steps in the integration of visible parasites on fish gills in operational welfare indicators as the non-invasive biomarker.

It's worth noting that the development and validation of non-invasive biomarkers for fish welfare are ongoing research areas. Exploring new techniques and biomarkers to improve the assessment of fish welfare while minimizing the impact on the fish is significant for sustainable aquaculture.

Conflict of interest: The author declares no conflict of interest.

Ethics committee approval: Ethical approval for this study was obtained from ANKARA UNIVERSITY (2018-16-101). The present study followed international, national, and/or institutional guidelines for humane animal treatment.

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Research Article

Length – Weight Relationships, Meat Yield and Morphometric Indices of Five Commercial Bivalve Species Collected from the Çanakkale Strait (Türkiye)

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ABSTRACT

This study was conducted to determine the meat yield, morphometric characteristics, length–weight relationships (LWRs) and their correlations with environmental variables of five commercial bivalve species collected monthly between 2014 and 2015 from the coastal waters of the Çanakkale Strait. A total of 8588 individuals were examined, and different ranges for both shell length (9.00-108.50 mm) and total weight (0.30-234.20 g) were determined according to species. The highest meat yields from *Donax trunculus* (16.40-23.34%), *Mytilus galloprovincialis* (14.89-34.35%) and *Ostrea edulis* (5.91-26.24%) were determined in spring, while *Ruditapes philippinarum* (10.80-29.53%) and *Chamelea gallina* (12.26-18.92%) had maximum yield in late summer and early autumn ($p<0.05$). Elongation index (SH/SL), compactness index (SW/SL), convexity index (SW/SH), and density indexes (TW/SL) were significant ($p<0.05$) and had high correlation coefficients ($r=0.806$-0.975). The mean value of the allometry coefficient (b) was 3.257 ± 0.168, ranging from 2.291 to 4.058. Four species had negative allometries, namely *D. trunculus* (2.738), *C. gallina* (2.889), *M. galloprovincialis* (2.597) and *O. edulis* (2.728), while *R. philippinarum* (3.137) displayed positive allometry. The morphometric indices show high morphological resemblances. As a result, it is thought that the data obtained in this study can both provide data in the fields of biology and ecology for current scientific studies on these species, and can be used as a resource for the sustainable production of these commercial species.

Keywords: Meat yield, morphometric indices, bivalve species, Çanakkale Strait

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INTRODUCTION

Bivalves are unique organisms in terms of ecological impact in the marine environment and as a nutritional food in many countries (Wijsman et al., 2019). Their economic value is directly proportional to consumer demand, and demand can cause significant population reductions in regions where stock–supporting activities such as aquaculture are not carried out (Wijsman et al., 2019). Türkiye has significant bivalve production areas from the Aegean Sea to the Black Sea, where the primary production method is based on fishing.

Bivalve fishing has been performed using various methods (hydraulic and mechanical dredges, hand dredges, SCUBA diving) since the 1970s in Türkiye, and socio–economically contributes to coastal communities (Çolakoğlu & Palaz, 2015). In the coastal waters of the Marmara Sea and the Çanakkale Strait (0–20 m), there are various bivalve species with high economic value and extensive stocks, such as wedge clam (*Donax trunculus* Linnaeus, 1758), striped venus (*Chamelea gallina* Linnaeus, 1758), venerid clam (*Ruditapes philippinarum* Adams and Reeve, 1850), Mediterranean mussel (*Mytilus galloprovincialis* Lamarck, 1819) and flat oyster (*Ostrea edulis*). Among them, the most produced (fished) bivalve species along the coasts of Türkiye in 2020 were reported to be *Mytilus galloprovincialis* (~4000 tons) and *Chamelea gallina* (~30 000 tons) (Turkstat, 2021).

The length–weight relationship (LWRs) and morphometric characteristics are important for biology and fisheries in population dynamics (Gaspar et al., 2002). This information is useful especially for predicting current conditions and stock assessment, as well as morphological comparisons of bivalve species (Gaspar, Santos & Vasconcelos, 2001; Vasconcelos et al., 2018). In relative studies conducted worldwide, LWRs, relative growth and shell morphometric relationships were assessed for different bivalve species (Charef, Langar & Gharsallah, 2012; Gaspar et al., 2001; 2002; Petetta et al., 2019; Vasconcelos et al., 2018). In Türkiye, different bivalve species caught along the coastal areas between the Aegean and Black Seas were analysed in terms of morphometric and population characteristics (Çolakoğlu & Palaz, 2014; Çolakoğlu & Tokaç, 2014; Dalgıç, 2006; Deval, 2009). Also, several studies in the southern Marmara Sea and the Çanakkale Strait focused on diverse subjects such as fishing, population dynamics, aquaculture, chemical and biological contaminants (Çolakoğlu et al., 2011; Künili, Çolakoğlu & Çolakoğlu, 2021a; Künili et al., 2021b). However, there is a limited number of studies to determine the meat yields and morphometric characteristics of these commercial bivalve species and to compare them with environmental parameters in the South Marmara Sea and Çanakkale Strait, where one of the densest populations is found (Çolakoğlu, 2011). The present study was performed to determine and compare the meat yield, morphometric relationships (between shell length, height, width, and total weight), morphometric indices (elongation, compactness, convexity and density) and relative growth (isometry vs allometry) of *D. trunculus*, *M. galloprovincialis, O. edulis, R. philippinarum,* and *C. gallina* collected along the Çanakkale Strait coast in Türkiye.

MATERIALS AND METHODS

Material, study area and sampling

The research materials were *D. trunculus, M. galloprovincialis, O. edulis, R. philippinarum, and C. gallina* collected from coastal areas containing both hard substrate and sandy bottoms along the Çanakkale Strait in the west of the Marmara Sea (Figure 1). The sampling locations were selected according to current information on bivalve harvesting and from local fishermen. Due to the living habits of research materials, two sampling methods were used for the two predetermined sampling groups. In the first group, *D. trunculus*, *C. gallina,* and *R. philippinarrum* samples were collected from 1–8 m depths by using a mechanical dredge towed parallel to the shoreline for 5 min at a constant speed of 1–2 knots (length of dredge width and height: 55 and 30 cm; the number of teeth and length: 25 and 16 cm; mesh size: 5 mm). For the second group, *M. galloprovincialis* and *O. edulis* samples found at 1–12 m depths around sampling locations were collected by hand during SCUBA dives. All samples were collected between September 2014 and August 2015. Samples were firmly packed with wire meshes and transported to the laboratory via an ice–cooled insulated box within 1–2 hours.

Environmental parameters of the sampling areas, such as Sea Surface Temperature (SST) (°C), salinity (ppt), dissolved oxygen (mg·L^{-1}), and pH, were measured in *situ* using a YSI 650 MDS multi–parameter water quality meter. Chlorophyll–a (Chl–a) concentrations in seawater samples obtained from the locations were determined according to the method described by the American Public Health Association (APHA, 1995).

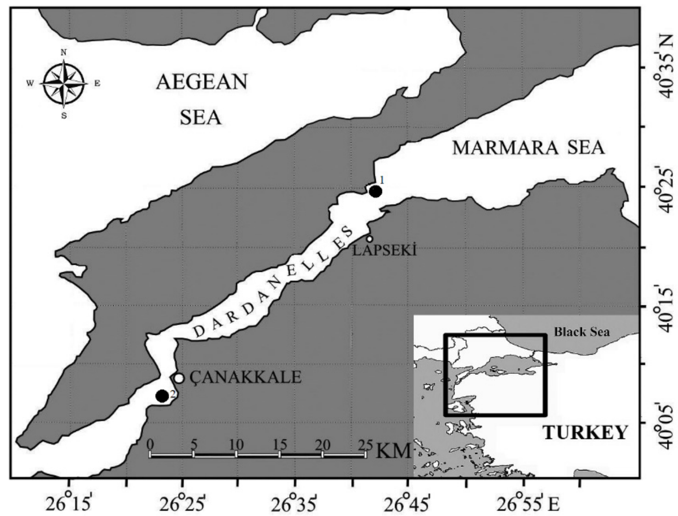

Figure 1. Map showing the study area along the Çanakkale Strait (Türkiye).

Data treatment and statistical analyses

The shell length (SL), shell height (SH), and shell width (SW) of individual specimens were measured using a digital vernier caliper (0.01 mm accuracy). The total wet weight (TW) and wet meat weight (MW) of each bivalve were measured using an electronic balance (d: 0.01 g, Max: 3100, Acculab, ALC-3100). Meat yield (MY) was calculated with the following equation: MY (%) = [(MW/TW) x 100]. Morphometric relationships were determined according to the allometric equation of Ricker (1973) $Y=a X^b$, where Y is SH, SW, or TW, X is the shell length (SL), *a* is the intercept, and *b* is the slope. The allometry coefficient is expressed by the exponent *b* in the linear regression equations. In these equations, in correlations between different types of variables and/or between different measuring units, the weight–length relationship reflects isometric growth when the exponent $b=3$ (Gaspar et al., 2002). To confirm whether the values of *b* obtained from linear regressions were significantly different from the isometric value ($b=3$) and described a negative ($b<3$) or positive ($b>3$) allometric relationship (Huxley & Teissier, 1936), the student t–test was applied with a confidence level of ±95% (Sokal & Rohlf, 1987).

Moreover, to characterize the morphology and growth shapes of bivalves, diverse morphometric indices including elongation index (SH/SL), compactness index (SW/SL), convexity index (SW/SH) and density index (TW/SL) were used (Vasconcelos et al., 2018; Caill–Milly et al., 2012, 2014). The significance for all statistical analyses was set at $P<0.05$ (Zar 1999).

RESULTS AND DISCUSSION

The present study evaluated the stock status of five commercial bivalve species from two stations in the Çanakkale Strait. Major environmental variables were measured and correlated with meat yield, as one of the most important economic properties, for complementary evaluation of the results. The results of the environmental parameters measured during the study period are summarized in Fig 2. The minimum and maximum values for SST (°C), salinity (ppt), DO (mg·L^{-1}), pH, and Chl–a (μg·L^{-1}) during the study period were determined as follows; 7.85 (Feb '15) – 23.85 (Jul '15) for SST, 22.00 (Aug '15)–26.00 (Mar '15) for salinity, 6.51 (Jul '15)–8.52 (Feb '15) for DO, 8.05 (Dec '14) – 8.40 (Mar '15) for pH, and 0.95 (Mar '15)–4.10 (Sep '14) for Chl–a, respectively.

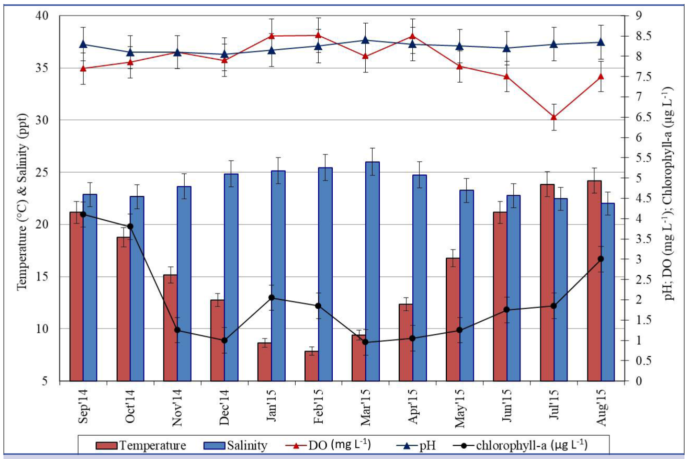

Figure 2. Summary of environmental variable data by months.

During the research period, 3394 individuals of *D. trunculus,* 2433 *C. gallina*, 1869 *R. philippinarum*, 580 *M. galloprovincialis* and 312 *O. edulis* were collected. The descriptive statistics and LWRs parameters of the samples are summarized in Table 1. The allometry coefficients (*b*) varied among species: *D. trunculus* =2.738, *C. gallina* =2.889, *R. philippinarum* =3.137; *M. galloprovincialis* =2.597 and *O. edulis* =2.728 ($P<0.05$). In general, regression analysis demonstrated a significant linear relationship for SH (range= 0.481–1.128) ($P<0.05$), SW (range=0.197–0.891) ($P<0.05$), and TW (range=0.323–2.159) ($P<0.01$) with SL. There were negative allometries (TW/SL) for four species *C. gallina* (*b*=2.889), *D. trunculus* (*b*=2.738), *M. galloprovincialis* (*b*=2.597) and *O. edulis* (*b*=2.728), while one bivalve species *R. philippinarum* (*b*=3.137) was observed to have positive allometries.

The morphometric indices, elongation (SH/SL), compactness (SW/SL), convexity (SW/SH), and density index (TW/SL) of the bivalve species studied are shown in Table 2. The morphometric indices of species were in the range 0.254–1.938 for SH/SL, 0.197–1.500 for SW/SL, 0.222–2.124 SW/SH, and 0.020–2.159 for TW/SL, respectively. In this study, four species had negative allometries, namely *D. trunculus* (2.738), *C. gallina* (2.889), *M. galloprovincialis* (2.597) and *O. edulis* (2.728), while *R. philippinarum* (3.137) displayed positive allometry.

According to the results, the lowest values were present for SH/SL in *M. galloprovincialis* and *O. edulis*; SW/SL in *O. edulis* and *D. trunculus*; SW/SH in *O. edulis* and *D. trunculus*, and TW/SL in *M. galloprovincialis* and *D. trunculus.* These values were found to be similar to those reported from Italian coasts for *M. galloprovincialis* (Orban et al., 2002), southern Black Sea for *D. trunculus* (Aydın, Tunca & Ersoy, 2020) and slightly different from the values from the Algarve coast, Portugal (Vasconcelos et al., 2018). In this study, mean values of SH/SL, SW/SL, SW/SH, and TW/SL were strongly correlated with SST and Chl–a ($P<0.05$). The morphology and physiology of bivalves are strongly influenced by fisheries and biomass (Gaspar et al., 2001, 2002), along with environmental conditions (Lucas et al., 1981) such as space competition for some species (Caill–Milly et al., 2014), differences in nutritional conditions and defence against predators (Caill–Milly et al., 2012; Tokeshi, Ota & Kawai, 2000; Watanabe & Katayama, 2010).

In comparison with previous reports, the allometric coefficient *b* (2.89) for *C. gallina* in this study was similar to values obtained from the Tyrrhenian Sea (2.74) and the Adriatic Sea (2.69) (Petetta et al., 2019), the West Marmara Sea (2.89) (Çolakoğlu & Tokaç, 2014), the North Sea (2.87) (Robinson et al., 2010), and the Algarve coast (Southern Portugal) (2.80) (Gaspar et al., 2001; Rufino et al., 2006), but higher than from the Mediterranean Sea (2.37) (Kasapoğlu & Düzgüneş, 2013). The *b* coefficient (2.74) value obtained for *D. trunculus* was similar to the values for the same species studied in the Tyrrhenian Sea (2.77) (Petetta et al., 2019) and

Table 1. Descriptive statistics, length–weight relationships and type of growth for five economic bivalves collected from the Çanakkale Strait (Türkiye).

Species	N	Length (mm)			Weight (g)			Morphometric relationship				Type of growth
		Min	Max	Mean±SD	Min	Max.	Mean±SD	a	b	SE (b)	r	
Dt	3394	13.00	40.50	28.78±0.07	0.36	7.69	2.93±0.02	0.0003	2.738	0.003	0.951	-A
Mg	580	15.05	84.00	59.02±0.39	0.30	50.17	16.06±0.28	0.0004	2.597	0.006	0.912	-A
Oe	312	53.00	108.50	75.73±0.54	17.30	234.20	72.52±1.78	0.0005	2.728	0.008	0.806	-A
Rp	1869	26.50	62.00	42.66±0.14	3.70	63.90	20.47±0.24	0.0001	3.137	0.003	0.932	+A
Cg	2433	9.00	38.00	23.65±0.11	0.30	17.76	4.67±0.07	0.0005	2.889	0.003	0.975	-A

Dt: Donax trunculus, Mg: Mytilus galloprovincialis, Oe: Ostrea edulis, Rp: Ruditapes philippinarum, Cg: Chamelea gallina, N:Number, SD: Standart Deviation, SE: Standart Error, a: Intercept, b: Slope, r: Correlation Coefficient;-A: Negative Allometry, +A: Positive Allometry the West Marmara Sea (2.69) (Çolakoğlu, 2014), and higher than in the Adriatic Sea (2.48) (Petetta et al., 2019) and the Algarve coast (Southern Portugal) (2.57) (Gaspar et al., 2001). The *b* value (3.14) for *R. philippinarum* was similar to exponential values obtained on the southern coast of the Marmara Sea (3.14) (Çolakoğlu & Palaz, 2014) and the Taehwa River, Ulsan (3.04) (Choi et al., 2011), but higher than for the coast of Yeongi in Tongyeong, Korea (2.99) (Cho, Jeong & Lee, 2008) and the Amurshy Bay, Sea of Japan (2.95) (Ponurovsky, 2008). The *b* (2.73) for *O. edulis* was lower than findings obtained in Mersin Bay, Aegean Sea (3.15) (Acarlı et al., 2011), but higher than in the Black Sea (2.46) (Aydın & Biltekin, 2020). The *b* value (2.60) for *M. galloprovincialis* was similar to the findings obtained in the Istanbul Bosporus (2.63) (Balcıoğlu & Gönülal, 2017) and Gökçeada Island, North Aegean Sea (2.73) (Keskin, Ekici & Serdar, 2020), but higher than in the Çanakkale Strait (2.33) (Balcıoğlu and Gönülal, 2017). Discrepancies in the value of *b* in LWRs could be affected by the fishing gear used and selectivity, and variations in environmental conditions such as ecological differences (water temperature, salinity, etc.) in the sampling areas, type of bottom and type of sediment, the intensity of predation, and lack or abundance of food (Gaspar et al., 2001; 2002; Çolakoğlu, 2020).

The meat yields of species were in the range of 16.40–23.34% for *D. trunculus*, 14.89–34.35% for *M. galloprovincialis*, 5.91–26.24% for *O. edulis*, 10.80–29.53% for *R. philippinarum*, and 12.26–18.92% *C. gallina*, respectively (Figure 3a). The highest meat yields for *D. trunculus*, *M. galloprovincialis* and *O. edulis* were determined in spring, while *C. gallina* and *R. philippinarum* had maximum yield in late summer and early autumn ($P<0.05$). The meat yields were found to be affected significantly by seasonal and species differences ($P<0.05$) (Figure 3a). The correlation of the meat yield with environmental variables and indexes is summarized in Figure 3b. The highest positive and negative correlations among meat yields of species and variables were observed as follows; *D. trunculus* had positive moderate correlations with salinity (r=0.598) and pH (r=0.630); *M. galloprovincialis* and *O. edulis* had strong–moderate positive correlations with Chl–a (r:0.827, r:0.597); and *C. gallina* and *R. philippinarum* had strong–moderate positive correlations with pH (r:0.545, r:0.731) ($P<0.05$). The highest negative strong correlations were observed as follows; *D. trunculus* with SST (r:–0.489); *M. galloprovincialis* with compactness index (C–I) (r:0.512), O. *edulis* with elongation index (E–I) (r:–0.518), *R. philippinarum* with DO (r:–0.356); and *C. gallina* with DO (r:–0.387) ($P>0.05$). The weakest correlations observed among species were determined as follows; *D. trunculus* with elongation index (E–I) (r:–0.069); *M. galloprovincialis* with density index (D–I) (r:0.014); *O. edulis* with density index (r:–0.077); *R. philippinarum* with salinity (r:0.062); and *C. gallina* with elongation index (E–I) (r:0.163, $P>0.05$).

In general, meat yield is an indicator of the condition status of bivalves which can change with seasons, reproduction period and food accessibility (Okumuş & Stirling, 1998; Orban et al., 2002). The highest meat yield of *D. trunculus, M. galloprovincialis*, and *O. edulis* were observed in the samples from the spring months, while it was at the highest level in the summer months for *R. philippinarum* and in the autumn months for *C. gallina*. Although the highest and lowest levels of meat yield slightly varied, the means of findings between months are similar to those reported in previous studies (Çolakoğlu & Tokaç, 2014; Vernocchi et al., 2007; Chen et al., 2020). Salinity was positively correlated with only *D. trunculus*, while pH was correlated with *D. trunculus, R. philippinarum* and *C. gallina* ($P<0.05$). A significant positive correlation for the most important parameter, Chl–a, was determined only for *M. galloprovincialis* and *O. edulis* (r:0.545–0.731; $P<0.05$). The living habits of both these bivalves (*M. galloprovincialis* and *O. edulis*) differs from other species (*D. trunculus*, *R. philippinarum*, and *C. gallina*) due to the requirements of hard substrates to adhere to in water columns (Wilcox & Jeffs, 2017; Potet et al., 2021). In seawater, the planktonic mass first increases in warmer months and then sedimentation and distribution occur to benthic and lower depths of the water column throughout seasons (Graf et al., 1982; Benedetti et al., 2019). This case may be an indicator that initially, meat yield increases in parallel with the planktonic bloom in the water column for *M. galloprovincialis* and *O. edulis*, then with the precipitation of the planktonic mass in late summer, other sub–benthic clams achieve higher meat yields. This is also most probably related to active feeding on these species from the spring to autumn as the Çanakkale Strait is characterized by SST and Chl-a level increases causing increased planktonic activity (Turkoglu, 2010), which is an important factor affecting food accessibility and growth of bivalves (Robinson et al., 2010). The information about meat yields in dif-

Table 2. Morphometric indices of five economic bivalves collected from the Çanakkale Strait (Türkiye)

Species	N	Elongation index (SH/SL)			Compactness index (SW/SL)			Convexity index			Density index (TW/SL)		
		Min	Max	Mean±SD	Min	Max	Mean±SD	(SW/SH)	Max	Mean±SD	Min	Max	Mean±SD
Dt	3394	0.71	0.50	0.59±0.001	0.42	0.27	0.33±0.001	0.78	0.36	0.56±0.001	0.20	0.03	0.10±0.001
Mg	580	1.94	0.25	0.37±0.003	1.50	0.37	0.52±0.003	2.12	0.42	1.45±0.006	0.60	0.02	0.26±0.004
Oe	312	1.13	0.48	0.81±0.006	0.89	0.20	0.34±0.004	0.96	0.22	0.43±0.006	3.99	3.14	3.58±0.008
Rp	1869	1.03	0.79	0.90±0.001	1.03	0.50	0.60±0.001	1.32	0.36	0.69±0.004	1.05	0.14	0.46±0.004
Cg	2433	1.07	0.77	0.92±0.001	0.59	0.39	0.51±0.001	0.67	0.42	0.56±0.001	0.48	0.03	0.19±0.002

Dt: Donax trunculus, Mg: Mytilus galloprovincialis, Oe: Ostrea edulis, Rp: Ruditapes philippinarum, Cg: Chamelea gallina, N:Number, SD: Standard Deviation, SL: Shell Length, SH: Shell Height, SW: Shell Width; TW: Total weight

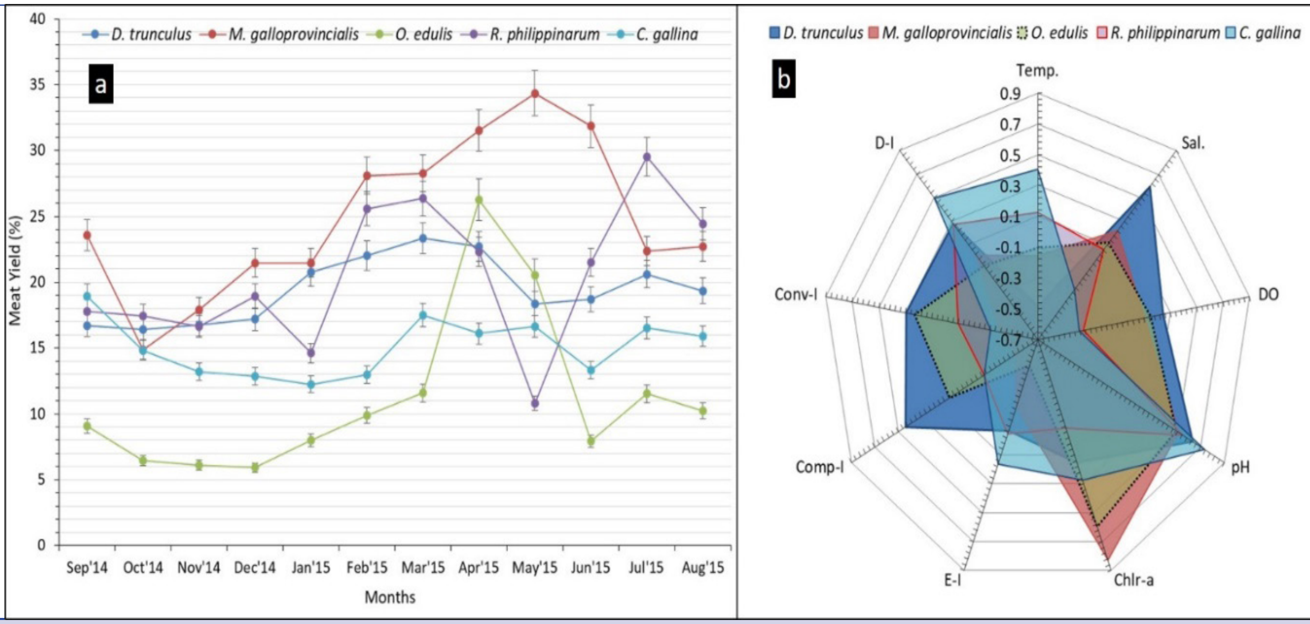

Figure 3. (a) Meat yields of bivalve species during study period (mean±SD); (b) correlations of meat yields with indexes and environmental variables.
Temp: SST, Sal: Salinity, DO: dissolved oxygen, Chlr–a: Chlorophyll–a, E–I: Elongation Index (SH/SL), Comp–I: Compactness Index (SW/SL), Conv–I: Convexity Index (SW/SH), D–I: Density Index (TW/SL).

ferent bivalves could be useful for maximizing catch and controlling the exploitation of bivalves in their natural beds in different geographical areas.

CONCLUSION

This study showed that condition, growth, and morphometric indices of *D. trunculus, C. gallina, R. philippinarum, M. galloprovincialis* and *O. edulis* could vary based on seasonal and environmental conditions. Although the LWRs, coefficient factors and morphometric indices showed good growth for the five species, the future of the populations of these species may be negatively affected since meat yield is one of the most important reasons for fishing due to consumer demand. The growth of species varied at a high rate with changing environmental factors, especially warmer seawater and planktonic abundance (Chl–a) by season. In further studies, commercial bivalve species should be monitored by including morphometric characteristics, growth, conditions indexes, as well as meat yield and environmental parameters, sustainability, and conservation of natural stocks in terms of fisheries management.

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Research Article

Records of Three Immature Gelatinous Specimens for the Turkish Mediterranean Coast with an Emphasis on Alternative Pathways

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ABSTRACT

From samples for the phytoplankton collected from the sea surface water of 67 stations along the entire Turkish Mediterranean coast during June-July 2019, three juvenile gelatinous organisms were recorded. Two cnidarian-hydrozoan species (*Podocorynoides minima* and *Gastroblasta raffaelei*) and one ctenophore (*Bolinopsis* cf. *vitrea*) occurred near Mersin Bay. The specimens were determined at the juvenile or eumedusoid stages. Referring to the literature, *Gastroblasta raffaelei* was presumably about 4-5-day old (1.05 x 1.56 mm in elliptical diameter), and *Podocorynoides minima* about a stage of liberated eumedusoid (0.327 x 0.316 mm in bell diameter x height). The specimen of *Bolinopsis* cf. *vitrea* was measured to be 11 mm in lobate (total) length and 8.6 mm in body width. Interestingly, all species were found at different locations close to each other on the set of the water rim current speeding easterly up to about 0.5 m s^{-1}. *Gastroblasta raffaelei* and *Podocorynoides minima* were first recorded on the Turkish Mediterranean coast. Early-staged specimens of these three species were described and discussed for their diagnostic structures with their occurrence in the Turkish Mediterranean Sea after the hydrozoans were reported in the Sea of Marmara, the ctenophore in the Black Sea, and other seas of the Mediterranean basin. The present study also discussed possible and presumable pathways of recent increased Turkish Mediterranean records of specimens that have been observed in the West Mediterranean Sea and the Adriatic Sea.

Keywords: New records, gelatinous species, Turkish Mediterranean waters

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INTRODUCTION

The Mediterranean Sea is specifically biologically well-diversified and significantly researched for its marine life (Vasilakopoulos et al., 2017). Although the Mediterranean Sea is recognized as a biodiversity hotspot (Coll et al., 2010), it has been threatened by pollution, over-exploitation, and global warming (Cuttelod et al., 2009). The eastern Mediterranean Sea, particularly the Levantine Sea is well open to the new records of the gelatinous organisms (Ctenophora and Cnidaria) besides other taxa. Most of them are invasive alien species (Galil, 2007). Many recent records (32 records since 2006, most of them since 2015) of zooplankton, especially gelatinous organisms, most inhabiting the West Mediterranean, and Adriatic Sea, to the Turkish marine system were noticed (Table 1). Furthermore, recent records have increased for other taxa (e.g., Patania & Mutlu, 2021; Garuti & Mutlu, 2021; Mutlu et al., 2023a). For all increased records there could be a vector for their transportation, such as water current among the other vectors, the straits, aquaculture, ship ballast water, etc.

Early staged-medusoid and juvenile ctenophore specimens are often encountered in the water samples for zooplankton and even phytoplankton. Their occurrences suggested that they could be established in the seas or trans-

Table 1. Recent records and sampling year of gelatinous zooplankton in the Turkish marine coasts. * is not a new record but is, at one location, extremely abundant specimens and ** is open water species but abundantly found at locations in Figure 1, which is found in the West Mediterranean Sea and recorded in the area symbolized in Figure 1.

Taxa	Regions	Year	Citations
Published specimen			
One ctenophore	Bosporus exit to Black Sea	2007	Öztürk, Mihneva, & Shiganova, 2011
Sixteen hydrozoans, One scyphozoan	Sea of Marmara, Aegean Sea	2006-2013	İşinibilir et al., 2015a; Isinibilir, Yilmaz, & Demirel, 2015b; İşinibilir, Ulucam, & Yüksel, 2019
One hydrozoan	Sea of Marmara	2015	Yılmaz et al., 2017
Two scyphozoans, One hydrozoan, One thaliacean	Sea of Marmara	2019-2020	İşinibilir et al., 2022
Two hydrozoans	Aegean Sea	2012, 2015-2016	Gülşahin, Tarkan, & Bilge, 2013; Gülşahin et al., 2016
One ctenophore	Aegean Sea	2015-2018	Killi, Abyzova, & Shiganova, 2019
One hydrozoan	Northernmost Aegean Sea	2021	Isinibilir, Yuksel, & Guresen, 2021
One lobat ctenophore	Turkish water	2015	Gülşahin and Türker, 2017
One new scyphozoan	Levant Sea	2018	Mutlu et al., 2020
One lobat ctenophore	Levant Sea	2020	Gokoglu and Galil, 2020
One cydippid ctenophore	Levant Sea	2019	Mutlu and Özvarol, 2022a
One hydrozoan	Levant Sea	2019	Mutlu and Ozvarol, 2022b
Unpublished specimens noticed			
One calanoid copepod (24 inds)	Levant Sea	2019	Duman, 2023
Four phytoplankton*	Levant Sea	2019	Karaca, 2023
One cheatognath**	Levant Sea	2019	Duman, 2023

ferred from one sea to another sea depending on their life longevity. In many cases, the literature is needed to identify immature gelatinous species as needed for the other taxa. To overcome such difficulties in the identification, laboratory studies were conducted to follow growth through different stages of the gelatinous species. This stage of growth could be differentiated by their diagnostic morphometries under different environmental conditions (Mayer, 1912; Oliveira et al., 2007; Gravili et al., 2007; Shiganova, 2020; Fabien Lombard pers. comm.).

Mediterranean plankton is easily drifted and moved from intra-seas of the Mediterranean Sea and inter-seas by water current induced by a variety of mechanisms of atmosphere-sea interactions over the Mediterranean Sea besides a variety of introduction vectors such as Suez Canal, ship ballast water, and aquaculture (Zenetos et al., 2012). Such events could change the temporal structure of ecosystems, fisheries, and surface water currents. Globally, the Mediterranean Sea is influenced by atmospheric actions, mainly North Atlantic Oscillation (NAO) (Raitsos et al., 2011) and slightly North Pacific Oscillation (NPO), Pacific Decadal Oscillation (PDO) and El Niño (Báez et al., 2022). Locally, the Mediterranean Sea is influenced by the Bimodal Oscillation System (BiOS) decadal-occurring around the Adriatic Sea and global warming (Poulain et al., 2013; Civitarese et al., 2023). The study area of the present study is one of the regions mostly affected by global warming and undergoes a process of tropicalization (Encarnação et al., 2019). All these events induced new aspects of the water current in the Mediterranean system and introduced new records of plankton among the Mediterranean

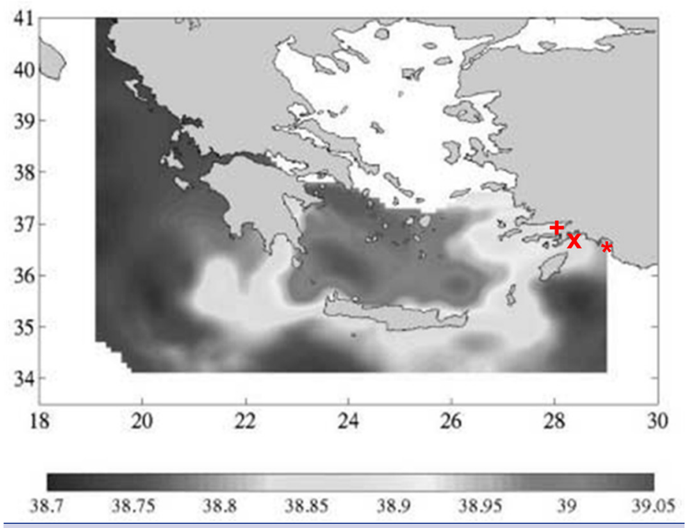

Figure 1. Water salinity at a deep layer having σt=29.18 (LIW) to show depth-wise changes of the layer along the different seas' interaction in 1991 (from Briand, Lascaratos, & Klein, 2000). + denotes records by Gülşahin, Tarkan, & Bilge, 2013; Gülşahin et al., 2016, x denotes records by Duman, 2023, Karaca 2023, and * denotes records by Mutlu and Özvarol, 2022a, b (see Table 1 for taxa observation and sampling dates).

seas, and ocean to the Mediterranean Sea. New records of the organisms particularly in the Turkish marine waters increased as the research was performed, or presumable new pathways could be developed in the Mediterranean Sea. Such introduction signaled the impulse of new records in the Turkish waters (Table 1).

Besides the endemic gelatinous species including medusoids of the hydrozoans of the Mediterranean Sea, the gelatinous alien species have altered the Mediterranean ecosystem (Coll et al., 2010; Dragičević et al., 2019); for instance, a new sea medusa, *Chrysaora pseudoocellata* Mutlu, Tulay, Olguner & Yılmaz 2020 was established in the eastern Mediterranean Sea (Dragičević et al., 2019; Mutlu et al., 2020; Douek et al., 2020). The records particularly the recent records coincided with decadal atmospheric and oceanic events, especially with a decadal period of BiOS which was first noticed in 1988. Furthermore, Duman (2023) and Karaca (2023) determined a new record of a calanoid copepod (specific to the Adriatic Sea) at one location and extreme-abundantly (more than at least 100-fold-higher at one location than other sampling stations of the present study) occurred phytoplankton species (three of four common in Adriatic Sea) at one location, respectively (Table 1, Figure 1). A delayed record could occur in the northern Turkish waters referring to southern Turkish waters. All species recorded in Table 1 originated from Atlanto-Mediterranean waters. Most of them were found in the western waters of the Levantine Sea. Depth-wise rise of a deep layer characterized by a water density of σ_t=29.18 (LIW) was observed along the Ionian Sea through the Aegean Sea to a part of the Levant Sea in 1991 (Briand et al, 2000). This event however coincided with the new records of some species for the Turkish waters (Table 1, Figure 1). Decadal events and this example (Figure 1) alert transportation of westerly originated species to the western coasts of Turkish waters beside the BiOS effect. Nevertheless, the BiOS induced acceleration of water velocity in the present study area (Figure 2).

A model that was recently conducted to simulate jellyfish dispersion in space showed a pathway of jellyfish distributional extension in time from the Egyptian (around the Suez Canal) and Israeli coast to the Turkish water on the set of the Atlantic water current (Edelist et al., 2022). Alternatively, BiOS is a water circulation process switching from cyclonic to anticyclonic or *vice versa* on decadal intervals via the North Ionian Gyre (NIG) (Civitarese et

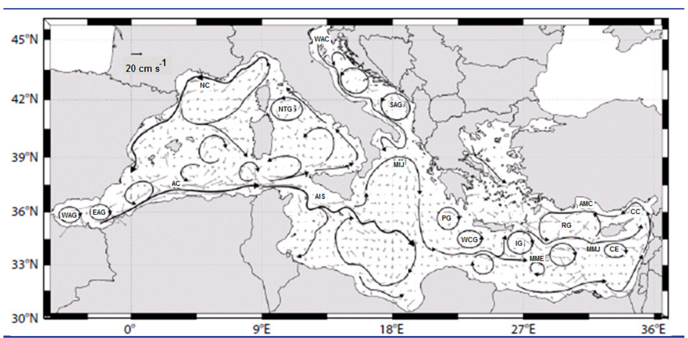

Figure 2. Mediterranean Sea surface water circulations affected by BiOS (Poulain et al., 2013).

al., 2010) and leads to changes in the thermohaline current structure in the Southern Adriatic (Civitarese et al., 2010), followed effect on Surface Water (LSW) and Intermediate water (LIW) in the Levant Sear (Ozer et al., 2017) via Ionian Jet (Figure 2). The Ionian jet then pumped the waters to the Levantine Sea (Poulain et al., 2013) when the NIG became cyclonic circulation. Studies have started to understand the effect of the NIG on the plankton communities, for instance on the phytoplankton in the Mediterranean Sea (Jasprica et al., 2022), and spatiotemporal benthic-pelagic coupling changes (Ricci et al., 2022) as the severity of the winter condition has changed globally yielding BiOS (Poulain et al., 2013).

Gelatinous biodiversity has increased in the Turkish marine environment. Öztürk, Mihneva, & Shiganova, (2011) contributed occurrence of one ctenophore, İşinibilir et al., 2015a, Isinibilir, Yilmaz, & Demirel, 2015b and İşinibilir, Ulucam, & Yüksel (2019) 16 hydrozoans and one scyphozoan, Yılmaz et al. (2017) one hydrozoan, Gülşahin, Tarkan, & Bilge (2013) and Gülşahin et al. (2016) two hydrozoans, and Gokoglu & Galil (2020) one ctenophore to the gelatinous biodiversity of the Turkish marine waters, followed by species records reported by Mutlu & Özvarol (2022a, b) and the present study (Table 1).

Therefore, the present study aims to mark the evidence of the occurrence of three gelatinous invertebrates in jelly form from a different region characterized with comprehensive environmental variables rather than other regions by the published studies and to discuss possible introductory pathways of western species-leveled intrusion undergoing the Mediterranean marine basin, particularly the Levantine Sea, and the Turkish coast.

MATERIAL AND METHODS

During a summer survey (June-July 2019) of acoustical studies on the vegetation along the entire Turkish Mediterranean coast (Taşucu Bay, the Mediterranean region, is the easternmost end – Datça Bay, the Aegean region is the westernmost end of the study area), water samples for a study of phytoplankton were collected from the surface waters of 67 stations (Figure 3). The locations of the sampling stations and the study area were described in detail in a study published by Mutlu et al. (2023b). The bottom depth of the stations varied between 5 m and 1000 m. One-third of the stations were in offshore waters and the rest were in coastal waters. Three major rivers (Goksu, Seyhan, and Ceyhan) flow into the eastern part, the coastal zone of which is eutrophic. On the contrary, Antalya and Muğla Bays are oligotrophic due to the absence of rivers compared to Mersin Bay. Sea surface temperature was at maxima (28 C in the Aegean part - 31 °C in the Mediterranean part), and salinity varied between 38 PSU and ~40 PSU in summer, respectively. Three of the stations were included in the present study because the species considered in the study were recorded only in the three stations (Table 1, Figure 1).

On board R/V “Akdeniz Su”, 100 ml of the water sample was taken from the sea surface at each station using a 5-l Nansen bottle. The water was then fixed in a dark bottle using a 1% glutaraldehyde solution. During the survey, samples for physicochemical and optical parameters were collected from the surface (prefix S) and near-bottom (prefix N) waters some of them were measured on board, and the rest were frozen for lab analyses. One board and one litter of the water were filtered through CF/C for the nutrients and total suspended matter, and another one liter through CF/F filters for chl *a*. The samples were then frozen at -20 C on board. These parameters were physical (T; temperature in °C, pH; pH, S; salinity in PSU) using a multi-parameter probe (YSI, Hi-Tech), optical (Secchi disk depth), and chemical parameters (nutrients; NO_2+NO_3, NH_4, and PO_4, SiO_2, chl *a* and TSM; total suspended matter).

Three immature and early-staged gelatinous species were observed during the lab microscopic study. Each specimen was recorded at the different stations of the three stations (Figure 3). These three stations were sampled on July 02, 2019. All species were found at their early stages. Diagnostic terms of hydrozoan followed the description made by Bouillon et al. (2006) The staged species were identified using the descriptions ascribed by Mayer (1910, 1912), Madin (1991), Oliveira et al. (2007). Gravili et al. (2007) determined the stage development of *Gastroblasta raffaelei* Lang, 1886, regarding the different lab conditions with water temperature, and Schuchert (2007) of *Podocorynoides minima* (Trinci, 1903) and species of the ctenophores (Chun, 1880; Mayer, 1912; Oliveira et al., 2007; Shiganova, 2020). Photos of the specimens were taken, and their size was measured under the microscope; elliptical diameters of *G. raffaelei* and maximum bell width and height of *P. minima*.

The nutrients were measured following the standard UV-spectrophotometric procedures described by APHA (1999). The values of the nutrients were then converted to a unit µM. Total suspended solids (the material was dried in an oven at 60 ^{C} for 24 h, and then weighed before the weight of the dried membrane was subtracted from the total dry weight), and chlorophyll *a* (chl-*a*) were determined following Lorenzen's method (1967).

RESULTS AND DISCUSSION

Three gelatinous organisms were determined along the Turkish Mediterranean coast. These are *G. raffaelei* found at 22E, *P. minima* at 25E (hydrozoans), and *Bolinopsis* cf. *vitrea* at 23E (the ctenophore) (Figure 3).

Environment of the study area

The 22E, 23E, and 25E were the coastal stations within inshore waters (Figure 3). The coastal stations had a bottom depth of 49 m, 48 m, and 52 m, respectively (Table 2). The Sechhi disk depth at all stations varied between 13 m and 15 m. The sea surface temperature was measured in a similar range of 26-27 °C. The surface and near-bottom water salinity varied between 36.4 and 37.6 (PSU) (Table 2).

The concentration of the sea surface chl-*a* was 2-fold lower at two coastal stations (23E and 25E) than that of another station (22E). However, the total suspended matter was measured in a similar range at all the stations (Table 3). Sea surface SiO_2 was minima at 22E and maxima at 23E, like NH_4 and contrasted to NO_2+NO_3. Sea surface water PO_4 varied between 1.41 µM at 23E and 3.39 µM at 25E (Table 2).

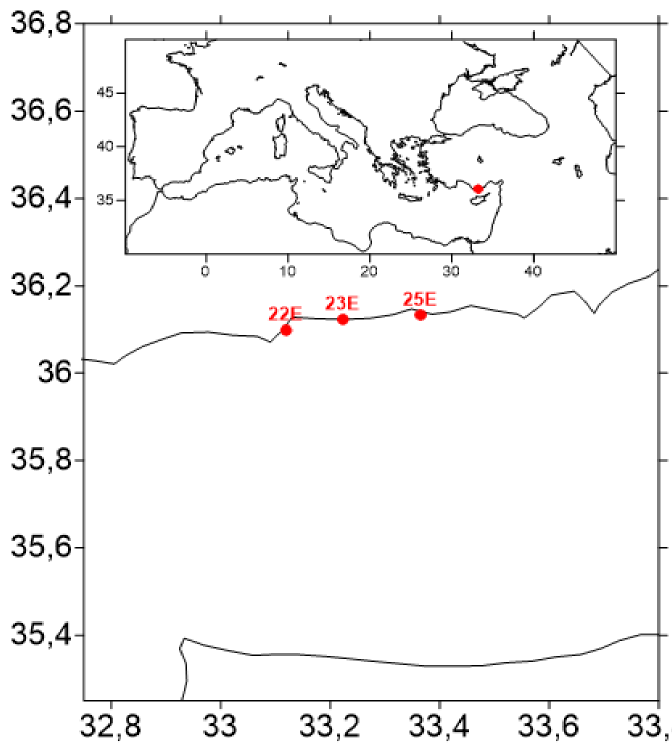

Figure 3. The study area (rectangle in color cyan) of the summer survey, and the area where the identified gelatinous specimens were found (red dot) at the sampling stations of the specimens (red dots labeled with station codes).

The stations were located between Turkey and the northern coast of Cyprus, which caused the derivation of the jet of the rim current. The current velocity was faster than 20 cm s^{-1} which is an average value for the Mediterranean Sea, and a maximum velocity of >50 cm s^{-1} was also measured along the Turkish Mediterranean coast (Poulain et al., 2013). These gelatinous planktons could be converged to the location by the rim current circumstance (Figures 2, 3).

Gelatinous species

Two species belonging to the phylum Cnidaria and one species to phylum Ctenophora were recorded for the first time along the Turkish Mediterranean coast. All three species were staged at a level of juvenile.

Species Gastroblasta raffaelei Lang, 1886

Phylum: Cnidaria
Class: Hydrozoa
Order: Leptothecata
Family: Campanulariidae
Genus: *Gastroblasta*

The material examined was an immature medusoid specimen collected from Tekeli (36° 05′ 91″ N, 33° 07′ 18″ E, station 22E, Mersin coast, Turkey, Eastern Mediterranean Sea) on 02 July 2019. Collection of the material was taken using a Nansen bottle from surface water at a seafloor depth of 49 m by Doğukan Kara-

Table 2. Distribution of the environmental parameters; bottom depth (depth), Secchi depth (SDD), surface (prefix S) and near-bottom (prefix N) water temperature (T), salinity (S), pH (pH), chl-a, total suspended matter (TSM), and the nutrients at stations of the present study. nm; not measured.

	22E	23E	25E
Depth (m)	49	48	52
SDD (m)	13	15	12.5
ST (°C)	26	27.1	26.5
NT (°C)	nm	nm	22.5
SS (PSU)	37.4	37.4	37.6
NS (PSU)	nm	nm	36.4
SpH	9.2	9.1	9.34
NpH	nm	nm	9.35
SChl-a (µg/l)	0.473	0.215	0.216
STSM (mg/l)	0.045	0.048	0.039
NTSM (mg/l)	nm	nm	0.054
$SSiO_2$ (µM)	8.83	10.51	9.39
$NSiO_2$ (µM)	12.18	nm	27.85
SNO_2+NO_3 (µM)	0.30	0.51	0.47
NNO_2+NO_3 (µM)	0.20	nm	0.28
SNH_4 (µM)	43.91	67.24	64.65
NNH_4 (µM)	422.36	nm	90.57
SPO_4 (µM)	2.07	1.41	3.39
NPO_4 (µM)	3.72	nm	3.72

ca. The specimen was measured to be 1.05 x 1.56 mm in the elliptical umbrella diameter.

Description: Umbrella (u) is much flatter, and elliptical and has a shorter axis of 1.05 mm and a longer axis of 1.56 mm (Figure 4A). There are eight tentacles (t) of which half of the completed tentacle is curly in length and the other half is straight with each originating from an incomplete pear-shaped tentacular bulb (TB) (Figure 4A, B). Manubria (m) were multiple urn-shaped and the central manubrium (m) was fully formed (Fig. 4) and the other two manubria were smaller (one just formed with two lips) than the central one. Developed two manubria had four lips (l) which were more pronounced in the central manubrium, developing manubrium has two lips (Figure 4). Two opposite weak radial canals (rc) were hardly observed crossing diagonally three manubria and a centripetal (circular) canal (cc) was developed. The velar opening (vo, velum) is rather wide (Figure 4).

Remarks: *Gastroblasta timida* resembles *G. raffaelei*, both having multiple manubria and centripetal radial canals, and differentiated in bell shape; *G. timida* in circular, and *G. raffaelei* in elliptical umbrella (Gravili et al., 2007). The specimen was about 4-5 days old medusa according to the description diagnosed by Gravili et al. (2007). *G. raffaelei* had different diagnostics under different temperature conditions (15 °C and 18 °C). Morphometrically, our specimen resembles a specimen of 4-5 days old *G. raffaelei* reared at 18 C by Gravili et al. (2007), but it is like stage reared at 15 C considering the size of the specimen.

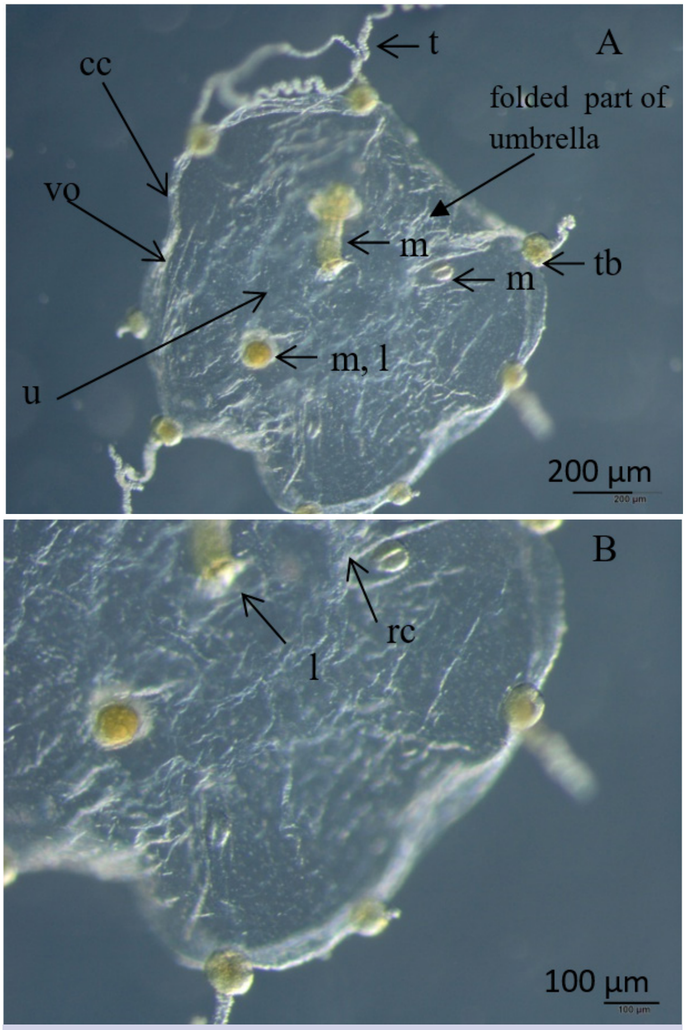

Figure 4. Oral view of *Gastroblasta raffaelei*: entire specimen (A), central manubrium, and newly occurred manubria at either side of the central manubrium (B). Umbrella (u), tentacles (t), tentacular bulb (tb) , manubria (m), central manubrium (m), lips (l), radial canals (rc), centripetal (circular) canal (cc), and velar opening (vo, velum).

Species Podocorynoides minima (Trinci, 1903)

Phylum: Cnidaria
Class: Hydrozoa
Order: Anthoathecata
Family: Rathkeidae
Genus: *Podocorynoides*

The material examined specimen was just released medusoid after the spawning and collected from surface water at a seafloor depth of 52 m off Akkuyu, Aydıncık (36° 07′ 98″ N, 33° 21′ 90″ E, station 25E, eastern Mediterranean Sea, Mersin coast, Turkey) on 02 July 2019, using a Nansen bottle by Doğukan Karaca. The size of the specimen was 0.327 x 0.316 mm in bell diameter x height.

Description: The body of the medusoid specimen is characterized by an incomplete sphere bell and untapered global pole of

Table 3. Previous occurrence, sampling year and location of three species in the Mediterranean Sea and adjacent seas.

Species	Region	Year	Location	Citation
Gastroblasta raffaelei				
	Tyrrhenian sea	1886	Gulf of Naples	Lang, 1886
	Ligurian Sea	1980	Portofino and Pontetto	Boero, 1980; Boero & Fresi 1986
	Balearic Sea	2003	Catalonian waters	Guerrero et al., 2018
	Ionian Sea, Adriatic Sea	2005	Otranto	Gravili et al., 2007
	Adriatic Sea		Northern coast	REGIONE DEL VENETO, SHAPE, 2013
	Marmara Sea	2008	İzmit Bay	Isinibilir, Yilmaz, & Demirel, 2015b
Podocorynoides minima				
	Ligurian Sea	1963-1964	Riviera-Corsica	Goy, 1972
	Red Sea	?		Schmidt, 1973
	Eastern Mediterranean		Lebanon water	Goy, Lakkis, & Zeidane, 1991
	Tyrrhenian Sea		Naples	Brinckmann-Voss, 1987
	Western Mediterranean	2004-2005	Tunisia	Touzri et al., 2012
	Marmara Sea	2006	Istanbul Bay	Isinibilir, Yilmaz, & Demirel, 2015b
	Adriatic Sea		Northern coast	REGIONE DEL VENETO, SHAPE, 2013
	Western Mediterranean	2014	Algeria	Kherchouche & Hafferssas, 2019
	Black Sea	2020	Romania	Mureşan, Teacă, & Begun, 2021
Bolinopsis vitrea				
	Aegean Sea	?	Turkey	Ergen, 1967
	Adriatic Sea	2003-2006	Italy	Shiganova & Malej, 2009.
	Black Sea	2007	Turkey	Öztürk, Mihneva, & Shiganova, 2011
	Adriatic Sea	2009	Montenegro	Lucic et al., 2012
	Adriatic Sea	2009-2010	Montenegro -Croatia	Branka et al., 2014
	Black Sea	2010	Bulgaria	Öztürk, Mihneva, & Shiganova, 2011; Öztürk, 2021
	Adriatic Sea		Northern coast	REGIONE DEL VENETO, SHAPE, 2013
	Levant Sea	?	Turkey	Çinar et al., 2014
	Adriatic Sea	2010-2019	Adriatic eastern coasts	Pestoric et al., 2021

the oral side and is shaped in a dome having thin global jelly and thicker apical jelly (Figure 5A). The subumbrella has a long gastric peduncle, cylindrical manubrium (m), elongated perradial lip (l) margins, and gonads (g) surrounding the manubrium (Figure 5A, B). The oral part has four radial canals (rc) extending to the apical pole, a narrow ring canal (rrc), four perradial tentacle bulbs (tb) pad-like, and tentacles (t) are very contractile (Figure 5A). Tentacles are first perpendicularly positioned (pp) to the umbrella or velum and then extended outward (Figure 5A).

Remarks: *P. minima* is distinguished by having a distinct, round trunk, and oral tentacles. The species resembles members of the family Rathkeidae as compared to that of the Hydractiniidae (Schuchert 2007). The initial parts of oral tentacles are positioned perpendicular to the oral velum (Figure 5A) unlike the oblique tentacles of *Lizzia blondina* Forbes, 1848 (Schuchert 2007).

Species Bolinopsis cf. vitrea

Phylum: Ctenophora
Class: Tentaculata
Order: Lobata

The material examined was a specimen at the stage of immature and collected from surface water at a seafloor depth of 48 m off Aydınçık, (36° 07′ 37″ N, 33° 13′ 39″ E, station 23E, Mersin coast, Turkey, eastern Mediterranean Sea) on 02 July 2019 using a Nansen bottle by Doğukan Karaca. The size of the specimen was 11.1 x 8.5 mm in the total (lobate) length x maximal body width. The specimen had also an aboral length of 9.6 mm, and an auricle length of 8.6 mm (Figure 6).

Description: Body shape from the tentacular plane is semi-ellipsoid oval, and laterally compressed. It has a blunt-aboral apex and wide oral lobes terminated between the apical location and

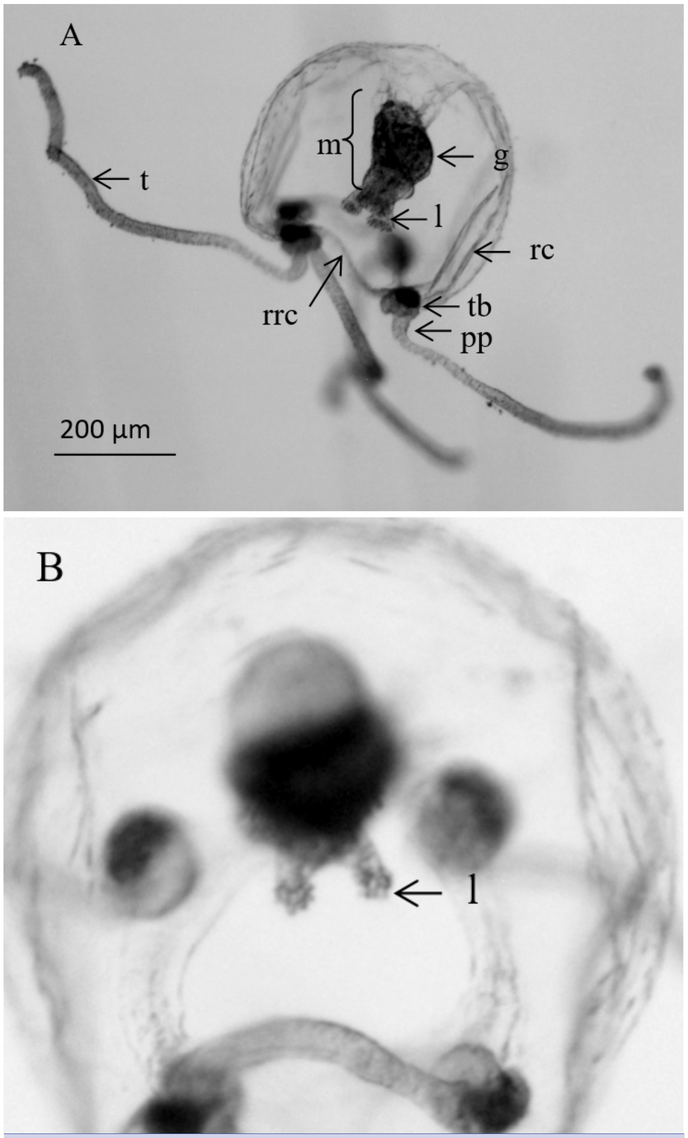

Figure 5. Liberated medusoid specimen of Podocorynoides minima; lateral view (A) and oral view focusing on the mouth lips (B). Manubrium (m), perradial lip (l), gonads (g), radial canals (RC), ring canal (rrc), tentacle bulbs (tb), tentacles (t), and tentacle in perpendicular position (pp) to velum.

mouth. Two poles of the specimen are about equal bluntness and lateral parts around the apical pole are characterized by multi-corners on the lateral of the body. The body surface has small granulation. Auricle (a) is developed and edged by ciliary combs (cc) and extends to just above the mouth. Tentacles (t) are very short and simple, initiated from tentacle bulbs (tb) located on each side of the mouth by extending to the bases of the auricles. Stomodaeum (s) is simple, long, and very narrow, has fine caliber chymiferous tubes, very simple meridional ventral tubes joined with the circumoral vessel, and two contrasted directional canals in "V" shape on each of two poles by joining to stomodaeum (Figure 6). Lobe (l) is initiated (li) in the middle part between mouth (m) and infundibulum (i)

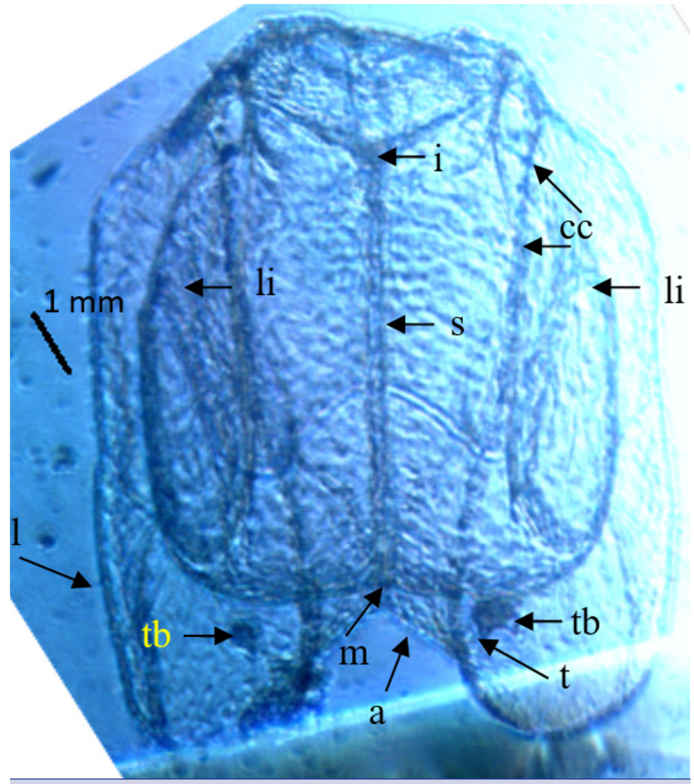

Figure 6. *Bolinopsis* cf. *vitrea*; view of the tentacular plane of its juvenile specimen. Auricle (a), ciliary combs (cc), tentacles (t), tentacle bulbs (tb), stomodaeum (s), lobe (l), initial region of the lobe (li), mouth (m), and infundibulum (i)

Remarks: According to descriptions performed by Mayer (1912), Oliveira et al. (2007), and Oliveira & Migotto (2006) for the adult specimen of *Bolinopsis vitrea* (L. Agassiz, 1860), all characters of a very simple and narrow stomodaeum, position of large tentacle bulbs, tip position of just developed auricle and oral lobe and mouth shape with reverse 'V' (Fig. 4) support that the specimen seems to be *B. vitrea*. Immature and mature *Mnemiopsis* specimens are distinguished as having auricle furrows (Oliveira et al., 2007), small tentacle bulbs positioned at the middle of the body and not acute and blunt aboral apex (Shiganova, 2020), and the specimen of the present study lacks the auricle furrows but having blunt aboral apex. Specimens of genus *Leucothea* pass to the stage of genus *Bolinopsis* which is closely related to *Leucothea* covered with small papillae on the whole outer surface (Mayer, 1912) as occurred in the present specimen with the very small pits-like granulates on the outer surface of the body. However, the present specimen lacks 2 remarkable blindly-ending sacs and the tentacle bulbs nearly to the level of the funnel. The tentacle bulbs are positioned at a level of mouth in the present specimen as differentiated by Oliveira et al. (2007). It is hereby remarked that the species could be *Bolinopsis vitrea* but was recognized as *Bolinopsis* cf. *vitrea* due to the following remarks: "I once got a juvenile like this and grown it in the lab (thinking it was either *Bolinopsis* or *Mnemiopsis*).... to turn out it was *Leucothea multicornis* once grown" (Fabien Lombard, pers. comm.) and "It is difficult with the small ones as the lobe morphology about statocyct change. What I can say for sure is that it does look like neither *Mnemiopsis* nor *Bolinopsis infundibulum*" (Cornelia Jaspers, pers. comm.).

Early stages of gelatinous specimens are very passive swimmers at movement and could be much more drifted by water currents than the adult specimens. Eumedusoid hydrozoan had a short stage passage to become adult (Gravili et al., 2007). This occurrence of such newcomers to a sea suggested that the specimen could be established in the region where they are found and could be passively moved from one sea to another sea via the water current depending on its velocity or via ship ballast water. This could induce a broad-scale distribution of the organisms in the Mediterranean Sea (Table 3).

Body morphometry and structure of gelatinous organisms were differentiated by the environmental parameters under lab conditions or from sea to sea worldwide (Gravili et al., 2007; Shiganova, 2020). Such differences led to the importance of local morphometry of the organisms reported to widen their descriptive knowledge. There are some studies published on such structural differences; Gravili et al. (2007) reared *G. raffaelei* under lab conditions in two different seasons and temperature conditions; 15 and 18 °C. The size of specimens at the same age is larger at 18 °C than at 15 °C. Our specimen fits with both sizes (4-5 days old) of specimen reared at 15 °C and the diagnostic structure of specimen reared at 18 °C rather lower than our sampling temperature of the water with higher chl-*a* concentration coincided with higher ammonium concentration than the other two stations (Table 2). Isinibilir, Yilmaz, & Demirel, 2015b showed a similar diagnostic structure of *G. raffaelei* in the Sea of Marmara, which is colder than the present study area, to the structure of specimen reared at 15 C (Gravili et al., 2007). Nutritional conditions as well as temperature could induce such differences between different Turkish and Italian waters. Near-bottom temperature was measured at around 22 C (Table 2). Similar structural differences were observed for a ctenophore species, *M. leidyi* in the European seas (Shiganova, 2020).

Gastroblasta raffaelei which endemic species to the Mediterranean Sea overspread the Mediterranean Sea, particularly the Adriatic, Tyrrhenian and Ligurian Seas (Bouillon et al., 2004; Gravili et al., 2007) and was first reported occurring in the Sea of Marmara for the Turkish waters (Isinibilir, Yilmaz, & Demirel, 2015b). Up to now, the species was however not reported for the Turkish Mediterranean coast (Çinar et al., 2014). *G. raffaelei* was recently recorded in the Catalan Sea (Guerrero et al., 2018) (Table 3). *Podocorynoides minima* has been distributed globally in the temperate waters of the world; Mediterranean, Atlantic Ocean, Indo-Pacific Ocean (Schuchert, 2007), Sea of Marmara (Isinibilir, Yilmaz, & Demirel, 2015b) and Black Sea (Table 3).

Regarding ctenophoran lobate distribution only in the Turkish waters, *M. leidyi* A. Agassiz, 1865 which was introduced first to the Russian waters of the Black Sea in 1987 (Vinogradov et al., 1989) was reported in the Turkish Black Sea in 1991 (Mutlu et al., 1994; Mutlu, 1999) and in the Mediterranean Sea, Mersin Bay in 1992 (Uysal & Mutlu, 1993; Kideys & Niermann 1993). *Bolinopsis vitrea* which is a cosmopolitan species in the world was reported in the Black Sea (Öztürk, Mihneva, & Shiganova, 2011), and *Leucothea multicornis* (Quoy & Gaimard, 1824), which is a cosmopolitan species in the world, in the Turkish Mediterranean Sea, Antalya Bay from a sample taken in 2020 (Gokoğlu & Galil, 2020). However, *B. vitrea* was generally observed in the eastern Mediterranean Sea and adjacent seas (Table 3). Recently, the occurrence of *Beroe mitrata* (Moser, 1907) was reported in the Turkish waters. (Killi, Abyzova, & Shiganova, 2019). However, Çinar et al. (2014) previously reported the species, *B. vitrea* from the Turkish Levant water. Nevertheless, *L. multicornis* was reported for the first observation in the eastern Mediterranean (Galil et al., 2014), followed by occurrences in the Syrian waters (Mamish, Durgham, & Ikhtiyar, 2019), and Greek waters (Digenis & Gerovasileiou, 2020).

Most of all three species records were performed from the western Mediterranean Sea and Adriatic Sea (Table 3). Regarding their Mediterranean-wide occurrence, a common location is Adriatic Sea with occurrence in our one sampling conducted in the summer of 2019. Furthermore, increased records have occurred in the Turkish marine waters (Table 1). This brings about speculation about the coincidence of the decadal formation of BiOS (Gacic et al., 2010) with three species found in the present study and other records (Table 1). The BiOS has the power to create the high-level variability of the Mediterranean Sea and the strong interconnection of its sub-basins by affecting the vectors of the saltier LIW, the LSW, and the fresher Atlantic Water (Civitarese et al., 2023). The BiOS which changed the cycle pattern of the Northern Ionian Gyre affected the Levant hydrographs in decadal periods. The Mid-Ionian Jet pumped the water toward the Turkish Mediterranean waters via two vectors: one directly to Rhodes gyre (RG) and the other to easternmost Levant water by the Atlantic current (CC) (Figure 2). The water of the study area had fewer saline waters and relatively high primary production (Table 2) as compared to the expected value (39 PSU) of the summer salinity in the Levant Sea (Lascaratos, Williams, & Tragou, 1993; Poulos, Drakopoulos, & Collins, 1997).

In comparison to the western vector introducing specimens to the Turkish Levant waters, Civitarese et al. (2023) monitored the decadal formation of the NIG circulation from 1996 to 2018. Regarding the records given in Table 1 and the present study, all physical data suggested an alternative way of water transportation by the BiOS or LIW derived by BiOS toward the Turkish waters (Figures 1, 2). Compared to regular summer measures existence of less saline and warmer water indicated that there was physical water transportation from somewhere to the present study area during the summer of 2019 as inferred from decadal surface water currents (Table 2, Figure 3).

In comparison to the eastern (Red Sea) vector introducing specimens to the Turkish Levant waters, Edelist et al. (2022) conducted a particle dispersal model to simulate jellyfish dispersion in space and showed a pathway of jellyfish distributional extension in time starting from the Egyptian (around Suez Canal) and Israeli coasts to the Turkish water on the set of the Atlantic water current path during 2017-2018 based on the sea surface current. Consequently, the particle arrived at İskenderun Bay which was far away from the record locations of the present study if the particles were released from the Egyptian coast. Subsequently, the particles arrived at Antalya Gulf which was on the set of record locations of the present study but far away from the record locations given in Figure 1 and locations of unpublished records given in Table 1 if the particles were released from the Israeli coast (Edelist et al., 2022).

Recently, Mutlu & Özvarol (2022a, b) and Uttieri et al. (2023) discussed possible introductory pathways of the zooplankton to the eastern Mediterranean Sea regarding the effect of the BiOS; three species (*Hormiphora plumosa* M. Sars, 1859, *Oceania armata* Kölliker, 1853 and *Pseudodiaptomus marinus*, Sato, 1913, respectively) are also observed in the Adriatic Sea and adjacent seas. Jasprica et al. (2022) related the BiOS caused by severe winter conditions to phytoplankton composition and distribution in the southern Adriatic Sea.

CONCLUSION

Early-staged specimens of hydrozoans and ctenophores could be morphometrically different under different environmental conditions and sea-to-sea. Description of these three immature gelatinous organisms was characterized for the Turkish Levant coast. However, records of the gelatinous organisms have increased in the Turkish waters. Discussing the possible vectors of their introduction to the Turkish coasts, water pumped by BiOS is bifurcated at Rhodes gyre. One branch was entrapped in Rhodes gyre, and another branch was joined to the Atlantic current, both affecting the Turkish Mediterranean coast (Figure 2) (Poulain et al., 2013). Therefore, it is possible that the species could have entered the eastern Mediterranean by the Atlantic and met BiOS through the western Mediterranean.

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Compliance with Ethical Standard: The authors declare that all applicable guidelines for sampling, care, and experimental use of animals in the study have been followed.

Conflict of interest: The authors have no conflicts of interest to declare that are relevant to the content of this article.

Ethical approval: The authors declare that all applicable guidelines for sampling, care, and experimental use of animals in the study have been followed.

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Research Article

Lakes of Turkey: Comprehensive Review of Lake Çıldır

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ABSTRACT

Lake Çıldır, situated in eastern Turkey, is one of the most noteworthy freshwater lakes in the region, distinguished for its aesthetic allure and its paramount ecological and socio-economic roles. This review thoroughly examines Lake Çıldır, beginning with an introduction that contextualizes its importance. The climatic conditions influencing the lake's dynamics, its hydrographic characteristics, and the physicochemical variables determining its water quality have been specified. Our review synthesizes findings about the lake's biological attributes, including its varied flora and fauna. Detailed sections are dedicated to the fish species, macrophytes, benthic macroinvertebrates, and phytoplankton that inhabit the lake. Using land cover data, temporal changes in the lake's environmental structure have been evaluated. The lake's trophic state and overall ecological health are critically discussed, shedding light on its current status. Furthermore, the potential and existing ecotourism practices around Lake Çıldır are explored, emphasizing its value as a tourist destination. In conclusion, the challenges faced by the lake are addressed, advocating for robust conservation and management initiatives. This comprehensive review of Lake Çıldır's complex ecosystem is a foundation for forthcoming research.

Keywords: Ecology, Ecotourism, Environmental structure, Lake Çıldır, Land cover, Trophic state

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INTRODUCTION

Situated in the northern region of East Anatolia, Lake Çıldır is located between the cities of Kars and Ardahan at 41° 00′ north latitude and 43° 12′ longitude in Turkey. Nestled within the provinces of Ardahan and Kars, Lake Çıldır, with its expanse of 123 km², stands as the second-largest lake in Eastern Anatolia. Positioned at an altitude of 1959 meters above sea level, it reaches a depth of 42 meters, encircled by a vast stretch of approximately 60 kilometers. The lake's genesis can be attributed to a confluence of a lava flow (Aykır & Fıçıcı, 2022). Lake Çıldır's hydrography is an intricate web of inflows and outflows. While myriad streams and springs replenish it, its sole drainage point is the Telek Creek, branching out to the Arpaçay near the Armenian frontier. Adjacent to the Akçakale ruins lies an island, further enhancing the lake's topography (Şimşek, 2019). Despite its limited vegetation, the lake's vicinities burgeon with pastures, underscoring the socio-economic dependencies on animal husbandry (Yerli, 1993). The regional economy is substantially bolstered by fishing, a year-round activity that persists even during icy winters. Predominantly, the mirror carp (*Cyprinus carpio*) is the prized catch (Yerli, 1997). Ecologically, the lake confronts adversities. Declining water levels in arid spells imperil habitats essential for fish reproduction, while unsustainable fishing practices exacerbate the strains on fish populations (Başçınar et al., 2009). Notably, the lake's northwestern fringes, adorned with wetlands and marshes, amplify its ecological heterogeneity. More than a mere geographic landmark, Lake Çıldır embodies a vibrant ecosystem, an economic linchpin, and a vault of regional heritage. Figure 1 offers a cartographic representation of its location, providing a spatial context;

according to the Turkish General Directorate of Mapping Data, maps were prepared using *ESRI* ArcMap 10.7 software. This paper holistically examines Lake Çıldır, advocating its preservation while spotlighting its multifaceted challenges in our evolving global context.

the connection between the Lake Çıldır basin and the Çıldır Plain, eventually fostering the formation of the lake, nurtured by streams originating from the encompassing terrains (Atalay et al., 2018). The prevalent soil types within the basin are chestnut-colored and basaltic (Karaoğlu & Çelim, 2018). The basaltic soils,

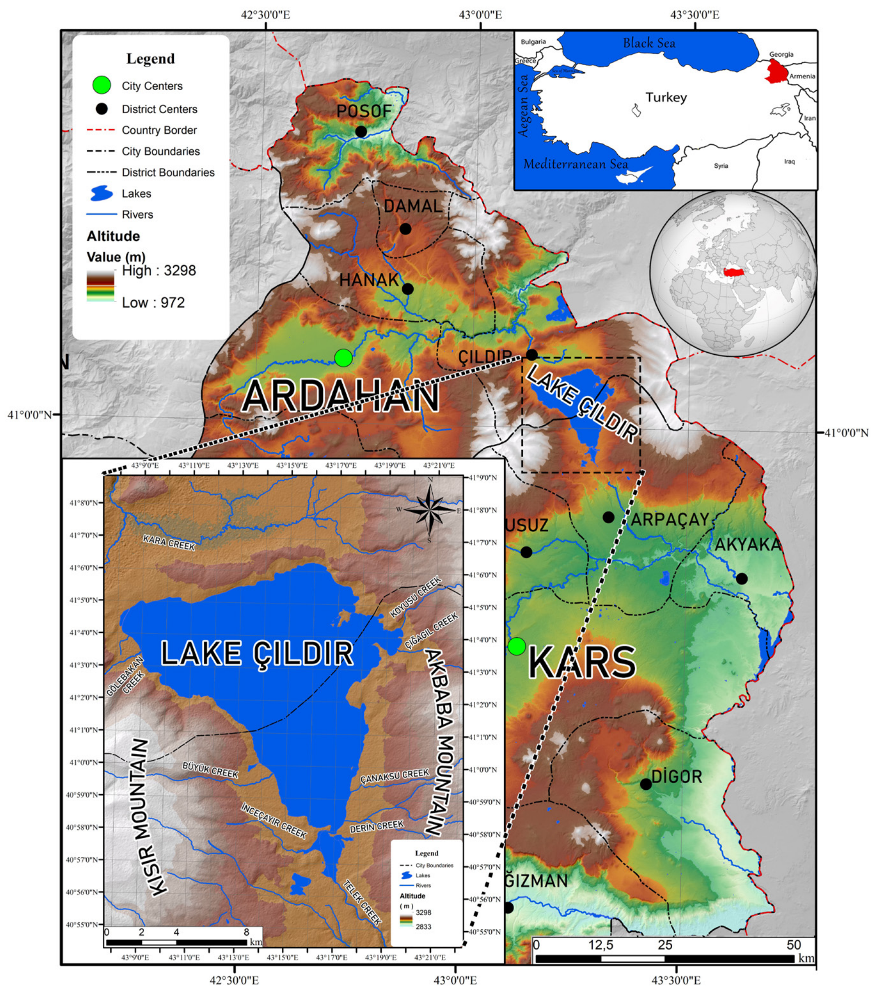

Figure 1. Geographical Position of Lake Çıldır in Turkey.

Geographical features

The geologic foundation of the Lake Çıldır basin is predominantly composed of volcanic tuff, block tuff, andesitic, and basaltic flows, supplemented by younger marl and conglomerates from the post-Oligocene period. During the Pliocene era, the region experienced significant vertical tectonic activities, precipitating block descents along the fault lines (Atalay et al., 2018). Historically, the Lake Çıldır basin and the adjacent Çıldır Plain existed as a unified depression, channeling its hydrologic flow towards the Kura River via the Çıldır Stream (N. E. Akbulut et al., 2022). However, subsequent lava flows partitioned this expansive basin into two distinct sections, creating an intermediary plain between the lava flow's forefront and the Kısır Mountain. Over time, the debris deposited from the Kısır Mountain culminated in the total occlusion of this corridor. This geologic evolution effectively severed

emerging from the volcanic bedrock, are characterized by their proficient drainage capabilities and clayey surface strata (Öztürk et al., 2015).

Climatic features

Situated in the northeastern part of the Eastern Anatolia Region, Lake Çıldır exemplifies the pronounced continental climate characteristic of the area. Due to its distinctive geographical position, the region is one of Turkey's most intensely affected areas by continental climate. This results in short, cool summers and bitterly cold winters (Şimşek, 2019).

The climatic characteristics of a region, encompassing temperature variations and precipitation patterns, are fundamental to comprehending its hydrological and ecological dynamics. Such knowledge is pivotal for formulating strategies for water resource management, agricultural activities, and the preservation of ecosystems. In this context, an analysis was conducted utilizing meteorological data collated by the Turkish State Meteorological Service from 1931 to 2023 for Kars and Ardahan. Figure 2 shows the precipitation and temperature averages for 2000-2023. As the temperature rises, the decrease in precipitation may pose a problem for species diversity and lake health. The findings indicate that the mean annual temperature for Kars is 4.8°C, reaching a peak in August at 17.8°C and a nadir in January at -10.8°C. Ardahan, on the other hand, exhibits a marginally lower mean annual temperature of 3.7°C, with the highest temperatures recorded in July and August at 16.0°C and the lowest in January at -11.2°C. Concerning precipitation, Kars records an annual average of 506.0 mm, while Ardahan experiences a slightly elevated average of 555.3 mm (Turkish State Meteorological Service, 2018). Notably, May is the month with the highest precipitation in both regions. This characteristic diverges from the typical precipitation patterns observed in other parts of Turkey, where summer is identified as the season with the highest rainfall. Conversely, the winter season is associated with the lowest precipitation levels in both Kars and Ardahan. This climatic information is crucial for developing effective strategies for water resource management, agricultural practices, and ecosystem conservation initiatives.

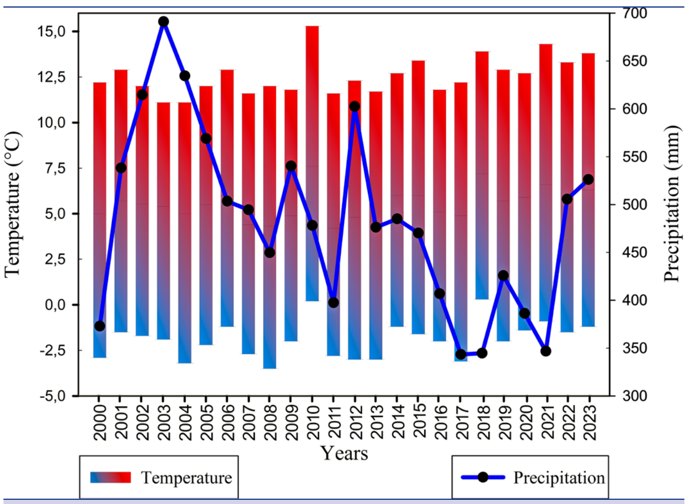

Figure 2. Temperature and precipitation with averages of Lake Çıldır.

Hydrographic features

Lake Çıldır, located in the catchment areas of the Aras and Kura rivers, holds a crucial hydrological role in the region, ultimately discharging into the Caspian Sea via these intersecting rivers in Azerbaijan's Sabirabad. The lake is predominantly replenished by snowmelt and several seasonal and perennial streams, originating from the Kısır Mountains in the west and the Akbaba Mountains in the east. Major tributaries on the western side include the Gölbelen, Kındırga, and Gölebakan rivers. In contrast, the eastern side receives contributions from the Gülyüzü River and smaller streams passing through the Göldalı and Çanaksu villages (Lahn, 1947). Lake Çıldır, characterized by its freshwater, has an overflow management mechanism that directs excess water through a narrow corridor near Taşbaşı Village to the Telek Creek and eventually to the Arpaçay. Additionally, the natural barrier between the Çıldır Plain and the lake basin facilitates the lake's assimilation of waters from various sources and streams originating from the plain. As a critical hydrological entity nourished by diverse geographical sources and feeding major river systems, Lake Çıldır's recognition is vital for informed water management and local ecosystem protection (Alkan et al., 2016).

Physicochemical variables

The health and function of lake ecosystems are intrinsically linked to their physicochemical properties, necessitating a comprehensive understanding of the interactions between these factors and their broader ecological consequences (Langdon et al., 2010). Parameters such as temperature, pH, dissolved oxygen, nutrient concentrations, and pollutants are diagnostic tools for assessing water quality and ecological status. These factors influence the metabolic rates of aquatic organisms, their life cycles, ecological interactions, nutrient solubility, productivity, and biodiversity (Brown et al., 2004). Furthermore, nutrient dynamics, particularly nitrogen and phosphorus, play a crucial role in lake ecology. Excessive anthropogenic inputs can lead to eutrophication, which reduces light and oxygen availability, adversely affecting aquatic systems; pollutants, heavy metals, and organic compounds pose significant risks, potentially leading to bioaccumulation and biomagnification in the food chain (Ali & Khan, 2019).

By integrating physicochemical data with environmental and biological indicators, we can comprehensively evaluate lake health and identify potential ecological disturbances (Beck & Hatch, 2009). Such insights are crucial for developing effective management strategies to restore and maintain the balance of lake ecosystems. Therefore, understanding and analyzing the interrelationships between physicochemical properties and biological dynamics is essential for preserving our precious freshwater resources. Table 1 indicates the physicochemical data of Lake Çıldır.

Lake Çıldır has been reported to show no signs of sewage and eutrophication, and it has been characterized as oligotrophic based on physicochemical parameters. However, due to population growth and the effects of pollution elements originating from residential areas and agricultural and livestock enterprises in its vicinity, Lake Çıldır is gradually becoming polluted (Çakır & Çiçek, 2015).

The heavy metal levels in the surface sediments of the northern littoral zone of Lake Çıldır have been studied, and it has been found that the lake is affected by the ecological effects of heavy metals. Additionally, the lake has been found to contain heavy metal levels, including Cu, Pb, Zn, Ni, Mn, Fe, As, Cd, Cr, and Hg, which may have ecological effects (Kükrer et al., 2015).

Biological feature

From a botanical standpoint, Turkey's Central and Eastern Anatolia regions are characterized by the presence of Iran-Turan flora, with a notable distribution in Northeast Anatolia. In contrast, the area surrounding Lake Çıldır dominates the European-Siberian

Table 1. Physicochemical variables in Lake Çıldır (Çelekli et al., 2020).

Variables	unit	Mean±SD	Minimum	Maximum
Temperature	°C	16.9±2.7	14.80	19.90
pH		7.94±1.29	6.47	8.86
EC	µS/cm	134±16	117.00	149.00
Salinity	ppt	0.10±0.05	0.07	0.15
DO	mg/l	8.86±0.29	8.53	9.05
TSS	mg/l	19.9±26.1	2.80	50.00
BOD_5	mg/l	4.7±1.4	3.70	6.30
COD	mg/l	19.6±5.0	14.50	24.40
TOC	mg/l	2.5±0.4	2.10	2.80
TN	mg/l	0.40±0.10	0.32	0.51
$N\text{-}NH_4$	mg/l	0.11±0.02	0.10	0.14
$N\text{-}NO_2$	mg/l	0.01±0.01	0.00	0.01
$N\text{-}NO_3$	mg/l	0.14±0.05	0.10	0.20
TP	mg/l	0.14±0.12	0.05	0.28
$P\text{-}PO_4$	mg/l	0.11±0.16	0.01	0.30
Secchi depth	m	3.00±1.32	2.50	4.00

The average and standard deviation (SD) of Lake Çıldır's environmental variables and their minimum and maximum values in brackets are provided. These variables include DO (dissolved oxygen), EC (electrical conductivity), TSS (total suspended solids), BOD5 (biological oxygen demand), TOC (total organic carbon), TN (total nitrogen), N-NO2 (nitrite), N-NO3 (nitrate), N-NH4 (ammonium), P-PO4 (orthophosphate), and TP (total phosphorus).

phytogeographic elements, reflecting the lake's unique geographic positioning (Atalay et al., 2018). Identifies four distinct vegetation types around the lake: steppe, meadow, rock, and tree-shrub. Salient species include willow variants (*Salix caucasica* and *Salix caprea*), shrubs (*Spiraea hypericifolia*, *Padus avium*), and wild plum (*Prunus spinosa*). The vegetation's diversity can be attributed to the area's short 4-5 months period and distinct geographical conditions. At Lake Çıldır, according to the International Union for Conservation of Nature, endemic plants are in danger. Some endemic plants found in the flora of Kars-Ardahan and Lake Çıldır are *Senecio integrifolius* subsp. *karsianus*, *Veronica thymoides*, *Chaerophyllum karsianum*, *Veronica gentianoides*, *Lathyrus karsianus* (Figure 3), *Festuca karsianum*, *Allium karsianum*, *Nonea karsensis* (Akgül & Aytaç, 2008; Armağan et al., 2017).

The aquatic ecosystem of Lake Çıldır is remarkably diverse. The lake is a habitat for native fish species such as the common carp (*Cyprinus carpio*) and the Caspian trout (*Salmo trutta caspius*). Additionally, the Prussian carp - (*Carassius gibelio*) and the crayfish (*Pontastacus leptodactylus*) are shown in Figure 4. There are also rainbow trout populations (*Oncorhynchus mykiss*) within the lake (Çiçek et al., 2022; Çiçek & Birecikligil, 2016; Koçyiğit & Önder, 2018; Zengin et al., 2012).

Avifauna around Lake Çıldır is also noteworthy. Regional data indicates the presence of 86 bird species, including significant ones like velvet ducks (*Melanitta fusca*) and pintails (*Anas acuta*). Two "bird islands" within the lake are hubs for dense bird populations, mainly gulls, providing a visually snow-like spectacle. Historically, pelican species nested here, with recent sightings suggesting potential breeding activity linked to neighboring Lake Aktaş (Kartzahi). The Dalmatian pelican (*Pelecanus crispus*), ruddy shelduck (*Tadorna ferruginea*), common crane (*Grus grus*), and Armenian gull (*Larus armenicus*) are bird species that meet global and/or, regional Important Bird and Biodiversity Area criteria. The area is a critical stopover for avian migration during spring and autumn. Notably, from mid-September onward, significant numbers of common buzzards (*Buteo buteo*) and lesser spotted eagles (*Aquila pomarina*) can be observed in the north-eastern and southwestern parts of the area. It is possible to monitor with the Bird Observation Tower in Lake Çıldır (Figure 5). However, there is no available data regarding the exact population sizes of these species (Azizoğlu & Adızel, 2020; Crivelli & Vizi, 1981; Özkoç, 2020; Şimşek, 2019; Yılmaz et al., 2018).

Figure 3. *Lathyrus karsianus* **a.** live plant (Savran, 2021) **b.** herbarium sample (POWO, 2023).

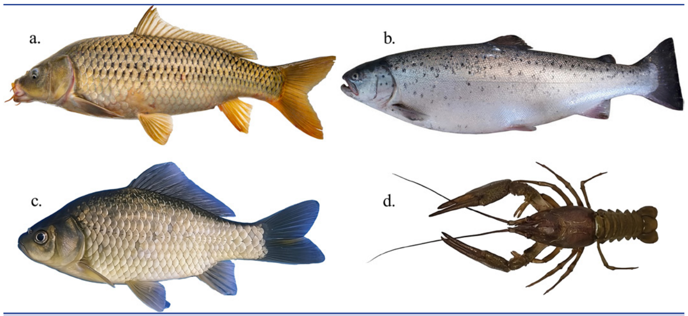

Figure 4. **a.** *Cyprinus carpio* (The Fishes of North Carolina, 2023), **b.** *Salmo trutta caspius* (Jouladeh-Roudbar et al., 2020), **c.** *Carassius gibelio* (Kalous et al., 2012), **d.** *Pontastacus leptodactylus.*

Figure 5. Photos from the Bird Observation Tower in Lake Çıldır (Çıldır District Governorate, 2023).

Lake Çıldır showcases a diverse macrozoobenthic community, with notable species such as *Armiger crista* and *Paratanytarsus lauterborni*. Recent research has highlighted the lake's rich aquatic oligochaete diversity, identifying 22 species, including new records like *Pristina synclites* and *Bratislavia palmeni*. Furthermore, a comprehensive analysis revealed that the lake's macrozoobenthic fauna encompasses 47 taxa, two unprecedented records for Turkey's Oligochaeta fauna (Arslan & Mercan, 2020; Mercan et al., 2022).

Lake Çıldır is characterized by a rich and varied phytoplankton community, reflecting its ecological complexity and significance in freshwater ecosystems. Phytoplankton play a crucial role in the global carbon cycle and are responsible for producing a significant amount of oxygen. Problems related to the carbon cycle are the causes of global warming and can cause various problems, up to human migrations (Çelekli et al., 2023). Their species diversity and abundance act as water quality indicators due to their different responses to environmental conditions (Çelekli et al., 2020). In addition, phytoplankton are of significant biotechnological importance; some are the removal of harmful dyes. (Zariç et al., 2022). As part of the research, 74 phytoplankton species have been identified from five lakes in the Aras River basin, including Lake Çıldır. *Cocconeis placentula*, *Ulnaria ulna*, *Cymbella affinis*, *Lindavia bodanica*, *Pseudopediastrum boryanum*, and *Ankistrodesmus arcuatus* were among the commonly found species; some of them indicated in Figure 6. Bacillariophyta was the dominant group in the lake based on their values of phytoplankton biovolume in all sampling times. The diatoms contributed (89%) to the total phytoplankton biovolume in Lake Çıldır (Çelekli et al., 2020). As mentioned above, the common diatom species of Lake Çıldır are also the most widespread and abundant in other lakes in Turkey. However, some species were found to be rare, such as *Coscinodiscus* sp., *Staurosirella martyi*, *Didymosphenia geminata*, *Stauroneis acute*, *Diatoma hiemale*, *Aulacoseira ambigua*, and *Ellerbeckia arenaria* (Akbulut & Yıldız, 2002).

In light of the above findings, it is evident that Lake Çıldır represents a complex nexus of biogeographic, ecological, and anthropogenic influences. Given its environmental significance, devising and implementing holistic conservation and management strategies is imperative.

Trophic state and ecological status of Lake Çıldır

Phytoplankton biomass, chlorophyll-a, total phosphorus (TP), and Secchi disk depth are essential indicators of lakes' trophic state and ecological status (Poniewozik & Lenard, 2022). Changes in water color intensity can affect a lake's physical, chemical, and biological parameters, including the concentration of TP and nitrogen and the biomass and composition of phytoplankton (Lenard et al., 2018). Secchi disk depth and TP values obtained during the study were used to determine the trophic status of ecosystems according to OECD criteria. Results of Carlson's trophic state index (Carlson, 1977) based on Secchi depth (TSI_{SD}) indicated that Lake Çıldır was mesotrophic characteristic (Çelekli et al., 2020). The modified phytoplankton trophic index-MPTI (Çelekli, 2016) and the Mediterranean phytoplankton trophic index-Med-PTI (Marchetto et al., 2009) were used to estimate the ecological status of the lake. The high ecological status in the Aras River basin was only found in Lake Çıldır (Çelekli et al., 2020). Although the lake's condition appears better than others, it could be due to its larger surface area compared to others. Continued conservation efforts are necessary because of the significant human impact on the lake.

The land cover changes Lake Çıldır

Lake Çıldır has undergone significant land cover transformations from 2000 to 2018. This was analyzed using CORINE Land Cover (CLC) (EAA,2018) data from the Copernicus Land Monitoring Service and visualized using *ESRI* ArcMap 10.7 software, as demonstrated in Figure 7 and their results are given in Table 2. This amalgamation of satellite, sensor network data, and land cover data allowed for a thorough examination and intuitive comprehension of the spatial distribution and transformations of various land cover types over the studied period.

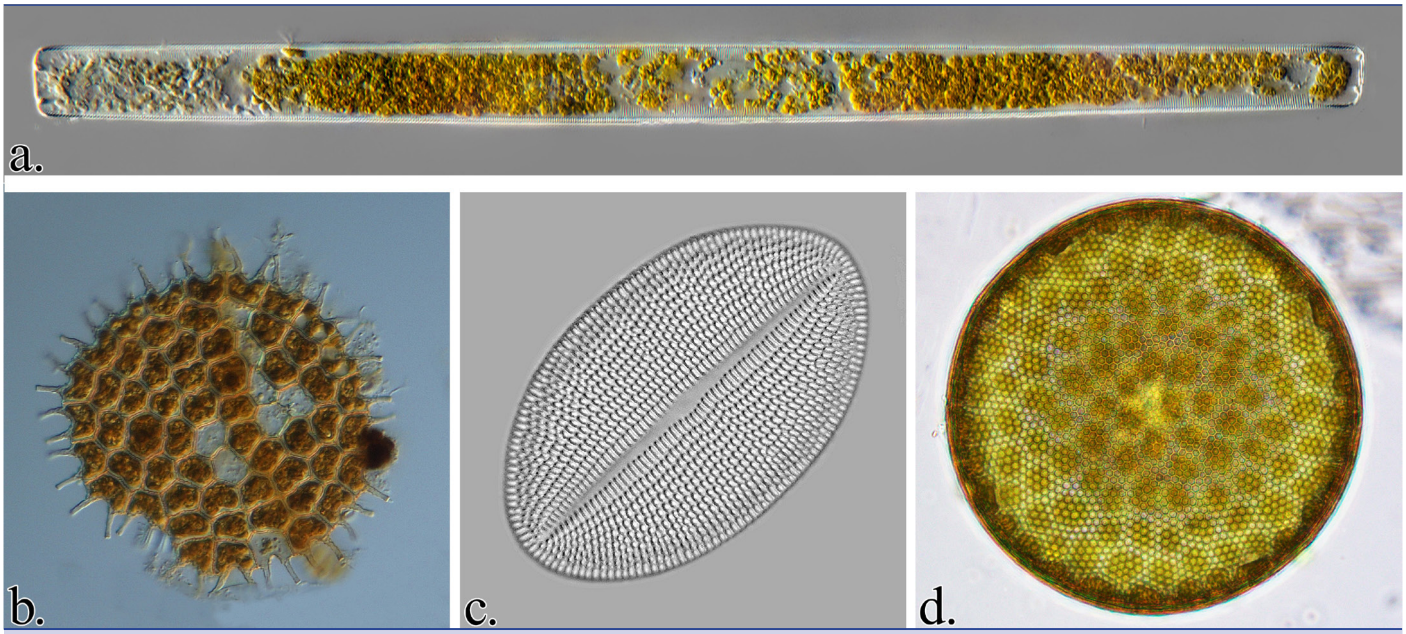

Figure 6. **a.** *Ulnaria ulna*, **b.** *Pseudopediastrum boryanum*, **c.** *Cocconeis placentula*, **d.** *Coscinodiscus* sp. (AlgaeBase, 2023).

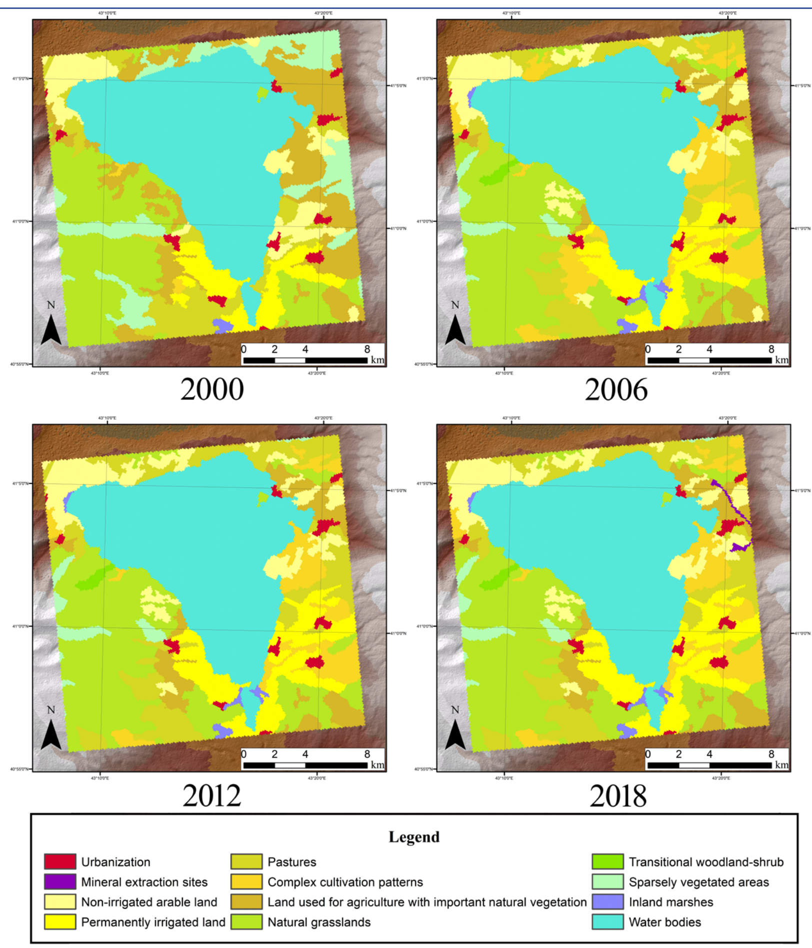

Figure 7. Lake Çıldır CLC Map (2000-2018).

Table 2. Land cover changes around Lake Çıldır from 2000 to 2018 (CLC, 2018).

Landcover (%)	2000	2006	2012	2018
Urbanization	0.49	0.45	0.55	0.56
Mineral extraction sites	0.00	0.00	0.00	0.14
Permanently irrigated land	2.39	3.22	3.36	3.42
Non-irrigated arable land	3.54	3.90	5.06	5.35
Pastures	2.90	20.31	24.26	22.46
Complex cultivation patterns	0.09	5.01	3.30	3.79
LUAINV	6.33	2.75	6.21	6.49
Natural grasslands	39.43	50.61	40.67	40.99
Transitional woodland-shrub	0.00	0.16	0.19	0.19
Sparsely vegetated areas	33.48	1.28	1.79	1.81
Inland marshes	0.10	0.24	0.22	0.23
Water bodies	11.24	12.06	14.39	14.58

LUAINV: Land used for agriculture with important natural vegetation

Lake Çıldır experienced considerable alterations in its land cover between 2000 and 2018, underscoring a significant anthropogenic impact on the region. This period saw an uptick in urbanization, indicating steady urban development near the lake. Concurrently, mineral extraction sites, which were absent until 2006, accounted for a notable proportion of the land use by 2018. Moreover, a marginal increase was observed in irrigated and non-irrigated agricultural lands, hinting at a broader expansion of agriculture in the region. Similarly, the expansion of pastures was observed, which is likely attributable to intensified livestock farming. Conversely, natural grasslands experienced a slight increment, and there was a discernible increase in water bodies, potentially attributable to land use modifications impacting water runoff. These transformations carry profound implications for the ecosystem of Lake Çıldır. The enlargement of agricultural lands and pastures may escalate nutrient runoff into the lake, thereby heightening the risk of eutrophication, a phenomenon detrimental to aquatic life due to the resultant depletion of oxygen levels in the water. Additionally, the advent of mineral extraction sites could adversely affect water quality, contingent on the extraction techniques employed. The augmentation of water bodies might precipitate modifications to the lake's hydrological regime, thereby influencing its water level and, subsequently, its ecological attributes. Furthermore, the marginal alterations in natural grasslands and the reduction in sparsely vegetated areas may engender shifts in the region's flora and fauna, impacting the lake's ecosystem.

In light of these findings, it is imperative to institute robust conservation and management measures to ameliorate the potential adverse repercussions on the Lake Çıldır ecosystem. Such measures should encompass implementing sustainable agricultural practices to curtail nutrient runoff, oversee and regulate mineral extraction operations, and execute land use planning and management strategies to safeguard natural habitats and biodiversity. Additionally, it is crucial to regularly monitor the lake's water quality and ecological attributes to identify and address any untoward alterations expeditiously.

Ecotourism of Lake Çıldır

Lake Çıldır offers significant potential for ecotourism, with its pristine natural beauty, diverse wildlife, and unique ecological characteristics attracting nature enthusiasts and adventure seekers. The establishment of nature trails around the lake allows visitors to explore the surrounding ecosystems, providing a close observation of its rich flora and fauna. The lake and its vicinity host diverse wildlife, including mammals, reptiles, and amphibians. Observation points facilitate the study and appreciation of these local species. Beyond its natural wonders, Lake Çıldır's ecotourism encompasses cultural experiences. Visitors can engage with local communities, learn about their traditional practices and the cultural significance of the lake, and experience artisanal showcases and traditional food tastings. One of the most unique experiences is walking on the frozen expanse of the lake during winter. However, visitors should be mindful of the conditions and prioritize safety. During the peak of winter, when temperatures plummet, the lake freezes over with ice reaching up to 80 cm in thickness (Çimen & Erginal, 2015). This provides a unique opportunity for ice fishing in an "Eskimo-style." The fish caught are then prepared and served, offering the region an authentic taste. Another popular winter activity is taking horse-drawn sleigh tours on the frozen lake, providing a traditional and delightful experience. Organized every February, the Lake Çıldır Festival sees participation from neighboring countries like Georgia, Azerbaijan, Turkmenistan, and Armenia. The Minstrel Festival coincides with this, making it a prime time for visitors to experience winter tourism and cultural festivities. This attraction resembles a frozen city, featuring an ice tower, green caves of pine leaves, a fish museum representing the Lake Çıldır fish, a fruit museum, a crystal cave, and a ghost house. Photographs from the lake Çıldır are shown in Figure 8. Visiting the Ice City, especially when illuminated at night, allows visitors to explore polarly (Çıldır District Governorate, 2023; Sezer, 2022).

Lake Çıldır is a testament to nature's splendor, rich cultural heritage, and diverse ecosystems. It presents a unique blend of ecotourism activities, from walking on its frozen expanse to delving into local traditions, making it an invaluable destination for na-

Figure 8. Lake Çıldır **a-c.** (Çıldır District Governorate, 2023), **d.** (Zariç, 2023).

ture aficionados and cultural enthusiasts. As the push for sustainable and immersive travel experiences continues to grow, Lake Çıldır undeniably offers an authentic and enriching journey that resonates with the core values of ecotourism.

CHALLENGES FACING LAKE ÇILDIR AND RECOMMENDATIONS FOR SUSTAINABLE MANAGEMENT

Lake Çıldır a pristine aquatic gem in eastern Turkey. Lake Çıldır has faced ecological challenges since the 1990s. Introducing invasive species, specifically the silver Prussian carp (*Carassius gibelio*) and the narrow-clawed crayfish (*Pontastacus leptodactylus*), has threatened the lake's natural balance. Moreover, attempts at introducing aquaculture, like rainbow trout (*Oncorhynchus mykiss*) farming, have further strained native species and raised sustainability issues. The situation has been aggravated by the operational expansion of the Arpaçay Hydroelectric Plant, which has negatively impacted the lake's shallow coasts, essential breeding grounds for native carp (Zengin et al., 2012). As tourism activities burgeon and exotic species make their mark, an exigent need exists to address these issues with sustainable and academically sound solutions. The following section delineates the challenges faced by the lake and provides recommendations for its sustainable management:

Preservation of current state and ecotourism enhancements:
Recommendation: Ecotourism activities around Lake Çıldır should underscore the importance of environmental education and conservation. It is crucial to instill in visitors the essence of ecosystem preservation, minimizing anthropogenic impacts, and ardently supporting local conservation endeavors. In tandem with this, developing eco-friendly accommodations, such as sustainable eco-lodges and camping sites, can significantly boost the sustainability quotient of ecotourism in the region. Infrastructure developments should be meticulously planned and executed, ensuring a minimal ecological footprint and a relentless commitment to conserving the natural landscape.

Exotic and invasive species

The lake has witnessed the incursion of invasive species, such as the Prusian carp and freshwater crayfish, corroborated by local accounts and other studies (Zengin et al., 2012). Prussian carp are recognized for their aggressive colonization of new environments and their ability to establish dominance, often outcompeting the majority of native species in the process. (Ruppert et al., 2017). Nonetheless, environmental, historical, and anthropogenic processes closely affect the alterations in the native fish population in the ecosystems (Anas & Mandrak, 2021). It is imperative to introduce stringent monitoring systems to track the population dynamics of these exotic species. Furthermore, awareness campaigns should be launched to educate the local populace about the detrimental impacts of introducing non-native species into the lake.

Environmental hygiene and cleanliness:
Robust sanitation and waste management protocols should be implemented. Regular underwater and on the shores, standard clean-up drives will help maintain the lake's pristine nature. Efforts should also be geared towards sensitizing visitors about the importance of leaving no trace.

Promotion of ecotourism activities
The winter charm of Lake Çıldır, characterized by its frozen expanse, offers many sports and recreational activities. The region can garner significant economic benefits by promoting ice tourism, which appeals to adventure enthusiasts and tourists driven by novelty-seeking motivations. This includes activities like sleigh rides, the Golden Horse Festival, ice skating, ice hockey, and ATV tours. However, it is paramount that these activities are conducted with environmental sensitivity to avoid undue strain on the lake's ecosystem.

Regulation and oversight of local enterprises
Stringent guidelines and regulations should be established for local businesses near the lake. Regular audits and checks can ensure compliance with environmental standards, ensuring the lake's health is not compromised for commercial gains.

Educating and empowering the local community
The local community plays a pivotal role in conserving Lake Çıldır. Organizing workshops, seminars, and interactive sessions can impart knowledge and skills to locals, enabling them to champion the cause of lake conservation. We can foster a collective responsibility toward its well-being by ensuring its stake in the lake's future.

In conclusion, while Lake Çıldır faces multifaceted challenges, with strategic interventions and a commitment to sustainability, it can continue to thrive as both a natural wonder and a hub of ecotourism.

CONCLUSION

Lake Çıldır, situated in Eastern Turkey, presents a significant confluence of ecological and cultural richness. The empirical evaluations underscore its superior environmental status within the Aras River basin. However, its pristine conditions have been incrementally perturbed due to anthropogenic intrusions, notably the introduction of invasive species and burgeoning tourism activities. The repercussions of these interventions are manifested not only in the fluctuating trophic state of the lake but also in its altered phytoplankton composition and biomass.

The juxtaposition of ecological challenges and the robust potential for ecotourism necessitates a paradigm shift in the lake's management strategies. To this end, a suite of recommendations has been advanced, pivoting on sustainable eco-tourism, vigilant monitoring of invasive species, enhanced environmental hygiene protocols, judicious oversight of local enterprises, and a pivotal emphasis on community education and empowerment.

It is paramount to accentuate that the sustainable management of Lake Çıldır not only bears implications for its intrinsic ecological integrity but also resonates with the broader tenets of sustainable ecotourism. Embracing a holistic approach that harmoniously integrates conservation with socio-economic aspirations is, thus, imperative for the lake's future trajectory.

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- Copyright Agreement Form,
- Author Contributions Form, and
- ICMJE Potential Conflict of Interest Disclosure Form (should be filled in by all contributing authors)

Preparation of the Manuscript

Title page: A separate title page should be submitted with all submissions and this page should include:

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- Name(s), affiliations, and highest academic degree(s) of the author(s) and ORCID ID (orcid.org)
- Grant information and detailed information on the other sources of financial support,
- Name, address, telephone (including the mobile phone number) and fax numbers, and email address of the corresponding author,

- Acknowledgment of the individuals who contributed to the preparation of the manuscript but who do not fulfil the authorship criteria.

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Keywords: Each submission must be accompanied by a minimum of three to a maximum of six keywords for subject indexing at the end of the abstract.

Manuscript Types

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Units should be prepared in accordance with the International System of Units (SI).

After the Conclusion section and before references list, information regarding conflict of interest, financial disclosure, ethics committee approval and acknowledgement are given. These information are to be provided in the author form which must be submitted togather with the manuscript.

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Ethics committee approval: Ethical committee approval is routinely requested from every research article based on experiments on living organisms and humans. Sometimes, studies from different countries may not have the approval of the ethics committee, and the authors may argue that they do not need the approval of their work. In such situations, we consult COPE's "Guidance for Editors: Research, Audit and Service Evaluations" document and evaluate the study at the editorial board and decide whether or not it needs approval.

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Tables

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.

Table 1. Limitations for each manuscript type

Type of manuscript	Page	Abstract word limit	Reference limit
Original Article	≤20	250	40
Review Article	≤25	250	60
Short Communication	≤5	250	20

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Figures, graphics, and photographs should be submitted as separate files (in TIFF or JPEG format) through the submission system. The files should not be embedded in a Word document or the main document. When there are figure subunits, the subunits should not be merged to form a single image. Each subunit should be submitted separately through the submission system. Images should not be labeled (a, b, c, etc.) to indicate figure subunits. Thick and thin arrows, arrowheads, stars, asterisks, and similar marks can be used on the images to support figure legends. Like the rest of the submission, the figures too should be blind. Any information within the images that may indicate an individual or institution should be blinded. The minimum resolution of each submitted figure should be 300 DPI. To prevent delays in the evaluation process, all submitted figures should be clear in resolution and large in size (minimum dimensions: 100 × 100 mm). Figure legends should be listed at the end of the main document.

All acronyms and abbreviations used in the manuscript should be defined at first use, both in the abstract and in the main text. The abbreviation should be provided in parentheses following the definition.

When a drug, product, hardware, or software program is mentioned within the main text, product information, including the name of the product, the producer of the product, and city and the country of the company (including the state if in USA), should be provided in parentheses in the following format: "Discovery St PET/CT scanner (General Electric, Milwaukee, WI, USA)"

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.

Limitations, drawbacks, and the shortcomings of original articles should be mentioned in the Discussion section before the conclusion paragraph.

References

While citing publications, preference should be given to the latest, most up-to-date publications. If an ahead-of-print publication is cited, the DOI number should be provided. Authors are responsible for the accuracy of references. List references in alphabetical order. Each listed reference should be cited in text, and each text citation should be listed in the References section. The reference styles for different types of publications are presented in the following examples.

Reference Style and Format

Aquatic Sciences and Engineering complies with APA (American Psychological Association) style 6th Edition for referencing and quoting. For more information:

- American Psychological Association. (2010). Publication manual of the American Psychological Association (6th ed.). Washington, DC: APA.
- http://www.apastyle.org

Accuracy of citation is the author's responsibility. All references should be cited in text. Reference list must be in alphabetical order. Type references in the style shown below.

Citations in the Text

Citations must be indicated with the author surname and publication year within the parenthesis.

If more than one citation is made within the same paranthesis, separate them with (;).

Samples:

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(Akyolcu, 2007)
Citation with two authors;
(Sayıner & Demirci, 2007)
Citation with three, four, five authors;
First citation in the text: (Ailen, Ciambrune, & Welch, 2000) Subsequent citations in the text: (Ailen et al., 2000)
Citations with more than six authors;
(Çavdar et al., 2003)

Major Citations for a Reference List

Note: All second and third lines in the APA Bibliography should be indented.

- **A book in print:** Baxter, C. (1997). *Race equality in health care and education.* Philadelphia: Ballière Tindall. ISBN 4546465465
- **A book chapter, print version:** Haybron, D. M. (2008). Philosophy and the science of subjective well-being. In M. Eid & R. J. Larsen (Eds.), *The science of subjective well-being* (pp. 17-43). New York, NY: Guilford Press. ISBN 4546469999
- **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)
- **An article in a print journal:** Carter, S. & Dunbar-Odom, D. (2009). The converging literacies center: An integrated model for writing programs. *Kairos: A Journal of Rhetoric, Technology, and Pedagogy, 14*(1), 38-48.
- **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)
- **Photograph (from book, magazine or webpage):** Close, C. (2002). *Ronald.* [photograph]. Museum of Modern Art, New York, NY. Retrieved from http://www.moma.org/collection/object.php?object_id=108890 (accessed 10.10.15)
- **Artwork - from library database:** Clark, L. (c.a. 1960's). *Man with Baby.* [photograph]. George Eastman House, Rochester, NY. Retrieved from ARTstor
- **Artwork - from website:** Close, C. (2002). *Ronald.* [photograph]. Museum of Modern Art, New York. Retrieved from http://www.moma.org/collection/browse_results.php?object_id=108890 (accessed 10.10.15)

REVISIONS

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Accepted manuscripts are copy-edited for grammar, punctuation, and format. Once the publication process of a manuscript is completed, it is published online on the journal's webpage as an ahead-of-print publication before it is included in its scheduled issue. A PDF proof of the accepted manuscript is sent to the corresponding author and their publication approval is requested within 2 days of their receipt of the proof.

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