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Original Article

Relationship Between by Catch Ratio of Sardine-Anchovy Targeted Purse Seine and Some Environmental Factors Based on a General Addictive Model in the Aegean Sea

Tevfik Ceyhan[1], **Zafer Tosunoğlu**[1]

Cite this article as: Ceyhan, T., & Tosunoğlu, Z. (2022). Relationship between by catch ratio of sardine-anchovy targeted purse seine and some environmental factors based on a general addictive model in the Aegean Sea. *Aquatic Sciences and Engineering, 37*(1), 1-7.

ABSTRACT

By catch is a serious conservation challenge for the fisheries whose viability is increasingly under threat. To approach maximum by catch reduction with minimum loss of targeted catch, fisheries need to have information on the environmental and anthropogenic factors in multi species seas like the Mediterranean. In this study, we used generalized additive models (GAM) to by the catch ratio of purse seine fishery to determine the effects of environmental variables. The data were collected during each fishing trip in the 2018-2019 fishing season that covers the time period between September 1st and April 15th. There were 26 species (66.216 mt in total) recorded as by catch and the rates of by catch species in the total by catch amount varied between 0.001% and 23.1%. In terms of habitat of by catch species, the total ratios of benthopelagic, demersal and pelagic species were 52% , 28% and 20%, respectively. Significant interactions observed indicate that the fluctuations in by catch ratios differed by depth and sea surface temperature, whereas the quarters of year and the moon phases were not found to affect by catch ratios significantly.

Keywords: Incidental Catch, Mediterranean, Small Pelagic, Depth, Moon Phase Interaction

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INTRODUCTION

The variety of definitions of the term "bycatch" (Alverson, *et al.*, 1994; Casale, 2011; D. C. Dunn, Boustany, & Halpin, 2011; Hall, Alverson, & Metuzals, 2000; Hall & Mainprize, 2005) might be still controversial but it becomes a serious conservation challenge for the fisheries whose viability is increasingly under threat because of overexploited stocks and low economic performance etc. (Maynou, 2020).

The studies on the effect of environmental, spatial variable gear design and sinking performance associated with incidental catch were generally modelled for tuna purse seine fisheries (Escalle et al., 2016; Hu, Harrison, Hinton, Siegrist, & Kiefer, 2018; Martínez-Rincón, Ortega-García, & Vaca-Rodríguez, 2012; Martinez-Rincon, Ortega-Garcia, Vaca-Rodriguez, & Griffiths, 2015; Tang, Xu, Wang, & Zhou, 2015; Tang et al., 2017). However studies on the by catch in small pelagic purse seine fisheries are rather limited (Arcos & Oro, 2002; Marçalo et al., 2015, 2010; Norriss, Fisher, & Denham, 2020; Teixeira et al., 2016; Tsagarakis, Vassilopoulou, Kallianiotis, & Machias, 2012; Tsitsika & Maravelias, 2006, 2008).

To approach maximum by catch reduction with minimum loss of targeted catch, fisheries need to have information on the environmental and anthropogenic factors in multi species seas like the Mediterranean. These studies are also essential to have ecologically based approaches for fisheries management. Because the evaluation of the environmental characteristics of high incidental catch and bycatch is expected to

contribute to this management goal. From this point forth, we used generalized additive models (GAM) to by catch ratio during purse-seine operations based on environmental variables to determine the environmental factors that influence the by catch ratio. GAMs have been widely used as a statistical modelling tool to analyse relationships between species distribution and the environment (Martínez-Rincón et al., 2012; Montero, Martinez-Rincon, Heppell, Hall, & Ewal, 2016). Besides, the using of non-parametric smoothing functions, statistical robustness of GAM allows flexible description of complex species responses to environment.

MATERIALS AND METHODS

Data collection

In this study, daily landing data for each operation were obtained from a commercial purse seiner (23.4 m LOA and 313.3 kW). The vessel was equipped with hydro acoustic systems (Two types of sonar - 107 kHz CH-84 model and 160 kHz CH-28, echo-sounder 50- 200 kHz freq.). The purse seine net consists of 5 bulk and 1 bunt, resulting in a length of 750m and a depth of 164m. Though the targeted fish are small and pelagic such as sardines and anchovies, the mesh size of the purse seine net is 13 mm. The data were collected each 174th fishing trip in 2018-2019 fishing season that covers the time period between September 1st and April 15th in Izmir bay by authors via participating in each operation. The major fishing areas were between the depths of 25-68 m. An 8000 watt light source over an auxiliary boat was used to bring together the sardine-anchovy shoals.

Classification of bycatch species

A mean 54% of total European pilchards, *Sardina pilchardus* (Walbaum, 1792), landing and 4.1% of total European anchovies , *Engraulis encrasicolus* (Linnaeus, 1758), landing of Turkey have been found in the Aegean sea for last 30 years (TurkStat, 2021). Therefore, European pilchards (*S. pilchardus*) and European anchovies (*E. encrasicolus*) were accepted as targeted catches for Aegean purse seiners. The species which were retained and sold but were not the targeted species were classified as by catch species. The species which were vulnerable species that were removed from the bunt of the net in the sea (i.e. rays, skates, sun fish), damaged fish, thrown from onboard, and elected small size fish from the grading sieve were named discard. The habitat classification of species were based upon Fishbase (Froese & Pauly, 2019).

The Estimation of by catch ratio

Targeted catch (TC), by catch (BC) and discard (D) were expressed as biomass (kg). The bycatch ratio (BCR) is mainly defined as the ratio of bycatch to total catch, whereby the total catch consists of the targeted catch, bycatch and discard for each haul in a fishing day. The formula is as below:

$$BCR = \frac{\sum BC}{\sum (TC + BC + D)}$$

The estimation of effects of variables on the BCR

The effect of variables on the BCR, was examined by means of Generalized Additive Modelling (GAMs) techniques (Hastie & Tibshirani, 1990). Generalized Additive Models (GAMs) with Tweedie family (Tweedie, 1984; Dunn & Smyth, 2005; Wood *et al.*, 2016) and log link function was used. Although, Tweedie is based partly on the Poisson family, Tweedie distributions are a family of distributions that include gamma, normal, Poisson and their combinations. This distribution is especially useful for modelling positive continuous variables with exact zeros. The Tweedie distribution is parametrized by variance power (p) and the p must be greater than 1 and less than or equal to 2. 1 would be Poisson, 2 is gamma (Tweedie, 1984; Dunn & Smyth, 2005). In this modelling p was chosen 1.01. Restricted maximum likelihood (REML) were also applied as a maximum likelihood-based smoothness selection procedures. The maximum degrees of freedom for each smoothing term, were set to 25 and 14 for Depth and the SST respectively. Therefore, the dimension of the basis used to represent the smooth term (k) were set as 26 and 15 in the GAM formulation. The test of whether the basis dimension for a smooth term was adequate (Wood, 2017) was done by k-index (the estimate of the residual variance based on differencing residuals) and p-value, computed by simulation. The QQ plot of the deviance residuals and the means of randomised quantile residuals were also plotted to check the model (Foster & Bravington, 2013; Pedersen, Miller, Simpson, & Ross, 2019).

Thus, the form of the GAM used was

$$BCR \sim a + s(Dh, k=26) + s(SST, k=15) + Q + MP + e$$

where, a is the intercept, Dh is Depth which is derived from echo sounder , SST is the Sea Surface Temperature (SST data were obtained from the General Directorate of the Meteorological Service (GDMS)), Q is quarter of year as factor variable (i.e. January, February and March are in Q1; April, May and June are in Q2; July, August and September are in Q3; October, November and December are in Q4), MP is the moon phase, which is a factor variable consisting of four periods; new moon, first quarter, full moon and last quarter, and s indicates the smoother function of the corresponding independent variable and e is a random error term.

Statistical inference was based on a 95% confidence level. The model fitting was accomplished using the "mgcv" library (Wood, 2003, 2004, 2017; Wood et al., 2016) under the R language environment (R Core Team, 2020). The "tidyverse" package (Wickham et al., 2019) was also required .

RESULTS AND DISCUSSIONS

There were 26 species (66.216 mt in total) recorded as by catch and the rates of by catch species in total bycatch amount varied between 0.001% and 23.1%. *Boops boops, Sardinella aurita, Sarda sarda* and *Mugil sp.* were the first four species, therefore the rest of them took a place which is lower than 1% (Figure 1). In terms of habitat of by catch species, the total ratios of benthopelagic, demersal and pelagic species were 52%, 28% and 20%, respectively.

A total of 179 BCRs were calculated during the fishing season. The BCRs were between 0.00125 and 1. The mean BCR is 0.157 ± 0.01. The fact remains that, the median value was recorded as 0.046. In the box plots of BCRs by moon phases, the high overlapping was shown clearly (Figure 2). In terms of quarters of the year, the median values of BCRs have been changing between

0.0204 and 0.0612. Although the median values are relatively the same, the interquartile ranges of Q3 and Q4 were limited according to Q1 and Q2 (Figure 3).

Besides, The QQ plot of the deviance residuals and deviance residuals vs. fitted plot (Figure 4), some diagnostic information about the fitting procedure and results were given in Table 1. As higher p values indicated that the basis dimensions used for smooth terms were adequate and the k-index showed that there were no missed patterns behind in the residuals (Table 1). The deviance residuals against approximate theoretical quantiles of the deviance residual distribution in Figure 4a also showed that the model distributional assumptions were met. Furthermore, the response data were independent, so the residuals appeared approximately as well (Figure 4b).

The analysis of the deviance table indicated that the depths and SSTs were significant (Table 2). Furthermore, the line was undulant in Figure 5. However, a horizontal line was observed in Figure 6.

We analysed data collected from a commercial purse seine vessel in Izmir bay, Aegean Sea to describe the relationship between the bycatch ratio and environmental factors. For the Aegean Sea, *S. pilchardus* and *E. encrasicolus* have been playing key roles as target species but the diverse catch composition of landed bycatch fish confirmed a low species selectivity of this fishery. In contrast to the two species comprising approximately half of the bycatch (~46%), the habitat classification confirmed the heterogeneity of the by catch and the low selectivity of the gear in this study. Tsitsika & Maravelias (2006) stated that 18 species which were 16 fish and 2 cephalopods were recorded as a taxonomic composition of the commercial purse seine catches in Pagasitikos Gulf (Greece) whereas the percentage of demersal fish species landings of the purse seiners had been changing between 1.3% and 8.1% from 1998 to 2007 in the Aegean Sea (Anonymous, 2008). Furthermore, Tsagarakis, Vassilopoulou, Kallianiotis, & Machias (2012) reported that five species (*S. pilchardus, E. encrasicolus, Sardinella aurita, B. boops and S. japonicus*) were considered as the target species for the purse seine fishery in the Aegean Sea, so they represented 97% of the marketable catch. The rest (3%) constituted of fifty five species. For the Black Sea, a total of 26 species including fish (24 species), gastropods (1 species), and crab (1 species) were recorded in the targeted purse seine fishery *E. encrasicolus* and *Trachurus mediterraneus* (Şahin, Ceylan, & Kalaycı, 2015). We think that all these findings could be accepted as normal, because the character of the Mediterranean fishery was described as multi-species catches by Lleonart & Maynou (2003).

In this study, The BCRs were determined between 0.00125 and 1. Şahin et al. (2015) did not calculate the by catch ratio for each operations but they gave the ratio of the by catch in total biomass of the operations targeted, *E. encrasicolus* as 2.1%. In spite of the priority of *E. encrasicolus* and *S. pilchardus* as target species (Anonymous, 2008), the ratio between the discards and the marketable fraction, considering all species, was reported as between <0.01 and 0.15 for the Greek purse seine fishery (Tsagarakis et al., 2012). However this information shows us only the ratios of species not sold to the marketable ones. Hall (1996) stated

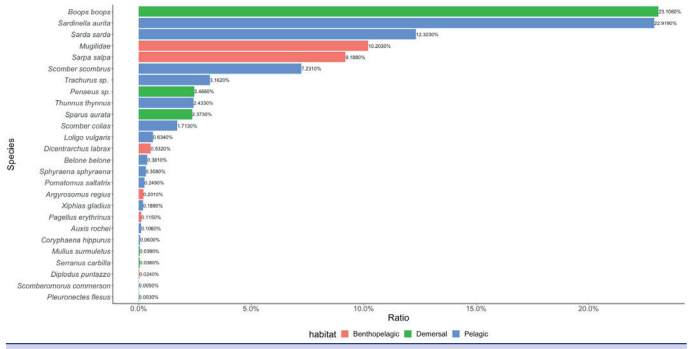

Figure 1. Species ratios in total by catch amount.

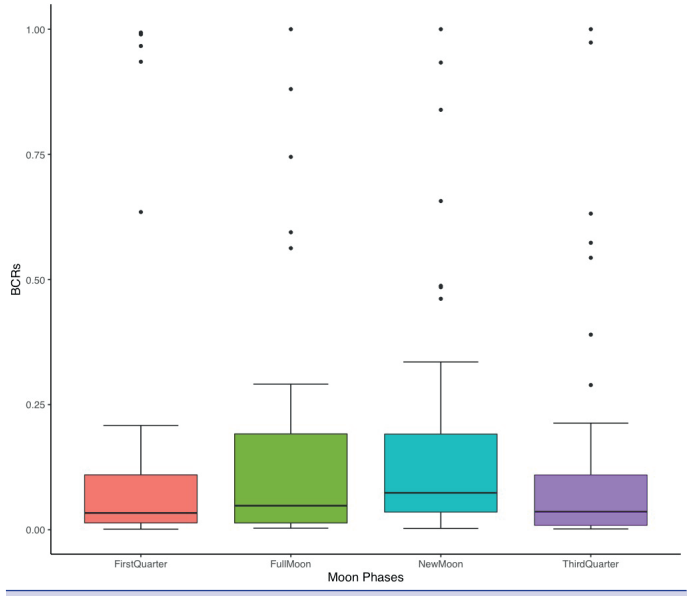

Figure 2. BCRs of purse seine fishery by Moon phase.

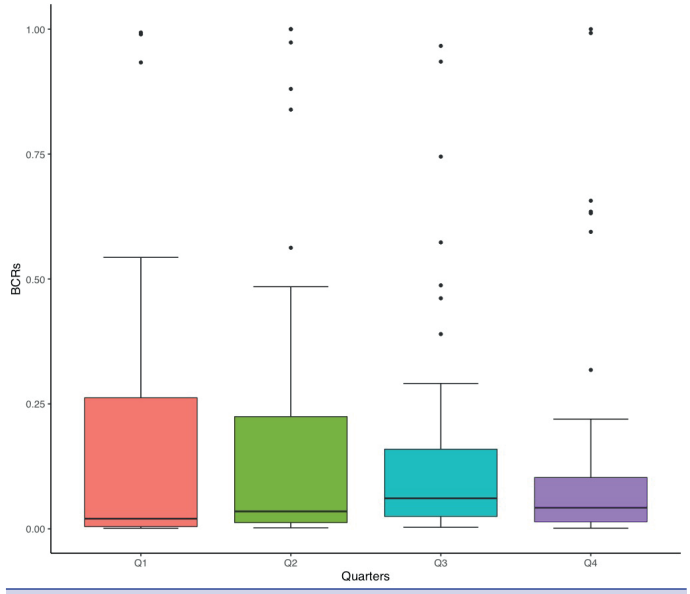

Figure 3. BCRs of purse seine fishery by quartiles of year.

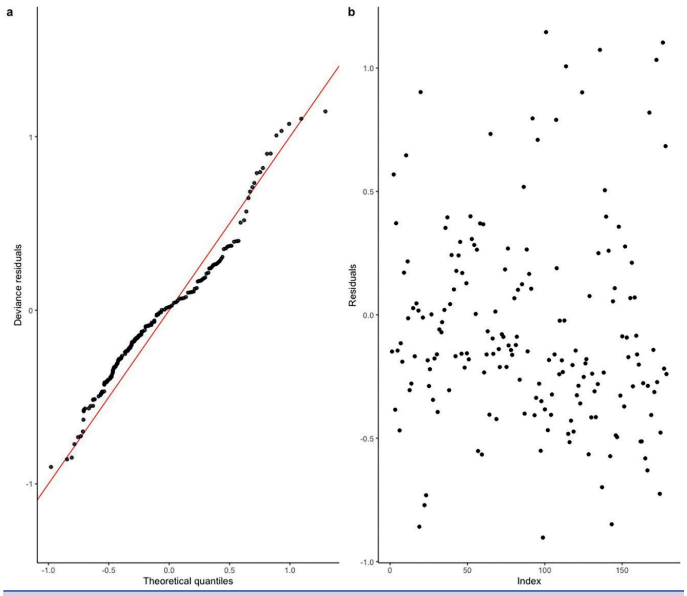

Figure 4. (a) QQ-plot of residuals (black). The red line indicates the 1–1 line. (b) means of randomised quantile residuals.

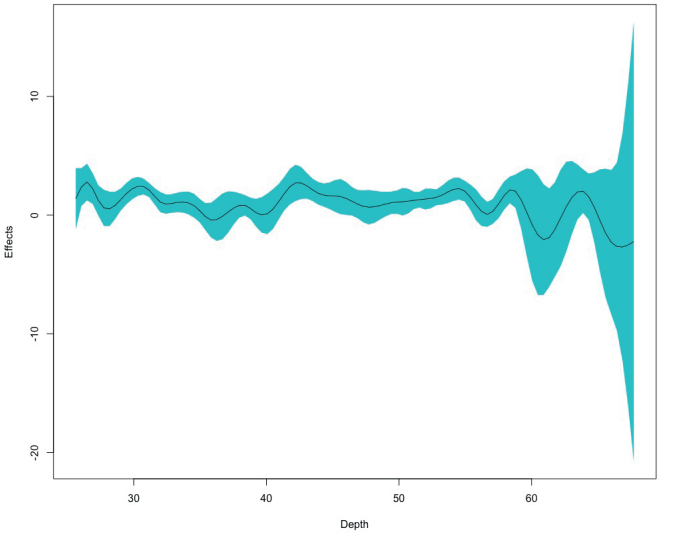

Figure 5. GAM estimated effect of Depth on BCRs for purse seine fishery (turquoise area corresponds to the 95% confidence intervals of the estimates).

Table 1. The result of basis dimensions of model.

Factor	k',	edf	k-index	p
s(Dh)	25	24.10	1.02	0.74
s(SST)	14	13.50	1.01	0.69

k'= upper limit on the degrees of freedom associated with an s smooth, edf= estimated degrees of freedom. , k-index = ratio of neighbour differencing scale estimate to fitted model scale estimate, p= p value.

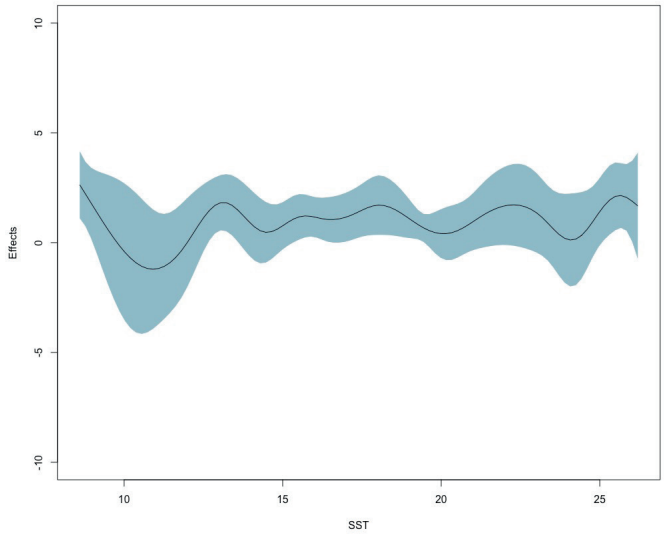

Figure 6. GAM estimated effect of SST on BCRs for purse seine fishery (blue area corresponds to the 95% confidence intervals of the estimates).

Table 2. Analysis of deviance table for the GAM model fitted to the BCR data of the purse seine fishery.

Factor	df	F	p
s(Dh)	24.83	1.748	0.0225*
s(SST)	13.94	1.985	0.0229*
Season	3	0.135	0.939
Moon phase	3	0.910	0.438

*<0.05; df = degrees of freedom, F = F-value, P = P-value, s(x) = smoother function of the corresponding independent variable, Dh= Depth, SST = sea surface temperature.

that the ratios of bycatch to target catch commonly are used as an argument to explain the ecological impact of a fishery. These calculations may help the decision makers and/or scientists to compare between various fishing techniques. Although this ratio is still using in propositions of some Non-Governmental Organizations (NGOs) to ban the tropical tuna purse seine fishery around Fish Aggregating Devices (FADs), by catch ratios might be high when the amounts of target catches were small, with the smallest class of catches responsible for the highest total portion of bycatch while only contributing negligibly to the total target catch (Dagorn et al., 2012). Here, we calculated the ratio or marketable fish (except targeted ones) to the all catch for each operation and we want to point out the margins of the by catch in the targeted fishery that especially are supported with the light located in auxiliary boat to bring together the sardine-anchovy shoals. We think that, these ratios of by catch are normal for each operations in multi species areas such as the Aegean Sea. In the case of the purse seine fishery, which has null mesh selectivity, modern echo-acoustic techniques to search for schools and light using (Colloca et al., 2013), BCRs arise. The inefficient use of re-

sources and changes in the abundance of both target and non-target species make the gear selectivity and an effort reduction beneficial for fisheries resources even on a small geographical scale where co-management can play an important role (Cook, 2010; Guidetti, Bussotti, Pizzolante, & Ciccolella, 2010).

Mannocci *et al.* (2020) developed a generalized additive model for silky sharks (*Carcharhinus falciformis*), oceanic triggerfish (*Canthidermis maculate*), rainbow runners (*Elagatis bipinnulata*), wahoos (*Acanthocybium solandri*), dolphinfish (*Coryphaena hippurus*) and for the Atlantic and Indian oceans relating by catch to a set of environmental covariates (depth, SST etc). Hereby, they stated that the depth and the SST were the most significant environmental covariates as did we. Furthermore, bycatch assemblages around FADs and free school sets from the tropical tuna purse seine fishery in the eastern Atlantic Ocean showed preferences for specific oceanographic characteristics and SST and month also play important roles in describing the diversity patterns of the by catch species (Lezama-Ochoa et al., 2018). Warmer SST contributes to the increased bycatch of sharks per set and high probability of incidental catch of the wahoo, *Acanthocybium solandri* in the tuna purse seine fishery, as well (Martínez-Rincón et al., 2012; Minami, Lennert-Cody, Gao, & Román-Verdesoto, 2007).

Regarding the effect of the moon phase, Jatmiko (2015) stated that the different light intensity in each moon phase may affect the fish in relation to the positive or negative phototaxis properties of light. In fact, Suharyanto *et al.* (2020) stated that the moon phases had no significant effect on the amount of catch, in spite of recording the lowest amount of catch in at the beginning of new moon phases. In contrast to no fishing activity in the lunar phase around a full moon in the Sardine purse seine fishery in Indonesia (Pet et al., 1997), the BCRs were almost the same regardless of the moon phases and there were no effect significant effects in the model of our study. Due to the use of artificial light, the purse seine fishery has been studied in all the lunar phases in the Mediterranean. As commonly known, a purse seine fishery with artificial lights is one of the most advanced and successful methods of increasing the catch rate (Arimoto, Glass, & Zhang, 2010; Dragesund, 1958; Fonteneau, Pallarés, & Pianet, 2000). However, there is a risk of causing an overfishing of the targeted species (Nguyen & Winger, 2019; Solomon & Ahmed, 2016). We think that the effect of artificial light may conceal the effect of moon light on fish species. There may be a need to develop eco-friendly light fishing technologies or fishing regulations to reduce the by catch ratio in purse seine fishery.

Tsitsika & Maravelias (2008) stated that depth was an important factor affecting catches and that most catches were recorded at seabed depths near 30–40m and visual stimuli in the water column is the main factor for the purse seine. Furthermore, Tsitsika & Maravelias (2006) reported that the depth of the purse seine was approximately 30m and most of the fish found within the first 30m of the water column were caught in their study. In fact, the ratios of benthopelagic and demersal species in by catch species were relatively high in our study. We argue that BCRs of the targeted purse seine fishery were affected by depth. Although, the target of purse seine fisheries is pelagic fish, the purse seines can be used to catch high commercial value demersal species such as sea breams (e.g., *Diplodus spp.*, *Pagellus spp.*, *Sparus aurata*) and *Dicentrarchus labrax* (Gonçalves et al., 2008). The high ratios of benthopelagic and demersal species occurred due to shallower fishing areas. However, there were too many peaks in the line presenting the effects of depth in our model. Environmental and geographical factors could play an important role in directing local distribution and variability in the presence of species (Muñoz, Pennino, Conesa, López-Quílez, & Bellido, 2013) but we believe that the high net depth (164m) could be the major reason. The purse seine could be used as demersal purse seine despite its own definition to the contrary.

CONCLUSION

In light of the knowledge of the decline in marine fish stocks and the fishery in the Mediterranean (Colloca et al., 2013; Demirel, Zengin, & Ulman, 2020; Maynou, 2020; Smith & Garcia, 2014; Ulman, Zengin, Demirel, & Pauly, 2020; Vasilakopoulos, Maravelias, & Tserpes, 2014), further studies need to be steered towards the decline in the ratio of the species which were retained and sold but were not the targeted species to the total catch amount in targeted fisheries, and new management arguments like Marine Spatial Planning (Bellido, Paradinas, Vilela, Bas, & Pennino, 2019) should be thrown out for achieving optimal economic-ecological benefits, as well.

Ethics committee approval: The research was approved by the Ege University Animal Experiments Local Ethics Committee in terms of sampling and use of experimental animals with the decision numbered 2018-004.

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Original Article

Monitoring of Biochemical Effects of Phenol in the Carp (*Cyprinus carpio*) Fry

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ABSTRACT

This study was conducted to investigate the possible side effects of phenol on biochemical parameters of carp (*Cyprinus carpio*) fry with an average weight of 0.474±0.04 g. Fishes were treated with 0 (control), 5, 10 and 20 ppm of phenol during 24, 48, 72, and 96 hours. We have tested the effects of phenol on the biochemical profile, i.e., the total protein, lipid and glycogen levels, in the whole body of the carp samples. They showed change as total protein ($p<0.05$), glycogen ($p>0.05$) and lipids ($p>0.05$) content in the whole body. In view of results, the present study reports metabolic dysfunction in response to phenol toxicity in carp.

Keywords: *Cyprinus carpio*, Phenol, Glycogen, Total protein, Lipid

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INTRODUCTION

One of the most important problems of recent years that is influencing all living organisms and retrograding natural resources is water pollution (Khan, et al., 2000; Inyinbor et al., 2018). A great number of chemical compositions that get into water ecosystems can cause dangerous impacts on freshwater and marine organisms (Stegeman et al., 1992; Garg et al., 2009). Random dismissal of industrial wastes into aquatic ecosystems without any pretreatment causes serious problems to the non-target organisms (Mishra & Poddar 2013). Draining of wastes into freshwater systems decreases the dissolved oxygen level which causes respiratory problems and consequent mortalities in fish (Black 1955; Abu Aita 2014).

Conversely, even less is known about the ecotoxicological impacts of organic pollutants on terrestrial, wildlife and aquatic organisms, and a comprehensive revisal on ecotoxicological impacts is missing. Aquatic organisms are especially important targets, as they are exposed to wastewater residuals over their all life. Standard intense ecotoxicity information has been reported for a number of natural pollutants, but, such data alone may not be appropriate for specifically addressing the question of environmental impacts, and afterwards in the risk and hazard assessment (Escher 2001; Traas & Van Leeuwen 2007; Ma et al., 2019).

Phenol and phenolic compounds are xenobiotics, which are derived from the aquatic environment as a result of anthropogenic factors and they are stressful environmental agents. Animals such as humans, fish, rabbits, mice, which were exposed to those compounds suffer from anemia and some other pathologies (Hori et al., 2006; Zaki et al., 2011). There were no published reports on the biochemical effects of phenol on carp fry. The aim of this study is to investigate the various biochemical effects of phenol in carp fry with an experimental study design.

MATERIALS AND METHODS

Fish and experimental design

Carp (*Cyprinus carpio*) samples (0.474±0.04 g mean weight) used in this study were obtained from the Keban Fish Breeding Unit of IX. Region Directorate, the State Hydraulic Works in Turkey. Two weeks prior to the experiments, the fish were acclimatized in glass aquaria aerated with

air stones. The fish were fed with a commercial diet daily to satiation. Exposure chambers were cleaned as needed. For the duration of the study, the dissolved oxygen (Intellical™ LDO101), pH (Thermo Scientific™ Orion Star™ A111 pH Benchtop Meter), temperature (Checktemp®1 Pocket Thermometer), hardness (Premium Water Hardness Test Kit), salinity (Extech EC170 Salinity Meter), nitrite and ammonia levels (100 UV Visible Spectrophotometer) were monitored and maintained within acceptable ranges. The water quality characteristics were determined according to the American Public Health Association guidelines (APHA, 2005).

The fish were divided equally into four groups (20 fish/per groups). The first group was maintained in tap water as a control group. The fish in groups 2, 3 and 4 were exposed to 5, 10 and 20 ppm of phenol respectively for 96 hours. The entire experiment was repeated two independent times; each replicate for each group contained ten fish, and a total of 160 carp individuals were used during the experimental stage of this study. No mortalities were observed during the experiment. At the 96th hour of the test, the fish were anaesthetized in 50 ppm benzocaine solution and the whole bodies were isolated, washed with physiological saline (0.9 % NaCl) and stored at -40 °C until the biochemical assays, which were performed within one month after extraction.

Biochemical assays

Total protein activity

Protein extraction was carried out according to Plummer (1971). Briefly, carp fry (one individual internal organs removed: 0.464±0.02 g) were homogenized in the homogenizer by adding 10 ml of 10% trichloroacetic acid solution. These fish samples (10.464 ml) were centrifuged at 3500 rpm for 15 minutes at room temperature. The supernatant in the tubes was discarded and 2ml of 96% ethyl alcohol was added. The tubes were then mixed slowly and centrifuged at 3500 rpm for 15 minutes. After centrifugation, the supernatant was discarded and the pellet was re-dissolved by adding distilled water (10 ml). As described by Lowry et al. (1951), the amount of protein was determined using the Folin-phenol reagent. Bovine serum albumin was used as the standard protein. Spectrophotometer was read with absorbance at OD_{695nm}.

Lipid level

Total lipid extraction was carried out according to Folch et al. (1957). Carp fry samples (one individual internal organs removed: 0.464±0.02 g) were homogenized in homogenizer by adding 10 ml of chloroform methanol (2:1, v/v). These fish samples were centrifuged at 1200 *g* for 5 minutes at room temperature to separate the lipid containing organic layer from the aqueous layer. The organic layer of each sample was left in pre-weighed and prepared boiling tubes. These tubes were then evaporated at 37 °C under nitrogen and allowed to dry. In desiccator was cooled dried tube containing lipid and reweighed. The weight of the crude lipid was calculated by subtracting the initial weight of the empty tube from the weight of the tube containing the dried lipid.

Glycogen content

Glycogen extraction was carried out according to the method reported by Joseph et al. (1961). Glycogen extraction was carried out. Carp fry samples (one individual internal organs removed: 0.464±0.02 g) were homogenized in the homogenizer by adding 10 ml of 10% trichloroacetic acid solution. The homogenizer bottle was placed in an ice water bath and the temperature of the contents was kept below 15 °C during the homogenization period. These homogenates were filtered through filter paper and the total volume of liquid passing through the filter was measured. In order to precipitate the glycogen, aliquots were left in the prepared centrifuge tubes and 5 volumes of ethyl alcohol were added. After the solution was kept in the oven set at 35-40 °C overnight. These samples to precipitate glycogen were centrifuged (10.464 ml) at 3500 rpm for 15 minutes at room temperature. After centrifugation, the supernatant in the tubes was discarded. Pellets were resuspended by adding 2 mL of distilled water. As described by Nicholas et al. (1956), the proportion of glycogen was determined using the anthrone method. Spectrophotometer was measured with absorbance at OD_{620nm} where the glucose solution was used as standard.

RESULTS AND DISCUSSION

The results of this experimental study, which was carried out to determine the biochemical effects of phenol on carp fry, showed that the biochemical profiles of carp fry change according to the exposure amount of phenol.

The effects of phenol on the total protein level of carp were shown in Table 1. A significant (p<0.05) decrease of the protein level in fish exposed to all the doses of phenol was observed to 0 and 1 ppm groups had higher protein activity than those the other experimental groups (p<0.05). In these exposed groups the total protein was found as 4.33±0.11, 3.83±0.14 and 3,14±0.13 mg/ml for all experimental groups at the 96th hour respectively.

Administration of phenol at the dose of 5 and 10 ppm showed a significant decrease in the total lipid after 96th hour. In total lipid activity, the minimum levels observed were 0.37±0.04, 0.26±0.04, 0.19±0.05 and 0.37±0.03 mg/g for the experimental and control groups, respectively (Table 2).

There was no significant difference (p>0.05) in the activity of glycogen in the whole body of 1ppm phenol treated fishes after the 96th hour of exposure as compared with the control group (Table 3). But, administration of phenol at the dose of 5 and 10 ppm showed a significant decrease in the glycogen content after the 96th hour.

Table 1. Effect of phenol on the total protein content in whole body of carp.

	Group	24 h	48 h	72 h	96 h
Protein (mg/ml)	Control	4.58±0.15	4.57±0.11	4.59±0.13	4.60±0.13
	1 ppm	4.39±0.13	4.38±0.14^{a}	4.29±0.10^{a}	4.33±0.11^{ac}
	5 ppm	4.32±0.14	4,10±0.14^{a,b}	3.71±0.14^{a,b}	3.83±0.14^{a,b}
	10 ppm	4.08±0.03	3,72±0.14^{a,b,c}	3.12±0.10^{a,b,c}	3,14±0.13^{a,b,c}

Table 2. Effect of phenol on the total lipid content in the whole body of carp

	Group	24 h	48 h	72 h	96 h
Lipid (mg/g)	Control	0.38±0.05	0.39±0.04	0.37±0.03	0.39±0.01
	1 ppm	0.39±0.03	0.38±0.03	0.38±0.04	0.37±0.04
	5 ppm	0.36±0.04^{a,b}	0.30±0.02^{a,b}	0.26±0.04^{a,b}	0.27±0.05^{a,b}
	10 ppm	0.31±0.02^{a,b,c}	0.23±0.02^{a,b,c}	0.19±0.05^{a,b,c}	0.24±0.02^{a,b,c}

Table 3. Effect of phenol on the glycogen content in the whole body of carp

	Group	24 h	48 h	72 h	96 h
Glycogen (µg/g)	Control	2.72 ±0.5	2.69±0.4	2.69±0.3	2.73±0.6
	1 ppm	2.70 ±0.4	2.71±0.5	2.70±0.5	2.69±0.5
	5 ppm	2.67 ±0.4	2.68±0.5	2.63±0.4	2.59±0.5
	10 ppm	2.08 ±0.3^{a,b,c}	1.96±0.6^{a,b,c}	1.99±0.3^{a,b,c}	1.82±0.6^{a,b,c}

As stated above, experimental exposure to phenol with various doses for 96 h caused some biochemical alterations in carp fry. The phenol could alter protein metabolism by changing the transamination rate of amino acids by increasing the activity of alanine aminotransferase (ALAT, EC 2.6.1.2) and aspartate aminotransferase (ASAT, EC 2.6.1.1) (Hori et al., 2006).

Administration of phenol at the dose of 5 and 10 ppm showed a significant decrease in the total lipid after the 96th hour. The main energy source in fish is lipids. Fish swimming induces high energy demands (Fernández-Vega et al., 2015). Catabolism of such amount of lipid in *Cyprinus carpio* reflects a higher demand for ATP as a consequence of the detoxification process and elevated stress (Sannadurgappa et al. 2007). The reduced glycogen content in carp exposed to phenol may cause a lower energy accumulation in fish, and these fish will probably swim slower.

Hori et al. (2006) and Abdel-Hameid (2007) reported that slow swimming is a general response observed in fishes exposed to phenol. Glycogen decrease was to attend energetic consumption caused by phenol. Due to the absence of increased glycogen in the white muscle of the fish, glycogen synthesis was not done to determine glucose reduction.

CONCLUSION

An attempt was made to assess the phenol toxicity on carp fry by performing acute bioassays. Results of tolerance limits and response of test fish towards phenol confirm that phenol has an impact during acute toxicity. The swimming behavior of fish is slowed down due to changes occurred in biochemical parameters like the swimming behavior of fish was due to changes occurred in biochemical parameters like proteins, lipids and glycogen. Our study claims that phenol can affect the metabolism of test fish by hindering biochemical activity. This experimental study showed the toxic potential of phenol on carp, but the induction pattern of antioxidant enzymes under long-term and high dose exposure conditions still needs to be investigated.

Conflict of interests: The authors have no conflicts of interest to declare.

Ethics committee approval: All animal studies were approved by the Animal Ethics Committee of Kahramanmaraş Sütçü Imam University, Faculty of Agriculture (KSÜZİRHADYEK) and Research Institute (Protocol number: 2016/1-1).

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Original Article

Biological and Reproductive Indices of *Mystus bleekeri* (Day, 1877) in Open Water Body (Dekhar Haor) of Bangladesh

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ABSTRACT

Some biological and reproductive aspects of the *Mystus bleekeri* from Dekhar Haor were investigated. From the commercial catches, a total of 600 sample collections were performed on a monthly basis from July 2017 to June 2018. Total length (TL, cm), body weight (W, g) and individual's sex were identified between 216 male (36%) and 384 female (64%) fish (ratio 1.00: 1.78). Mean TL and W of samples were 14.85±3.38 cm and 27.54±15.76 g, respectively. The TL of male and female ranged from 6.9-21.3 and 7.80-24.60 cm, respectively; and W ranged from 5.3-63.3 and 6.70-76.40 g, respectively. In length–weight relationship of this fish depicted a negative allometric growth (*b*=2.408) with a condition factor (*K*n) of 1.04±0.11. Fish of 16.00-16.90 and 10.00-10.90 cm length groups demonstrated the highest (1.45±0.23) and lowest (0.89±0.18) *K*n values, respectively which was significantly varied among different length groups. Monthly study of gonadosomatic index showed two peaks in the month of May-June and November for both sexes. The results of this study provide baseline data on some biological aspects of *M. bleekeri*, which would be useful in predicting the responses of *M. bleekeri* populations in Dekhar Haor.

Keywords: Condition factor, Gonadosomatic index, Length-weight relationship, *Mystus bleekeri*, Sex ratio

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INTRODUCTION

Mystus bleekeri (Day, 1877), a member of the Bagridae (Siluriformes) family locally known as gulsha, is widely available in the tropical countries like Bangladesh, Myanmar, Pakistan, Cambodia, India, Malaysia, Sri Lanka, Vietnam, Cambodia, and Laos (Froese & Pauly, 2006). This species inhabits rivers, canals, khals, beels, lakes, swamps, and other freshwater bodies in Bangladesh (Rahman, 2005). Being a dominant catch in Dekhar Haor (northeastern Bangladesh), it has become an important target species for small-scale fishermen (Alam et al., 2017; Bhattacharjee et al., 2017). Similar to other small indigenous fish species of Bangladesh, this species also possessed high nutritional factors like protein, micronutrients, vitamins, and minerals (Sultana et al., 2019). Due to its nutritive values but also taste and palatability, the demand and price of this fish is increasing day by day. Development of seed production technology of this species is the prime need to protect and conserve natural stock. Investigation and securing of research knowledge on some important biological aspects of fish like length-weight relationships (LWRs), sex ratio, condition factor (*K*n), and gonadosomatic index (GSI) are the prerequisite strategies for successful production and stock management (Mazumder et al., 2020).

Length–weight relationships (LWRs) are needed to estimate weight from length because direct weight measurements can be time-consuming in the field (Hossain et al., 2006a) as well as these parameters are important in fish biology and can provide information on the stock condition (Gonzalez Acosta et al., 2004). In fish, *K*n reflects the physiological status, health condition, and well-being of stock directly interrelated to future sustainability. This parameter also acts as an indicator of life cycle status of a species, including management practices and equilibrium condition of an ecosystem (Lizama & Ambrosio, 2002). An index of gonad size relative to fish size is termed as GSI, and gradual increment of GSI value indicates the gonadal development of fish reaching to the spawning in the monsoon and late monsoon period (Amtyaz et al., 2014). However, there is scarcity of literature on the biological parameters of *M. bleekeri* in the Haor wetland ecosystems of Bangladesh. Only, in the previous study, the author checked the seasonal effect on trophic level, stomach contents, LWRs, and condition factor of *M. bleekeri* (Mazumder et al., 2021). Subsequently, this study aimed to investigate the biology of *M. bleekeri* in terms of LWRs, *K*n, GSI and sex ratio to provide an updated knowledge that can be utilized for captive spawning, wild stock management, successful farming, and environmental management of this species.

MATERIALS AND METHODS

Dekhar Haor was selected for the present study as it is one of the largest and most important haors in Bangladesh. This wetland is positioned between 24°34′N to 25°12′N and 90°56′E to 91°49′E latitude and longitude, respectively (Figure 1). In Sunamganj district, this waterbody covers Sunamganj Sadar, Dakshin Sunamganj, Dowarabazar, and Chhatak upazilas. It is made up of 36 small, medium and large interconnecting beels, canals, rivers, and crop lands. During the monsoon, Haor looks like an inland sea full of water, but in the dry season it becomes almost dry except for some deeper beels. This Haor contributes to fish production for the local and regional demand, livelihood for small scale fishermen, place of aquatic biodiversity, and a source of natural fish seed supply for the local fish culture (Pandit et al., 2015).

Mystus bleekeri specimens were sampled from July 2017 to June 2018 from three geographical locations as Pagla Bazar, Sunamganj Fish market and Dowarabazar. These three sites were major fish landing zones of Dekhar Haor. Each month 50 fish samples were obtained, meaning 600 samples in 12 months. The fish were caught using three layered trammel net, cast net, scoop net, and different sized traps. In stretched condition, mesh sizes of the trammel nets were 4.2, 6.5 and 7.5 cm in three different layers, and cast nets were 2 cm. Specimen identification was carried out in the field according to Talwar and Jhingran (1991). After collection, specimens were confirmed to the species level, preserved by date in plastic jars with 10% (w/v) formalin and transported to the laboratory for further study (Alam et al., 2018).

TL and W of all fish were measured in laboratory condition by using a centimeter scale and weighing balance to the nearest centimeter (cm) and gram (g), respectively. Then, the specimen was dissected, gonads were removed and weighted. For size structure measurement, length measurements were pooled into

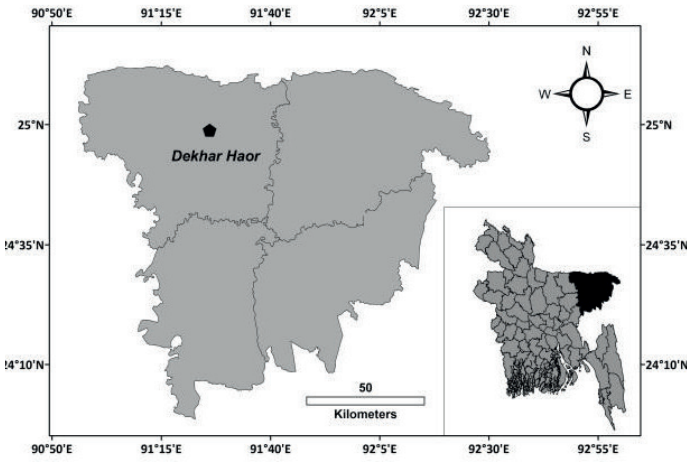

Figure 1. Map depicting the location of Dekhar Haor, Sylhet, Bangladesh (Map modified by Mazumder et al., 2021).

groups of 1 cm length intervals. Throughout the examination of the gonad, sex of the individual fish was determined; sex ratio was calculated as total number of males relative to total number of females depicted as M: F (males: females) (Oliveira et al., 2012). The chi-square (χ^2) was employed to quantify the significant difference between the sex ratio as commonly expected value is 1:1(Sokal & Jamesrohlf, 1987).

The LWR was estimated with a nonlinear regression equation (Froese, 2006):

$$W = a \text{ x } TL^b$$

Here *W*, *a*, TL and *b* indicate body weight (g), total length (cm); intercept in the y-axis, and the regression coefficient (isometric growth value is 3), respectively. Analysis of covariance in both slope and intercept to quantify the level of significance.

*K*n of each individual fish was estimated according to the equation of Le Cren (1951).

$$Kn = \frac{W}{(a\ x\ TL^b)}$$

Where, the meaning of *a* and *b* is the scaling coefficient for the weight at a certain length and body shape parameter, respectively. Obtained values of *a* and *b* from the LWR equation were also employed in this equation.

Digital balance (Model: AND Gulf EK600, country of origin: China) with an accuracy of 0.01 g was used to measure individual fish and its removed gonad weight. Then, individual fish GSI (%) was calculated using an equation developed by Bagenal & Tesch (1978).

GSI (%) = Gonad weight (g) /body weight (g) x 100

All the data sets were analyzed by using Microcalc Origin™ v 8.0 software employing Student's *t*-test. A *p* value less than 0.05 ($P < 0.05$) was considered as the level of significance.

RESULTS AND DISCUSSION

Among the 600 specimens, most of the fish belongs to 11-18 cm size groups. The size pattern demonstrated a normal distribution (Figure 2), and the TL of largest mature male and female were 21.30 and 24.60 cm, respectively.

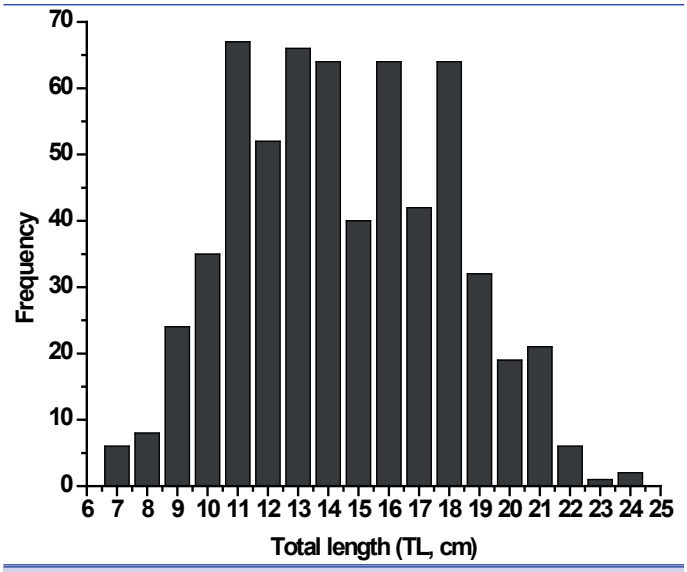

Figure 2. Length frequency distribution of *M. bleekeri* from Dekhar Haor.

Average length and weight (±SD) for all fish were 14.85±3.38 cm TL and 27.54±15.76 g, respectively. The TL of males and females ranged from 6.90–21.30 and 7.80–24.60 cm, respectively and weighed between 5.30–63.30 g and 6.70–76.40 g, respectively. The reported maximum TL of *M. bleekeri* was 17.7 cm for male (Hossain et al., 2017). However, the highest TL found in this study was higher than the reported TL. LWRs regression slope *b* valued at 2.41 (Figure 3) was not different ($P > 0.05$) from the mean exponent 3.

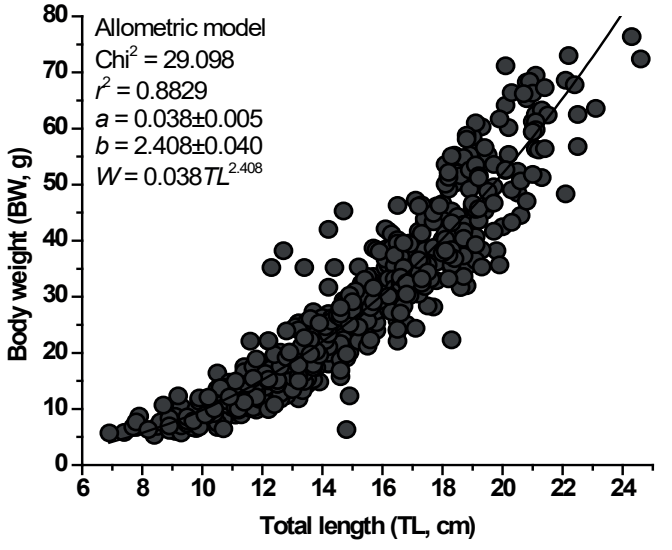

Figure 3. Length–weight relationship of *M. bleekeri* from Dekhar Haor (n=600).

Fish LWRs differences can be governed by different influential factors like physico-chemical parameters of living environment, season of the year, degree of feed availability, sex variations and gonadal maturity, normal growth pattern, length ranges of specimens, and number of fishes investigated (Cherif et al., 2008). Findings of this study demonstrated the allometric coefficient (*b*) is 2.41, which was positioned between the expected range of 2.00 to 4.00 (Bagenal and Tesch, 1978). If the slopes or exponents *b* remain between 2.00 to 4.00, the growth pattern (relationships between length and weight) of fish remain in the acceptable limit. A high value of correlation coefficient r^2 indicated a high degree of relation between length and weight of this species. Table 1 depicted the LWRs of different *Mystus* species from different parts of the world, indicating these parameters can differ due to locations and species variations. Seasonal fluctuations of value (*b*) of LWRs are directly related to the weight affected by ecological factors such as temperature, food supply, spawning conditions, and other factors, such as sex, age, fishing time, and area and fishing vessels (Kalaycı et al., 2007; Subba and Adhikaree, 2011). Changes in (*b*) value can also be attributed to factors such as overfishing and competition for food (Alam et al., 2018; Bagenal & Tesch, 1978; Mazumder et al., 2016; Mir et al., 2012). Thus, LWRs of fish are affected by above-mentioned factors although none of them was considered in current research.

Among different size groups, mean *K*n differs significantly ($P < 0.05$), highest (1.45±0.23) and lowest (0.89±0.18) TL values were documented in 16.00-16.90 and 10.00-10.90 cm fish groups, respectively (Table 2). It was also observed that the number of individuals caught was also higher with length groups from 11.00 cm to 18.00 cm. The mean *K*n values that were ≥ 1 in all the size groups except in 10.00-10.90 are the indication of stable physiological condition of studied fish species (Mazumder et al., 2016). The *K*n values of the studied species in the present study varied between 0.89 and 1.45 as shown in Table 2. These values suggested a state of wellbeing for the species evaluated. A number of factors that affect the growth condition including reproductive cycles, availability of food as well as habitat and environmental factors (Morato et al., 2001). The variation of *K*n from 1 divulges information regarding the differences in the availability of food and the consequence of physicochemical features on the life cycle of fish species (Le Cren, 1951). On the other hand, gonad weight and *K*n having proportional relation meaning of *K*n value elevated with the increasing of gonad weight just before the spawning. Moreover, after spawning, *K*n decreased because of releasing gonadal materials (Gupta & Banerjee, 2013). It should be noted that, prevalence of parasites, food availability and physiological status of fish influence the *K*n values (Simon et al., 2013) might be the probable causes of differences with the previous findings.

Fish physiological status depends on their different food and feeding habits, which is also relevant to year-round food supply. Nonetheless, aquatic climatic conditions and their correlation matrix might have played essential roles for the shaping of fish *K*n in Dekhar Haor. The adult *M. bleekeri* in 16.00-16.90 cm TL group demonstrated higher *K*n than the lower size classes, and better foraging tactics may be responsible for these kinds of outcomes (Fagade et al., 1984).

Table 1. Comparison of length-weight relationships of the genus *Mystus*.

Species	Sex	LWR parameters			Growth type	Locality	References
		a	*b*	r^2			
M. baramensis	B	0.004	2.88	0.92	(-) ve	Malaysia	Martin-Smith 1996
M. vittatus	M	0.023	2.96	0.96	(-) ve	Bangladesh	Hossain et al. 2006b
M. vittatus	F	0.018	3.13	0.97	(-) ve	Bangladesh	Hossain et al. 2006b
M. vittatus	B	0.020	3.06	0.96	(-) ve	Bangladesh	Hossain et al. 2006b
M. pelusium	B	0.028	3.00	0.99	I	Iran	Heydarnejad 2009
M. cavasius	B	0.012	2.91	0.96	(-) ve	India	Sani et al. 2009
M. bleekeri	M	0.013	2.64	0.987	(-) ve	Pakistan	Naeem et al. 2012
M. bleekeri	F	0.014	2.70	0.986	(-) ve	Pakistan	Naeem et al. 2012
M. bleekeri	B	0.015	2.62	0.892	(-) ve	Pakistan	Naeem et al. 2012
M. bleekeri	B	4.310	2.816	0.99	(-) ve	India	Maurya et al. 2018
M. bleekeri	B	0.026	2.54	0.98	(-) ve	Bangladesh	Hossain et al. 2016
M. cavasius	B	0.007	3.10	0.969	(+) ve	Bangladesh	Hossain et al. 2016
M. gulio	B	0.009	3.11	0.975	(+) ve	Bangladesh	Hossain et al. 2016
M. tengra	B	0.016	2.80	0.958	(-) ve	Bangladesh	Hossain et al. 2016
M ystus vittatus	B	0.017	2.77	0.960	(-) ve	Bangladesh	Hossain et al. 2016
M. bleekeri	B	0.038	2.41	0.88	(-) ve	Bangladesh	This study

*M=male, F=female, B=both sexes, (-) ve = negative allometric growth, I = isometric growth

From the total of 600 fish studied, 384 (64%) were females and 216 (36%) were males, giving an indication of male and female sex ratio at 1:1.80 (Table 3). Significantly higher numbers of females over males were found from January to April ($P<0.01$) and May to June ($P<0.05$). Fish sex ratio can be varied with season among different size and age groups although overall female number is higher relative to male. Investigation results of this study showed female *M. bleekeri* dominance in that population compared to the male group, indicating deviation from 1:1 as the expected value. These results are very much similar to the previous reports of Bhatt (1971), Rao & Sharma (1984) and Roy & Hossain (2006), in which the number of female *Mystus* species was higher than males. Similar types of deviation in male and female number (sex ratio) were also found in two archer fish: *Toxotes chatareus* and *T. jacularix* (2012). Reason of *M. vittatus* female dominance in the stock is not clear, but Fagade et al., (1984) described it as a natural way of population dynamics and stock regulation for fish species. During the period of spawning, mortality

Table 2. Condition factor (*Kn*) of *M. bleekeri* in relation to size classes.

TL (cm)	n	Mean	SD	95% CI of *b*	P-Value
<8.00	6	0.97	0.09	-0.17, 4.99	0.060
8.00-8.90	10	1.03	0.18	-1.93, 7.19	0.220
9.00-9.90	24	1.03	0.23	-0.71, 5.39	0.126
10.00-10.90	35	0.89	0.18	0.46, 6.39	0.025
11.00-11.90	67	1.00	0.19	-0.09, 4.71	0.058
12.00-12.90	52	1.00	0.31	-2.51, 8.45	0.281
13.00-13.90	66	0.99	0.21	-0.32, 7.32	0.072
14.00-14.90	64	1.07	0.27	-7.25, 4.10	0.581
15.00-15.90	40	1.01	0.14	0.52, 9.88	0.030
16.00-16.90	63	1.45	0.23	-5.65, 4.77	0.868
17.00-17.90	43	0.99	0.14	-0.93, 10.61	0.098
18.00-18.90	54	1.01	0.18	-1.08, 13.49	0.093
19.00-19.90	32	1.17	0.19	-12.54, 7.66	0.625
20.00-20.90	19	1.00	0.16	-9.58, 23.07	0.395
21.00-21.90	16	1.00	0.08	-16.35, 18.80	0.883
22.00-22.90	6	1.00	0.13	-66.3, 65.8	0.991
>23.00	3	0.99	0.04	-43.07, 57.54	0.319
Overall	600	1.04	0.12	-	-

Table 3. Monthly variation of sex ratio in *Mystus bleekeri*.

Month	No. of Fish	Male (Observed Value)		Female (Observed value)		Ratio of male and female	χ^2	d. f.	*P*	Remark
		No.	%	No.	%					
July' 17	50	24	48	26	52	1:1.10	0.08	1	0.77	NS
August' 17	50	22	44	28	46	1:1.30	0.72	1	0.39	NS
September' 17	50	18	36	32	64	1:1.80	3.92	1	0.06	NS
October' 17	50	26	52	26	48	1:1.10	0.00	1	1.00	NS
November' 17	50	20	40	30	60	1:1.50	2.00	1	0.15	NS
December' 17	50	22	44	28	46	1:1.30	0.72	1	0.39	NS
January' 18	50	14	28	36	72	1:2.60	9.68	1	0.00	S**
February' 18	50	12	24	38	76	1:3.20	13.52	1	0.00	S**
March' 18	50	14	28	36	72	1:2.60	9.68	1	0.00	S**
April' 18	50	12	24	38	76	1:3.20	13.52	1	0.00	S**
May' 18	50	18	36	32	64	1:1.80	3.92	1	0.04	S*
June' 18	50	16	32	34	68	1:2.10	6.48	1	0.01	S*

NB: χ^2 = Chi-square test d.f. = degrees of freedom, P = probability, NS = Non-Significant; S** = Significant at 1% level; S* = Significant at 5% level

of males is greater than that of females as male possess higher metabolic strain than the same aged females of a specific population (Banik et al., 2012; Parvin et al., 2011). In different *Mystus* species, early maturation of males relative to females was previously reported by Bhatt (1971) and Rao & Sharma (1984).

Up to the present time, fishery of *M. bleekeri* is capture-based. As in the present study, *M. bleekeri* was found with a sex ratio of 1: 1.80 (male: female) therefore, two females for one male can be stocked for captive breeding to get success. The present study reveals that the right time to collect the brooders from nature was September-October for *M. bleekeri*. However, proper strategies to conserve the fish species in its natural habitat are required.

The monthly changes in GSI of both female and male mature fish were shown in Fig. 4. GSI of female fish showed a steady increase from minimum in August (1.28±0.71) to maximum in November (8.00±1.88). In December, it fell to a low level; indicating that spawning had occurred. However, from December, the GSI again started to increase up to April, and then gradually decreased (Figure 4). Likewise, GSI in male fish decreased abruptly in August to reach the lowest level (0.39±0.21) and from September it gradually increased up to November (2.59±1.67). From November, it again dropped sharply to a low level, and it increased again in January and February. Nevertheless, after that, it fell in March during spawning and gradually increased up to June. The mean monthly GSI values indicate that *M. bleekeri* might spawn twice a year between April-July and October-November. The high GSI values for female fishes were observed in April and November whereas for male it was observed in May to July and November. GSI value is related to the maturation of gonad and becomes maximum just before the spawning. After completing spawning, the GSI value decreases abruptly (Simon et al., 2012). In monthly basis study, in which GSI value(s) reach at the peak indicating the spawning duration and for any particular fish species higher GSI reading depicts the breeding periodicity (Kiran & Puttaiah, 2003). In case of maturation, GSI values of females are much higher than males as a result of greater proportion of body reserve deposition in the gonads of females (Chatzifotis et al., 2004).

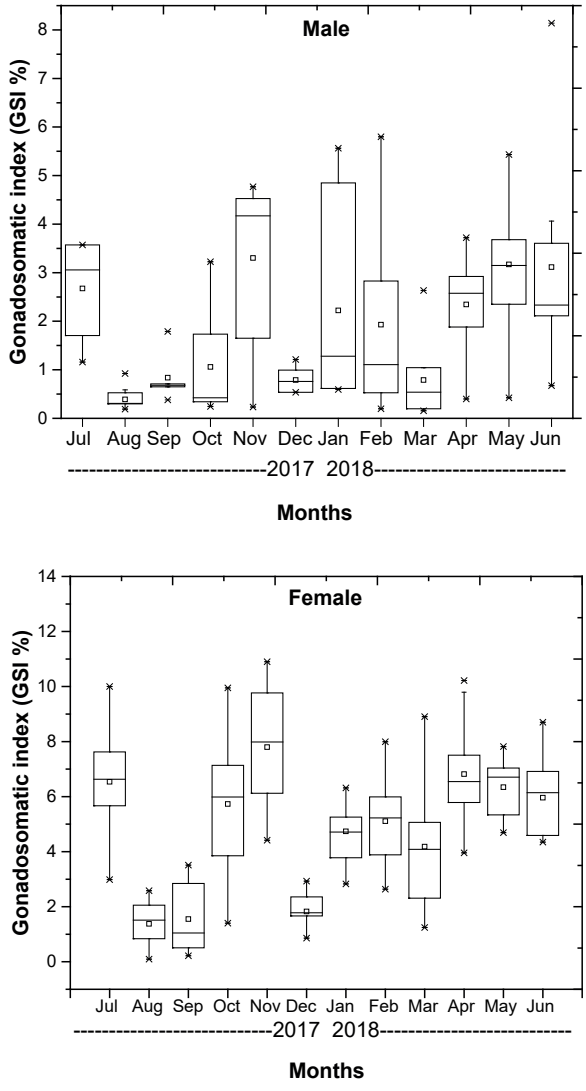

Figure 4. Mean monthly gonadosomatic index for male and female *M. bleekeri*.

CONCLUSION

Production of *M. bleekeri* is completely dependent on natural collection, and it is very important to start artificial breeding to initiate captive culture. Findings of present study reveal that brood fish size at 15-17 cm TL should be collected from wild stock. In breeding seasons, *M. bleekeri* male: female sex ratio becomes 1:1.50; therefore, to achieve artificial breeding success three females for two males should be stocked. However, it is necessary to investigate human interventions (exploitation) and natural environmental alteration for the modulation of fish biological parameters, which will be supporting evidence for the policymakers regarding sustainable exploitation and stock management of *M. bleekeri* in Dekhar Haor.

Conflict of interests: The authors declared no conflict of interest.

Ethics committee approval: Ethics committee approval was not required.

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Original Article

New Records of Diatoms (Bacillariales, Rhopalodiales & Surirellales) with Ultrastructure Details from the Black Sea Coast of Turkey

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ABSTRACT

Diatoms associated with the order Bacillariales, Rhopalodiales and Surirellales are well known to be present in marine and brackish waters. In this study, diatoms in the western Black Sea coasts of Turkey were investigated with ultrastructure details. Species belonging to the Bacillariales, Rhopalodiales and Surirellales were subject to light microscopy (LM) and scanning electron microscopy (SEM) analysis, and a total of twenty-four taxa were investigated. This study examines details on the morphology and biogeography of the taxa with remarks on their distribution in Turkey. The results revealed that four species were reported on the Turkish coasts for the first time. These species were *Nitzschia liebethruthii, N. volvendirostrata, Epithemia guettingeri* and *Campylodiscus scalaris*.

Keywords: Bacillariophyceae, biodiversity, diatoms, scanning electron microscopy, the Black Sea

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INTRODUCTION

Diatoms make essential contributions to primary productivity and the photosynthesis rate (Salleh & McMinn, 2011; Witkowski, Bohaty, Edgar & Harwood, 2014). Benthic diatom taxonomy studies determine the flora of the observed area and are useful for further research, e.g. ecology and monitoring. The Black Sea has brackish water characteristics like the Baltic Sea and the Caspian Sea. Unlike the Caspian Sea, the Black Sea and Baltic Sea show some morphological similarities. The Baltic Sea is connected to the North Sea via the Kiel strait; the Black Sea is connected to the Mediterranean via the Dardanelles and the Bosphorus straits; however, water flow is relatively lower. The low rate of saline water flowing through the straits to the Black Sea is one reason for this habitat to show brackish characteristics. However, it has a high rate of river discharge along its coasts. Compared to waters with similar characteristics, the Baltic Sea has been the most studied in recent years. Diatom distribution in the Baltic Sea has been studied by several researchers (Witkowski, 1991; Vilbaste, Sundback, Nilsson & Truu, 2000; Hällfors, 2004).

There have been several studies in marine habitats from the Black Sea. One of the pioneering studies was performed by Mereschkowsky (1902) and Proshkina-Lavrenko (1955, 1963). However, in the last decade, there have been more papers published on the composition and distribution of diatoms of the Black Sea. Most of these studies were performed in the northern part of the Black Sea (Petrov, Nevrova, Terletskaya, Milyukin & Demchenko, 2010; Nevrova, Witkowski, Kulikovskiy, Lange-Bertalot & Kociolek, 2013; Snigireva & Kovaleva, 2015; Nevrova, 2016). Along the southern coasts, studies were limited to a few published papers (Baytut, Gönülol & Koray, 2005; Kaleli, Kulikovskiy & Solak, 2017).

The latest studies reported significant numbers of diatom taxa in the southern Black Sea. However, there is still insufficient data. The diatom flora of the Baltic Sea is an example of taxa that can be used as a comparison for the south

Black Sea. Nevertheless, brackish species have been a challenge for researchers in the past. Some taxa may be confused as being marine, brackish or even as a freshwater species. Monographs, including the brackish taxa (Krammer & Lange-Bertalot, 1988; Witkowski, Lange-Bertalot & Metzeltin, 2000; Hofmann, Werum & Lange-Bertatlot, 2011) are good ways of identifying these taxa. According to some authors, certain environmental parameters (e.g., salinity, temperature) are necessary to identify the taxa. Ultrastructural studies can contribute to the knowledge of the diatom structure in the observed area, even though data is lacking in the Black Sea apart from a few studies in the north (Witkowski, Kulikovskiy, Nevrova, Lange-Bertalot & Gogorev, 2010; Nevrova et al., 2013; Nevrova & Petrov, 2019). When compared to the illustrated fine structure of the freshwater diatoms, marine diatoms have slightly less available data.

Members of the Bacillariaceae family have the highest number of taxa with the Naviculaceae (Kociolek et al., 2021); *Nitzschia*, *Rhopalodia*, and *Surirella* making up many brackish taxa. These brackish conditions, e.g. low saline coasts or electrolyte-high waters, can show high biodiversity. Therefore, diatoms from the Bacillariaceae family are of interest in this paper. Here we present some Bacillarioideae diatoms through the fine structure from the Black Sea coast of Ereğli and report 24 taxa from the *Nitzschia*, *Surirella*, *Rhopalodia*, *Tryblionella*, and *Psammodictyon* genera. This paper focuses on the species which are known to be found in marine-brackish waters. This paper discusses taxa on morphology data provided with Scanning electron microscopy (SEM) analysis and comments on their biogeography.

MATERIAL AND METHODS

Ereğli Bay in Zonguldak province is one of the important industrial zones on Turkey's Black Sea coast. The material used in this study was collected from Ereğli Bay (41°16'56" N 31°24'48" E), during benthic marine diatom sampling of the Black Sea coasts in August 2017 (Fig. 1). Sampling was performed by scraping the submerged stones and rocks at the port.

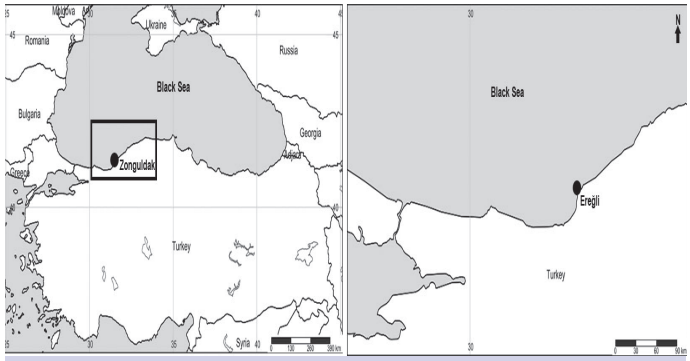

Figure 1. Sampling site location, Ereğli, Zonguldak province, Turkey.

Samples were treated with 10% HCl, then with H_2O_2 to remove the organic material and were then washed with distilled water four times. Permanent slides were prepared with air-dried clean valves and mounted with Naphrax®. Observations in the light microscope (LM) were performed with an Olympus BX-51 microscope at Kütahya Dumlupınar University. SEM observations were performed by Tescan Mira3 XMU at the Sivas Cumhuriyet University Advanced Technology Application and Research Center. Valve measurements were made using ImageJ software. Taxonomic classification and nomenclatural updates followed that of Maraşlıoğlu & Gönülol (2021), Guiry & Guiry (2021) and Kociolek et al., (2021).

RESULTS AND DISCUSSION

Bacillariales Hendey
Cylindrotheca Rabenhorst

Cylindrotheca closterium (Ehrenberg) Reimann & Lewin
Fig. 4: 26, 27

Basionym: *Ceratoneis closterium* Ehrenberg

Synonym(s): *Ceratoneis closterium* Ehrenberg, *Nitzschiella curvirostris* var. *closterium* (Ehrenberg) De Toni, *Nitzschia longissima* var. *closterium* (Ehrenberg) Peragallo & Peragallo, *Nitzschiella tenuirostris* Mereschkowsky.

References: Hasle & Syvertsen (1996)

Dimensions: Length 52 µm, width 3.9 µm.

Remarks: *Cylindrotheca closterium* was found to be cosmopolitan in marine phytoplankton. The species is also known to be harmful algae (Hasle & Syvertsen, 1996).

Distribution & biogeography: The taxon was identified in different locations in Turkey (Maraşlıoğlu & Gönülol, 2021), it is a cosmopolitan and widespread species of marine coasts (Guiry & Guiry, 2021).

Nitzschia Hassall

Nitzschia amabilis H. Suzuki **Fig. 3: 16-19**

Synonym: *Nitzschia laevis* Hustedt 1939

References: Witkowski et al. (2000), Suzuki et al. (2010)

Dimensions: Length 10-12.1 µm, width 4.6-4.9 µm, striae 38-40 in 10 µm, costae 14-16 in 10 µm.

Remarks: Valves are elliptic, the median fibula is distinct, internally the raphe is slightly undulated, terminal raphe endings extend to the apices. In external valves, hymenes were observed internally.

Distribution & biogeography: Hustedt (1939) identified taxa from the coasts of Germany as *Nitzschia laevis* (non *N. laevis* Frenguelli). It is a widespread species (Guiry & Guiry, 2021) and has been reported and documented on the Aegean coast of Turkey (Kaleli, et al., 2020).

Nitzschia incognita Legler & Krasske **Fig. 2: 4**

References: Legler & Krasske (1940), Witkowski et al. (2000).

Dimensions: Length 48 µm, width 3 µm, costae 12 in 10 µm.

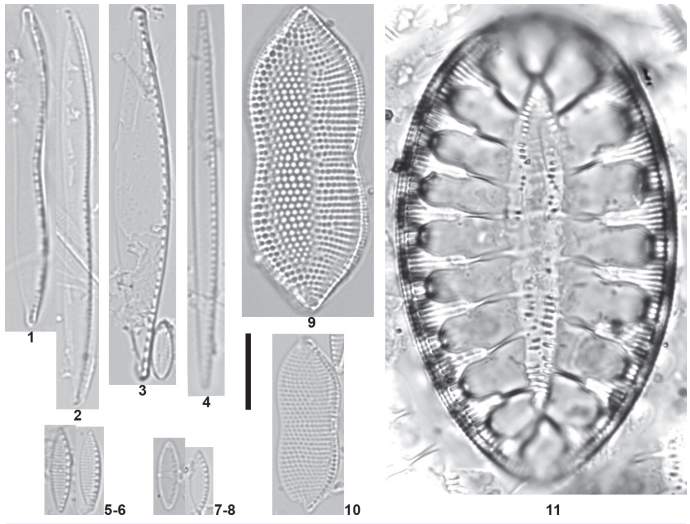

Figure 2. Scale bar: 10 µm. 1, 2. *Nitzschia* cf. *traheaformis*; 3. *N. lanceolata*, 4. *N. incognita*, 5, 6. *N. liebethruthii*; 7, 8. *N. inconspicua*, 9. *Psammodictyon panduriforme* var. continuum; 10. *Tryblionella coarctata*, 11. *Surirella fastuosa*. Scale bar: 10 µm.

Remarks: Striae are indiscernible in LM, median distant fibula exist, valve endings are narrowed apiculate.

Distribution & biogeography: *Nitzschia incognita* is a brackish water species that was identified in Lake Van, Turkey (Legler & Krasske, 1940), and observed in the temperate waters of Europe (Guiry & Guiry, 2021) and the Mediterranean Sea (Witkowski et al., 2000).

Nitzschia inconspicua Grunow **Fig. 2: 7, 8**

Synonym(s): *Nitzschia frustulum* var. *inconspicua* (Grunow) Grunow, *Homoeocladia inconspicua* (Grunow) Kuntze, *Nitzschia perpusilla* var. *inconspicua* Grunow

References: Rovira et al (2015).

Dimensions: Length 7.2-12.5 µm, width 2.3-2.9 µm, costae 14-20 in 10 µm.

Remarks: The taxon resembles *N. frustulum*; however, transapical striae are indistinct in LM, and the valves are broader-lanceolate than the former taxa. It is a widespread brackish-freshwater species (Guiry & Guiry, 2021).

Distribution & biogeography: Species was reported and documented from the Aegean streams and is also common in Turkey (Maraşlıoğlu & Gönülol, 2021). *Nitzschia inconspicua* is a widespread freshwater species also occurring in brackish water (Guiry & Guiry, 2021).

Nitzschia lanceolata W. Smith **Fig. 2: 3**

Synonym: *Homoeocladia lanceolata* (W. Smith) Kuntze

References: Witkowski et al (2000).

Dimensions: Length 47.5 µm, width 7.1 µm, costae 8 in 10 µm.

Remarks: One valve of *Nitzschia lanceolata* was observed. Valves are broadly lanceolate; fibulae are unevenly distributed.

Distribution & biogeography: *Nitzschia lanceolata* was identified in streams in the Aegean and the Black Sea region; however, the taxon was not documented before in marine coasts of Turkey. It is a cosmopolitan marine-brackish species that was reported in the Mediterranean and the Black Sea (Witkowski et al., 2000; Guiry & Guiry, 2021).

Nitzschia liebethruthii Rabenhorst
Fig. 2: 5, 6; Fig. 3: 15

Synonym: *Homoeocladia liebethruthii* (Rabenhorst) Kuntze

References: Krammer & Lange-Bertalot (1988); Witkowski et al. (2000).

Dimensions: Length 11.4-12.4 µm, width 2.7 µm, fibulae 16-18 in 10 µm.

Remarks: Valves are lanceolate, striae are distinct in LM. *N. liebethruthii* valves were observed with higher fibulae density (Witkowski et al., 2000; 11-12 in 10 µm). The taxon is close to *N. frustulum*, and small valves resemble *N. inconspicua*.

Distribution & biogeography: The species was reported inland of the Antalya and Euphrates regions. This is the first time the species was identified in Turkish coasts. It is a cosmopolitan species

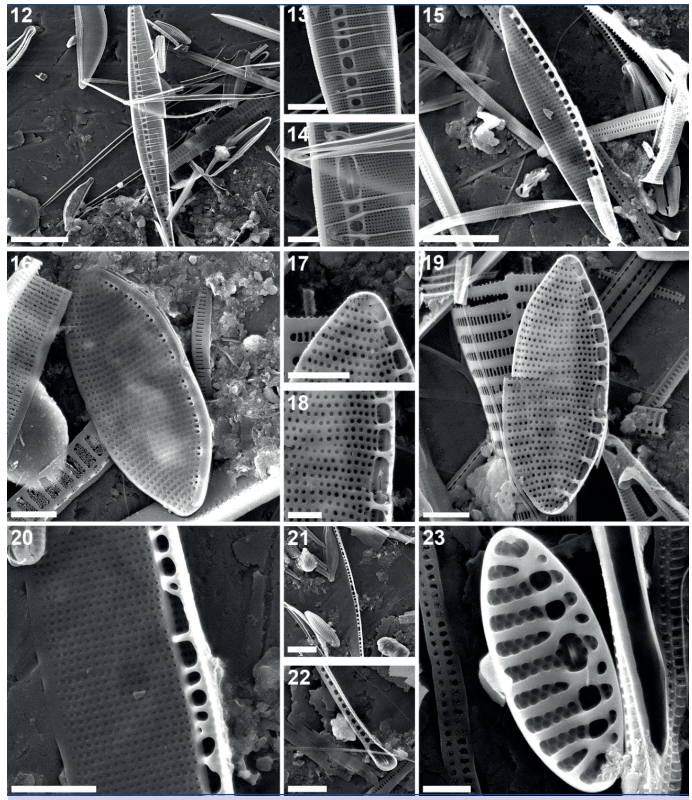

Figure 3. 12-14. *Nitzschia ventricosa*; 15. *N. liebethruthii*; 16-19. *N. amabilis*; 20-22. *N. reversa*; 23. *N. valdestriata*. Scale bars: 17,18,23: 1 µm; 13,14,16,20,22: 2 µm; 15,19,21: 5 µm; 12: 10 µm.

reported in the Mediterranean Sea (Witkowski et al., 2000) and also occurs in brackish-freshwater habitats (Guiry & Guiry, 2021).

Nitzschia* cf. *traheaformis Chunlian Li, Witkowski & Yu
Fig. 2: 1, 2; Fig. 4: 30

References: Witkowski et al. (2016).

Dimensions: Length 28.7-36.3 µm, width 3.6-3.9 µm, striae 34-40 in 10 µm.

Remarks: Valves are slightly constricted in the middle with apiculate apices. Specimens are similar in valve outline to the following species; however, observed specimens were smaller in size compared to *N. hybrida* (48-103 µm) and *N. pellucida* (55-70 µm; Witkowski et al., 2000). The valves showed more resemblance to *N. traheaformis,* which was 17-54 µm long and 3-4.7 µm wide (Witkowski et al., 2016), and more dubiatae than the other species. Here the valves have higher striae than *N. traheaformis* (32-34 in 10 µm). Therefore we commit the taxa as *N.* cf. *traheaformis* for further observation.

Distribution & biogeography: *Nitzschia traheaformis* was found in the Red and Yellow Sea and the Indian Ocean. It is necessary to observe the taxon in the Black Sea in detail to confirm before addressing and expanding its biogeography.

Nitzschia reversa Smith **Fig. 3: 20-22**

Synonym: *Nitzschia longissima* var. *reversa* Grunow

References: Snoeijs & Potapova (1995), Witkowski et al. (2000).

Dimensions: Length 89.8 µm, width 4.2 µm, striae 52 in 10 µm, costae 16 in 10 µm.

Remarks: *Nitzschia reversa* can be distinguished by its apices which deflect in opposite directions. It is a cosmopolitan species, found in phytoplankton, identified in inland saline waters (Witkowski et al., 2000; Kociolek et al., 2021).

Distribution & biogeography: The taxon was identified by Ersanlı & Gönülol (2003) in Simenit Lake; however, this is the first time it was documented in Turkish coasts. It is cosmopolitan and widespread (Guiry & Guiry, 2021), found in marine phytoplankton (Witkowski et al., 2000).

Nitzschia ventricosa Kitton **Fig. 3: 12-14**

Synonym: *Homoeocladia ventricosa* (Kitton) Kuntze

References: Witkowski et al. (2000), Lobban et al. (2012).

Dimensions: Length 160.6 µm, width 6 µm, striae 48 in 10 µm, fibulae 8 in 10 µm.

Remarks: Valve is long with rostrate endings, fibulae and costae are irregular, central nodule exits, central raphe endings form helictoglossae.

Distribution & biogeography: This is the first time this species was observed in the coasts of the Sea of Marmara in Turkey (in print). Species is widespread in the warm waters of the oceans and observed in the Mediterranean Sea (Guiry & Guiry, 2021).

Nitzschia vidovichii (Grunow) Grunow **Fig. 4: 24, 25**

Basionym: *Homoeocladia vidovichii* Grunow

References: Navarro (1982), Navarro & Lobban (2009).

Dimensions: Length 103.8 µm, width 7.6 µm, striae 28 in 10 µm.

Remarks: The specimen observed here conforms with the micrographs of Navarro (1982), Navarro & Lobban (2009). Taxon resembles *Nitzschia scalpelliformis*, showing similar dimensions; however, raphe is centrally formed.

Distribution & biogeography: It is a widespread marine species (Guiry & Guiry, 2021). Species was reported from Homa Lagoon in the Aegean Sea (Çolak-Sabancı & Koray, 2010).

Nitzschia volvendirostrata Ashworth, Dabek & Witkowski
Fig. 4: 28

References: Witkowski et al. (2016).

Dimensions: Length 12.1 µm, width 2.8 µm, striae 52 in 10 µm, costae 10 in 10 µm.

Remarks: Valve has a linear outline and rostrate apices, fibulae irregular. The taxon resembles *Nitzschia nanodissipata*, in terms of its outline. Witkowski et al. (2016) described the species with the dimensions of 7-11.5 µm length and 3-3.5 µm width. Here, valve dimensions are slightly higher and resemble *N. volvendirostrata* (striae 52-54 in 10 µm, 8-9 costae in 10 µm) in terms of striae numbers.

Distribution & biogeography: Taxon was identified in the Red Sea (Witkowski et al., 2016). This is the first time it has been identified in Turkish coastal waters.

***Psammodictyon* D.G. Mann**

Psammodictyon panduriforme* var. *continuum (Grunow) Snoeijs
Fig. 2: 9

Basionym: *Nitzschia panduriformis* var. *continua* Grunow

Synonym: *Nitzschia panduriformis* var. *continua* Grunow

References: Krammer & Lange-Bertalot (1988), Snoeijs & Balashova (1998).

Dimensions: Length 18.5-22.5 µm, width 7.5-8 µm, striae 18-19 in 10 µm.

Remarks: Taxon is slightly panduriform, valves are smaller than *Tryblionella coarctata* and have higher striae numbers in the observed material.

Distribution & biogeography: It is a brackish-marine waters species identified in the Baltic Sea (Snoeijs & Balashova, 1998). Species was formerly observed in the Aegean Sea coasts in Turkey (Kaleli et al., 2020).

***Tryblionella* W. Smith**

Tryblionella compressa (Bailey) Poulin **Fig. 4: 32, 33**

Basionym: *Pyxidicula compressa* Bailey

Synonym(s): *Pyxidicula compressa* Bailey, *Dinopyxis compressa* (Bailey) Stein, *Exuviaella compressa* (Bailey) Ostenfeld, *Nitzschia compressa* (Bailey) C.S. Boyer, *Prorocentrum compressum* (Bailey) T.H. Abé ex J.D. Dodge.

References: Poulin et al. (1990), Witkowski et al. (2000), Witon & Witkowski (2006).

Dimensions: Length 33.1 µm, width 21.9 µm, striae 9 in 10 µm.

Remarks: Valve is lanceolate, slightly inflated in the middle, with produced apices. Striae are punctate and equal to fibulae numbers.

Distribution & biogeography: *Tryblionella compressa* is a marine, brackish species and widespread on the coasts (Guiry & Guiry, 2021).

Tryblionella coarctata (Grunow) Mann **Fig. 2: 10**

Basionym: *Nitzschia coarctata* Grunow

Synonym(s): *Nitzschia coarctata* Grunow, *Homoeocladia coarctata* (Grunow) Kuntze, *Nitzschia punctata* var. *coarctata* (Grunow) Hustedt

References: Snoeijs & Balashova (1998), Witkowski et al. (2000).

Dimensions: Length 31.6-40 µm, width 14-15 µm, striae 12 in 10 µm.

Remarks: Valves are panduriform with apiculate endings. *Tryblionella coarctata* possess punctate aerolation similar to *Psammodictyon*. Furthermore, *T. coarctata* differs from *P. panduriforme* var. *continuum*, by the hyaline valve face in one half of the valve in LM micrographs. Also, *P. panduriforme* var. *continuum* has lower striae density.

Distribution & biogeography: It is a cosmopolitan species inhabiting marine (Guiry & Guiry, 2021) and brackish waters (Snoeijs & Balashova 1998). It was formerly reported from the Mediterranean Sea (Witkowski et al., 2000) and the Dardanelles Strait (Yıldız, 2018).

Tryblionella* cf. *coarctata* var. *densestriata Álvarez-Blanco & S. Blanco **Fig. 4: 31**

References: Blanco & Blanco (2014).

Dimensions: Length 10.7 µm, width, 5.1 µm, striae 34 in 10 µm.

Remarks: One small specimen was observed, the valve is slightly panduriform with a higher striae count (Blanco & Blanco, 2014; 22-30 in 10 µm). Blanco & Blanco (2014) listed the striae numbers from different authors, and here the specimen was closer to the valves observed from Murcia.

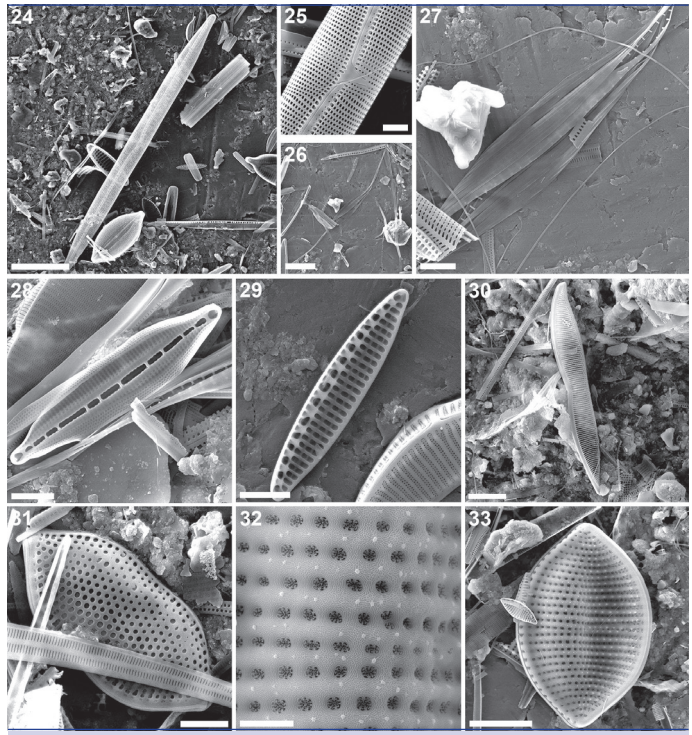

Figure 4. 24, 25. *Nitzschia vidovichii*; 26, 27 *Cylindrotheca closterium*; 28. *Nitzschia volvendirostrata*; 29. *Nitzschia*. sp.1; 30. *N.* cf. *traheiformis*; 31. *Tryblionella* cf. *coarctata* var. *densestriata*; 32, 33. *Tryblionella compressa*. Scale bars: 25,27,28,29,31,32: 2 µm; 30: 5 µm; 24,26,33: 10 µm.

Distribution & biogeography: Taxon was described in the Mediterranean Sea (Blanco & Blanco, 2014).

Rhopalodiales D.G. Mann

***Protokeelia* C.W. Reimer & J.J. Lee**

***Protokeelia* sp. Fig. 5: 36, 43**

Dimensions: Length 7.2-11.2 µm, width 2.5-3.5 µm, striae 32-36 in 10 µm, 35 coastae in 10 µm.

Remarks: Valves are semi lunate to weakly convex and some valves are constricted slightly in the middle, almost straight at the ventral margin. Valve endings are acute and rounded, not produced, areolation are biseriate, raphe terminates slightly before the apices, and is expanded. Central endings are near, slightly bent on the dorsal side in the external valve. In the internal view, fibulae are regular and rounded. Central median fibulae exist with an open gap, however, just one valve was observed and more valves might reveal if it is an opening or broken part of the fibulae. The ventral margin girdle band is placed with two rows oblong of areolae. Taxa resemble genus *Protokeelia* with its characteristics. Some differences were found though, like the ventral side of the specimens resemble mostly *P. hottingeri* (Reimer & Lee, 1984; Lee et al., 1989); the structure of the keel and biseriate areolae are similar, although fibulae structure are different, or ventral margin of *P. spinifera*, *P. aculeata* and *P. cholnokyii*

are concave (Round & Basson, 1995a,b), while *Protokeelia* sp. has straight margins. Therefore we commit the taxa to *Protokeelia* sp. for further studies.

Epithemia Kützing

Epithemia guettingeri (Krammer) Lobban & J.S. Park **Fig. 5: 37-39**

References: Krammer (1988), Park et al. (2018).

Dimensions: Length 19.9-26.5 µm, width 8-8.3 µm, 40 striae in 10 µm, 11-16 costae in 10 µm.

Remarks: Valves are dorsiventral, and straight at the ventral margin. Apices are acute and rounded, raphe terminates at the apices. Central endings are bent on the dorsal side of the valve. Costae are regularly distributed, and striae are biseriate. In the specimens, flabelli-like structure was observed on the areolae. Fibulae are irregular, and median central raphe was observed. Apical costae in the middle of the valve were observed in the external valve view; however, in the internal view, it was not observed, which is in line with Krammer (1988). Park et al. (2018) indicated *Epithemia guettingeri* and *E. pacifica* resemble, and the median costae differs, both existed and were observed in internal and external valves in *E. guettingeri*, while in *E. pacifica* it was observed in the internal valve. Here, areolae presence on the apical band conforms with SEM images of Park et al. (2018). Taxon has recently been proposed to be transferred back to the genus *Epithemia* (Park et al., 2018).

Distribution & biogeography: It is a marine species which was reported in temperate waters of the oceans (Guiry & Guiry 2021). It is reported for the first time in the Turkish coasts.

Epithemia pacifica (Krammer) Lobban & J.S. Park **Fig. 5: 40-42**

References: Lange-Bertalot & Krammer (1987), Witkowski et al. (2000).

Dimensions: Length 40 µm, width 8.7 µm, striae 18 in 10 µm, costae 8 in 10 µm.

Remarks: Valve is strongly dorsiventral and slightly concave at the ventral margin. Areolae are circular, striae are biseriate, raphe terminates at the produced rostrate apices. In the fine structure of the broken valve, the median apical hyaline band was observed. Recently taxa were proposed as *Epithemia pacifica* (Park et al., 2018).

Distribution & biogeography: It is a marine species described from the Pacific Ocean (Guiry & Guiry, 2021) and also was observed in the Mediterranean Sea (Hafner et al., 2018). It is observed for the first time in the Sea of Marmara coasts in Turkey (in print).

Rhopalodia **O. Müller**

Rhopalodia* cf. *brebissonii Krammer **Fig. 5: 34, 35**

References: Lange-Bertalot & Krammer (1987), Witkowski et al. (2000).

Dimensions: Length 19 µm, width 4.3 µm, 30 striae in 10 µm, 5 costae in 10 µm.

Remarks: Valve is dorsally convex, ventrally straight. Areolae are square-oblong, here apices were not produced and acutely apiculate. In Lange-Bertalot & Krammer (1987) valves have produced endings and are bent at the ventral margin, differed from the specimen observed and striae 30 in 10 µm (Lange-Bertalot & Krammer, 1987; 17-22 in 10 µm) are higher than described. Ruck et al. (2016) proposed the transfer of *Rhopalodia* species into *Epithemia*, therefore, after further analysis to confirm specimen belongs to *R. brebissonii* it can be proposed to move into *Epithemia*.

Distribution & biogeography: It is a freshwater taxon (Guiry & Guiry, 2021), reported from the Baltic Sea (Snoeijs & Balashova, 1998).

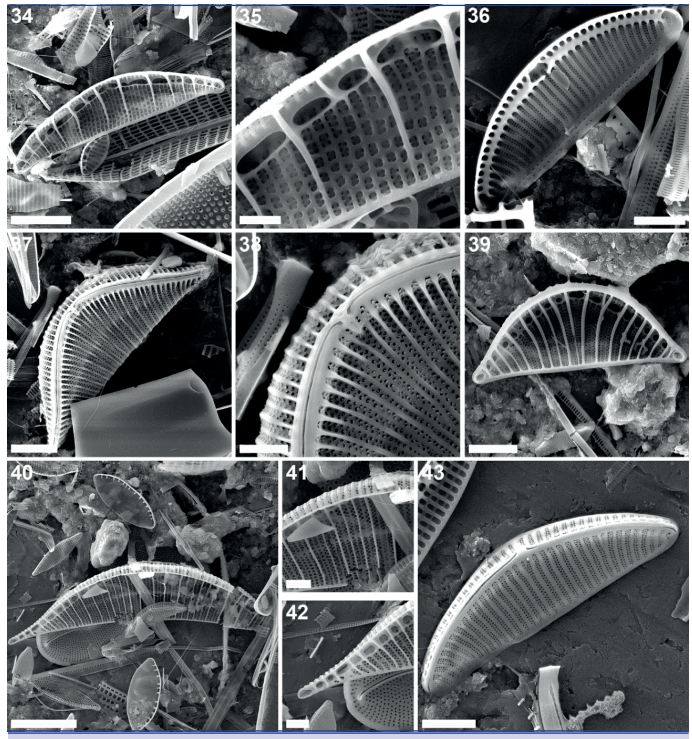

Figure 5. 34, 35. *Rhopalodia* cf. *brebissonii*; 36. *Protokeelia* sp.1; 37-39. *Epithemia guettingeri*; 40-42. *E. pacifica*, 43. *Protokeelia* sp.1. Scale bars: 34,37,39: 5 µm; 35: 1 µm; 36,38,41,42,43: 2 µm; 40: 10 µm.

Surirellales D.G. Mann

Campylodiscus **Ehrenberg ex Kützing**

Campylodiscus scalaris (Giffen) Lobban & Park **Fig. 6: 44-46**

Basionym: *Surirella scalaris* Giffen

References: Giffen (1967), Navarro (1983), Lobban et al (2012), Park et al. (2018)

Dimensions: Length 22.2 µm, width 22 µm.

Remarks: SEM images of *Campylodiscus scalaris* showing that external valve is depressed, the central area is connected to margins by canals, double branched near the margin. In internal view, areolae were small, oblong, and formed a mixed distribution. Warts cover the branches and the occluded area in between. Giffen (1967) described taxa as *Surirella scalaris*, Lobban et al. (2012) proposed a transfer of the species into *Campylodiscus* (see, *C. scalaris* non Tempére & Brun 1889 for different species).

Distribution & biogeography: This is the first record for the Turkish flora, reported from the Pacific Ocean and the Adriatic Sea (Guiry & Guiry, 2021).

Campylodiscus* aff. *bicostatus W. Smith ex Roper **Fig. 6: 47**

Synonym: *Campylodiscus clypeus* var. *bicostata* (W. Smith ex Roper) Hustedt

References: Peragallo & Peragallo (1897-1908)

Dimensions: Length 22.1 µm, width 19.3 µm.

Remarks: Valve consists of a large hyaline area in the centre and irregular punctae are located at the margin between the ribs. Drawings of Peragallo & Peragallo (1897-1908) and Hustedt (1985) resemble the valve found here. However, more specimens should be observed to dedicate the taxa to *C. bicostatus*.

Distribution & biogeography: *C. bicostatus* was reported from different locations, formerly reported from the Sarıkum lagoon and freshwater lakes (Maraşlıoğlu & Gönülol, 2021 and references therein). It is a cosmopolitan freshwater species (Guiry & Guiry, 2021).

Campylodiscus* cf. *thureti Brébisson **Fig. 6: 54**

References: Romagnoli et al. (2014), Kulikovskiy et al. (2016), Schmidt (1874-1959)

Dimensions: Length 32 µm, width 24.5 µm.

Remarks: Valve is oval, composing triseriate striae crossed by ribs and emerges near the margin. Ribs are more further apart, similar to Romagnoli et al. (2014). Our specimen resembles *C.* cf. *thuretii* in Romagnoli et al. (2014). Before committing the specimen into *C. thuretii*, more valves should be observed.

Distribution & biogeography: *C. thuretii* has not been recorded in Turkey before, it has been reported from the temperate waters of marine coasts (Guiry & Guiry, 2021) and the Mediterranean Sea (Romagnoli et al., 2014).

***Surirella* Turpin**

Surirella* cf. *brebissonii* var. *kuetzingii Krammer & Lange-Bertalot **Fig. 6: 51-53**

References: Krammer & Lange-Bertalot (1987), Schmidt (1874-1959).

Dimensions: Length 28.3-34.9 µm, width 12.1-13 µm, 31striae in 10 µm.

Remarks: Valves are wedge-shaped and broadly rounded in headpole and narrower and rounded in the foot pole. Striae are parallel to radiate at the endings and biseriate. Taxon resembles *Surirella brebissoni* var. *kuetzingii* in terms of its valve outline (Krammer & Lange-Bertalot, 1987), however in SEM *S. brebissonii* var. *kuetzingii* composed triseriate-quadriseriate striae (English & Potapova, 2011). In LM striae are discernible in the centre of the valve face. Species also resemble *S. suevica* Zeller (Krammer & Lange-Bertalot, 1987; fig. 46,47) and Schmidt (1874-1959; pl.23: 60-65). Further observation is needed from the material.

Distribution & biogeography: It was reported from different regions of inland waters. (Guiry & Guiry, 2021).

Surirella fastuosa (Ehrenberg) Kützing
Fig. 2: 11; Fig. 6: 48-50

References: Peragallo & Peragallo (1897-1908), Witkowski et al. (2000).

Dimensions: Length 60.1 µm, width 34.9 µm.

Remarks: *Surirella fastousa* shows a wide range of valve morphology; valves are elliptic-ovate with rounded apices in general, due to this character taxon is similar to *S. armoricana* in valve outline. Our specimen resembles *S. armoricana* Peragallo, with wider costae at the margin. Illustrations of Hendey (1974, Pl. 40:6) and Foged (1975, Pl.30:5) match our specimen; however, the drawings and description of *S. armoricana* in Peragallo & Peragallo (1897-1908) revealed that the central area is panduriform. Therefore we commit our specimen to *S. fastousa*.

Distribution & biogeography: Taxa was reported from temperate waters of the coasts of oceans (Guiry & Guiry, 2021).

In this study, members of the Bacillariales, Rhopalodiales and Surirellales from the coast of the Black Sea were investigated, which have a more complex structure such as fibula or keel. A total of 24 species were observed and of these, 22 taxa were investigated with fine structure. Taxa were discussed with regards to taxonomy and biogeographical distribution. Fifteen species were observed from the Bacillariales including *Cylindrotheca*, *Nitzschia*, *Psammodictyon* and *Tryblionella*; while four taxa from the Rhopalodiales showed distribution with *Protokeelia*, *Rhopalodia* and finally the Surirellales were composed of five taxa belonged to *Campylodiscus*, and *Surirella*. Taxa observed here have marine-brackish habitat preferences. Some taxa (*Tryblionella compressa*, *Surirella* cf. *brebissonii* var. *kuetzingii*, *Rhopalodia* cf. *brebissonii*) presented here showed a wide range of distribution in the coasts and lakes of Turkey (Maraşlıoğlu & Gönülol, 2021).

Amongst the species, several of them were formerly reported from the Black Sea, including *Cylindrotheca closterium*, *Nitzschia liebethruthii*, *N. inconspicua*, *N. liebethruthii*, *Psammodictyon panduriforme* var. *continuum*, *Surirella fastuosa*, *Tryblionella compressa* and *T. coarctata* (Nevrova, 2016; Nevrova & Petrov, 2019). In the Aegean Sea coasts, common species were *Campylodiscus scalaris*, *Epithemia guettingeri*, *Nitzschia amabilis*, *N. liebethruthii*, *N. inconspicua* (Louvrou, 2007). Similarly, species

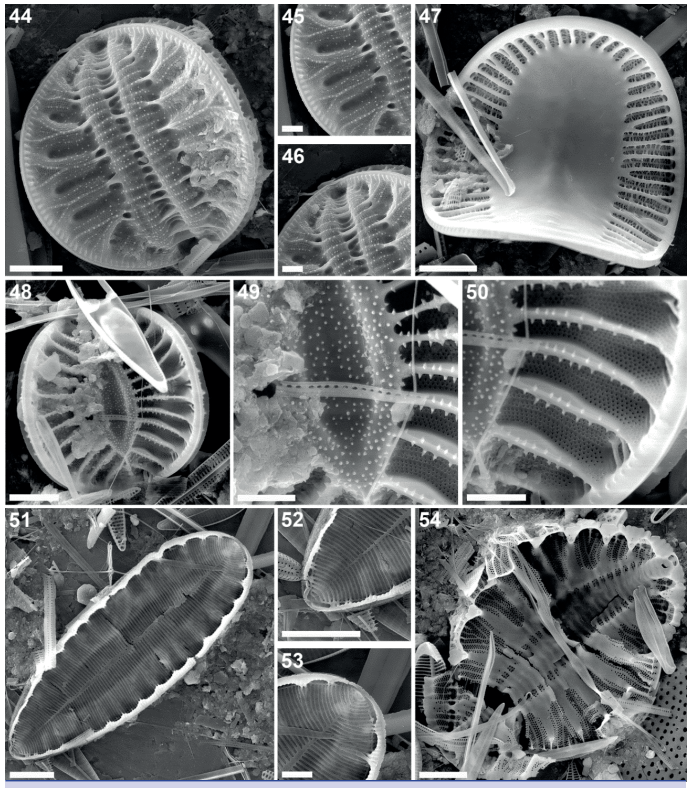

Figure 6. 44-46. *Campylodiscus scalaris*; 47. *C.* cf. *bicostatus*; 48-50. *Surirella fastuosa*; 51-53. *Surirella* cf. *brebissonii* var. *kuetzingii*; 54. *Campylodiscus* cf. *thuretii*. Scale bars: 45,46,49,50,52,53: 2 µm; 44,47,48,51,54: 5 µm.

numbers were higher in the lower saline waters of the Black Sea (Nevrova & Petrov, 2019). While marine diatom ecology has been given less attention, and limited data is present on the autecology of the species, in *Campylodiscus scalaris* an example which was described from the South African coasts, our findings in the Black Sea expanded the biogeographic range of the species in lower saline waters of the Black Sea. This could suggest that with the help of the ultrastructural view, more species could be introduced to the Black Sea flora and contribute to their habitats.

Marine benthic diatoms are important components of the coastal areas; nevertheless, low salinity levels could yield such challenging environments and could be suitable both for brackish and freshwater taxa to survive (Petrov & Nevrova, 2007; Barinova et al., 2019). Recently benthic diatom communities in the Black Sea have become intensively studied in the north due to observing the diatoms' adaptation to environmental changes. On the other hand, Black Sea flora consisted mostly of the presented taxa, particularly in the Northern Black Sea (Petrov & Nevrova, 2007; Nevrova & Petrov, 2019) showed the high biodiversity presence of Bacillariaceae. Although diatom biodiversity of diatoms from the Turkish coasts has not been studied in depth before, some of the previous studies (Baytut & Gönülol, 2016; Kaleli et al., 2017) devoted to benthic diatom flora found that biodiversity could be even higher than that which has been investigated so far on the Black Sea coasts. Also previously, freshwater taxa were reported from the coasts or lagoons in the Black Sea (Soylu et al. 2011; Kaleli & Akçaalan, 2021 and references therein).

The current study represented some of the taxa with SEM. Although the ultrastructure of the valves is a more reliable way of identification, some taxa (*C. thuretii*, *C. bicostatus*, *R. brebissonii*) should be investigated further from the location. Due to the lack of specimens, some taxa could not be defined at the species level. Therefore, for those, there is a need to observe more valves in LM and SEM. Finally, ultrastructure analysis revealed that *Nitzschia liebethruthii*, *N. volvendirostrata*, *Epithemia guettingeri* and *Campylodiscus scalaris* were reported for the first time in the coastal waters of Turkey and contributed to the knowledge of marine benthic diatoms.

CONCLUSION

Eregli Bay, located on the southwestern coasts of the Black Sea, is a moderately populated industrial zone. The taxa discussed above contributed to the knowledge of Turkish diatom flora as well as the Black Sea. The characteristics of the Black Sea could allow a wide range of diatom communities in terms of habitat preference (Nevrova, 2016; Barinova et al., 2019). In addition to this, some studies in the coastal lakes and lagoons in the Turkish Black Sea coast indicated that the diatom community in these areas could be also composed of several freshwater forms (Soylu et al. 2010, 2011). This study brings comprehensive data on the marine and brackish forms which could grow on the hard substrates of the bays in the Southern Black Sea coasts. Further studies would provide data both for the taxonomical aspect and extend the biogeography of the taxa for the Black Sea. Even when the species are taken into consideration under family or order classification, the Turkish coasts of the Black Sea presented a high level of biodiversity, and this could be useful to understand the biodiversity of diatoms and provide data for further studies in the region.

Conflict of Interest

The authors declare no conflict of interest.

Ethics committee approval: Ethics committee approval is not required.

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Original Article

Optimization of Culture Conditions for Total Carotenoid Amount Using Response Surface Methodology in Green Microalgae / *Ankistrodesmus convolutus*

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ABSTRACT

Commercial carotenoids of green microalgae have become significant especially for their applications in the cosmetic, pharmaceutical, food and feed industries. Effects of physical and chemical parameters on carotenoid contents in isolated microalgal species have been investigated. The variables of shaking rate, nitrogen concentration and light intensity affect biomass production and the synthesis of carotenoids in the green microalgae were investigated using the statistical design by Box-Behnken (BBD) employing Response Surface Methodology (RSM). Furthermore, the optimized cultivation conditions using BBD for *Chlorella vulgaris, Ankistrodesmus convolutus, Dunaliella salina, Tetraselmis striata* were determined using the spectrophotometric method to enhance carotenoid concentration. *A. convolutus* within the green algae was detected with the highest carotenoid concentration. The optimum conditions results indicated that the growth of *A. convolutus* (0.55 mg/L) and production of total carotenoids (25.1138 mg/g biomass) were found at the stirrer rate of 100 rpm under the light intensity of 100 µE/m^{2}s, and in the nutrient component of 8.82 mM $NaNO_3$. These conditions were validated experimentally for total carotenoid yield (24.13 mg/g biomass). After that the production was performed in a flat-plate photobioreactor with a volume of 6L based on the optimized conditions and the carotenoid profile was defined by HPLC-DAD using standards such as violaxanthin, astaxanthin and β-carotene. This study proposes that the RSM approach can be used to define optimal conditions for large-scale production of carotenoids by *A. convolutus*.

Keywords: *Chlorella vulgaris*, *Ankistrodesmus convolutus, Dunaliella salina*, *Tetraselmis striata*, Carotenoid, Optimization, Photobioreactor

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INTRODUCTION

Microalgae has a number of natural bioactive molecules, especially carotenoids. Furthermore microalgal carotenoids can provide lots of properties as anti-virals, antimicrobials , anti-inflammatory, anti-oxidant, provitamin A and anti-cancer (Cezare-Gomes et al., 2019). Additionally, microalgae cultivation can be preferred compared to plant cultivation when as algae has many abilities of such as higher biomass production, faster growth and requiring a smaller area of crop (Coelho et al., 2019). Microalgae can create two types of carotenoids: primary carotenoids as photosynthetic apparatus, and secondary carotenoids whenever cells are exposed to stress conditions (Sun et al., 2018; Novoveská et al., 2019). Under various stress conditions such as light intensity, salinity, temperature, nutrient limitation promotes the carotenoid production for microalgae cultivation (Ambati et al., 2018). The demand for natural products, health protecting biomolecules due to healthy and functional food and nutraceuti-

cals bazaar has enhanced increased in the recent years. Many microalgae species are usually identified as safe by the US Food and Drug Administration (GRAS; www.fda.gov). Therefore, the global carotenoid market is anticipated to increase with an annual buy hold gain of 3.9% from 2014 to 2019 (Di Lena et al., 2019). Carotenoids can be divided into two groups: carotene (α-carotene, *β*-carotene and lycopene) and phylloxanthin (astaxanthin, lutein, fucoxanthin and zeaxanthin) and are used in different industries as natural colorants and for their bioactive molecule properties in nutraceuticals, food, feed and cosmetics. For this reason, the investigation of novel natural sources of carotenoids is increasingly resultant in demand (Di Lena et al., 2019).

Response surface methodology (RSM) is both the design of mathematics and statistics concerned with assessing issues due to dependence on several independent variables with an objective to maximize the process variables for approaching optimum response (Eren Şenaras, 2019). Response surface design, which has a center point involved for each independent variable along with the high and low points, requires three trials for each independent variable. In general, a central composite design (CCD) and a Box-Behnken designs (BBD) are the two most reliable and efficient techniques utilized in RSM (Şahin et al., 2019). RSM has decreased the number of experiment runs as a result of saving time, material and labour (Bajwa et al., 2019).

This study had three aims : (I) to cultivate different green microalgae and determine the growth curve, (II) to investigate the carotenoid concentrations of algae enhanced by statistical methods (III) to determine carotenoid profile using HPLC-DAD.

MATERIAL AND METHODS

Cultivation of microalgae and measurement of growth rate

All microalgae species were isolated from Turkish fresh and marine water. Strains were added to the culture collection for long term preservation. Green microalgae were provided by the Ege University Microalgae Culture Collection EGEMACC (https://egemacc.com/cultures.php). The species of microalgae and culture collection codes were given as *Chlorella vulgaris* Beyerinck [Beijerinck] 1890 EGEMACC-53, *Ankistrodesmus convolutus* Corda 1838 EGEMACC-52, *Dunaliella salina* (Dunal) Teodoresco 1905 EGEMACC-84, *Tetraselmis striata* Butcher 1959 EGEMACC-42.

Both *C. vulgaris* and *A. convolutus* were cultivated in Blue-Green Algae Medium-BG-11 (https://utex.org/products/bg-11-medium?variant=30991786868826) (17.65 mM $NaNO_3$ sodium nitrate) and in a medium of 300 mL flask at 100 rpm in a shaker incubator at 20±2 °C under 20 μE/m^2s light intensity while *T. striata* and *D. salina* were grown in DBG-11 (Sea salt added BG-11) and Daigo's IMK Medium (Nihon Pharmaceutical Co., Ltd) media made with artificial seawater for dissolving 22 g/L sea salt (Marinium reef) with the same cultured conditions, respectively. The inoculum was added in a bubble-column photobioreactor (2 L) and cultivated under the light intensity of 100 μE/m^2s, at 20 ± 2 °C, and with the aeration system of 3 L/min. The growth of microalgae was pursued by counting microalgae cells, optic turbidity (at 600 nm), chlorophyll and carotenoid analysis at 2-day intervals over the period of 20 days. Then, cells upon reaching the stationary phase were harvested with centrifuge (ProResearch, By Centurion Scientific Ltd, UK), resuspended with fresh medium and cell concentration were counted with hemocytometer, and used as inoculum at 10^7 cell/mL for further experiments. The inoculum of microalgae was incubated under the RSM conditions for 11 days in a shaker incubator.

The microalgae specific growth rate (*μ*) was calculated from the initial logarithmic phase of growth curve for at least 3 days and the cell death was neglected as

$$\mu = (lnX2 - lnX1)/(T2-T1)$$

Where X_2 is the last cell concentration at time T2, X_1 is the beginning cell concentration at time T1 (Demirel et al., 2018). Doubling time (DT) was also calculated as $DT=\frac{ln2}{\mu}$

Dry weight was defined via filtering a 5 mL culture through preweighed Glass Microfiber filters, grade GF/C (GF-3, Macherey-Nagel), then washed with distilled water and dried at 65±2 °C to a constant weight.

Spectroscopic determination of carotenoid

10 mL of culture was centrifuged for 15 min at 4000 g and then the pelted was suspended in Dimethyl sulphoxide (DMSO) for 45 min in an ultrasonic bath at 45°C in darkness, followed by centrifugation. The optic turbidity of the supernatant was determined at 665, 649 and 480 nm. Chlorophyll a & b and carotenoid concentration was calculated using the equation according to Wellburg (1994).

Statistical analysis

The optimization of the carotenoid process was identified by Response Surface Methodology (RSM) based on Box-Behnken Design (BBD) by the aid of software package Design Expert (version 7.0.0; StatEase Inc., Minneapolis, MN, USA). BBD analysis was used to implement the impact on three independent parameters (shaking rate between the range of 100-250 rpm, light intensity between the range of 100-400 μE/m^2s, and nitrate ($NaNO_3$) concentration between the ranges of 0-17.65 mM) in 15 runs.

Flat-plate photobioreactor (PBR) for Ankistrodesmus convolutus

Flat-plate photobioreactor (PBR) composed of plexiglas enclosed in a rigid metal frame with a volume of 6L was equipped with an online controller (Biosis, Pikolab, Turkey). A temperature-dissolved oxygen probe and pH probe were located in the upper part of the PBR. The culture pH and dissolved oxygen were measured by a sensor, whereas temperature was maintained at 21±2 °C in the temperature-controlled incubator. PBR made of stainless steel was autoclaved at 121 °C for 15 min prior to use. *Ankistrodesmus convolutus* culture was prepared using 11.06 mM nitrogen concentration in BG-11 and mixed by only air at an aeration rate of 4 L/min and controlled by a flow meter (RST electronic Ltd, LZM-6T Turkey). Illumination was provided on the frontal plate by a light emitting diode lamp (LED-Cata 10W CT-5254) with a light intensity of 200.94 μE/m^2s.

After 11 days, biomass was harvested by centrifugation. Paste biomass was stored in a freezer at –20°C and then dried using a freeze-dryer (Christ Alpha 1-2 LD plus, Germany).

Determination of carotenoid profiles

0.02 g microalgae powder and 0.02 g $CaCO_3$ were used with methanol:dichloromethane (MeOH:DCM) (3:1) for the extraction of carotenoids from *A. convutulus*. The cell was lysed using 1 mm diameter glass bead at maximum speed for 10 minutes. The solution was centrifuged at 6000 rpm for 20 min. The residue was repeatedly extracted until the pellet became colorless. The supernatants were collected and combined. The supernatant was then pushed through a 0.45 µm PTFE filters and carotenoid profiles was analyzed by HPLC-DAD (Thermo Scientific Ultimate 3000, Diode Array Detector) using Carotenoid column (YMC C_{30}, 5 mm, 250 x 4.6 mm ID) manufactured by Waters (Milford, MA, USA). The column temperature was set at 20.0 °C. For the carotenoid and chlorophyll assay, Solvent A DCM: MeOH: acetonitrile (ACN): water (5:85:5.5:4.5) and Solvent B DCM: MeOH: ACN: water (22:28:45.5:4.5) were used as the mobile phase and run at a flow rate of 1 mL/min. The gradient conditions were 100% A and 0% B for 8 min, decreased to 0% A and increased to 100% B in 12 min, 100% B maintained for 30 min. The total analysis period was 50 min. Under these conditions, standard chlorophyll-a, chlorophyll b, β-carotene, violaxanthin, astaxanthin (Sigma-Aldrich) were given maximum absorbance at 400-480 nm. For calibration, a standard (0.1-20 mg/L) was prepared from the stock solution and later the standard curve defined concentration of standard chemicals (Erdoğan et al., 2015).

RESULTS AND DISCUSSION

This study was realized in three step: (I) green microalgae was cultivated and growth kinetics were determined, (II) the carotenoid concentration of algae was evaluated to enhance various growth conditions using response surface methodology (RSM) (III) microalgae including maximized carotenoid was grown in flat-plate photobioreactors and carotenoid profile was defined using HPLC-DAD.

Growth of microalgae

The green microalgae was cultivated over the period of 20 days for the determination of growth rates. Fresh water microalgae *Chlorella vulgaris* and *Ankistrodesmus convolutus* reached faster doubling times than marine or brackish water microalgae *Dunaliella salina*, *Tetraselmis striata* (Table 1). Maximum total carotenoid of microalgae (9.27 mg/L) was found for *C. vulgaris* with the highest cell concentration, although the highest dried weight was obtained for *A. convolutus*. The maximum cell concentration of $2x10^7$ cell/mL of *C. vulgaris* was obtained in the BG-11 medium containing 17.65 mM nitrogen concentration under light intensity of 100 µE/m^2s on the 18th day of cultivation while the lowest specific growth rate of *T. striata* was reached in the DBG-11 media containing the same nitrogen concentration and under the same light intensity, as shown in Fig. 1. Fig. 1A and 1B were used to state the specific growth rate of the algae. The microalgae cells determined the stationary phase to use as inoculum for A Box-Behnken design experiments.

Determination of carotenoid concentration and statistical analysis

A Box-Behnken design (BBD) is a three-level full factorial (low, medium and high) design that is implemented to state the nature of the response surface in the trial area. BBD with 15 sets ensures saving on time in comparison with 20 sets of central composite design due to fewer factors of the design determination (Keskin Gündoğdu et al., 2016). The BBD was preferred to study the interaction effects of the parameters. . This experimental design was created to obtain the highest containing carotenoid concentration in the shortest time. This study was enhanced to evaluate carotenoid concentration at different shaking rates, light intensities, and sodi-

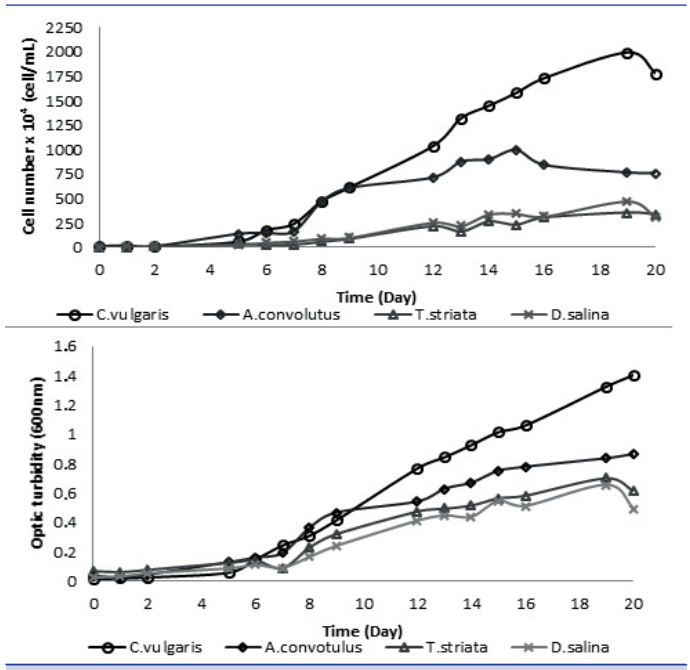

Figure 1. Growth curves of green microalgae in a bubble-column photobioreactor under the light intensity of 100 µE/m2s at 20 ± 2 °C A: cell number and B: optic turbidity.

Table 1. Growth kinetics of microalgae.

Microalgae	Specific growth rate (day^{-1})	Doubling time (day)	Dry weight (mg/L)	Max total carotenoid (mg/L)	Max chlorophyll-a (mg/L)
C. vulgaris	0.33	2.10	0.47	9.27	19.35
A. convolutus	0.44	1.58	0.55	6.54	19.86
T. striata	0.20	3.47	0.33	7.83	17.44
D. salina	0.21	3.30	0.38	8.28	14.57

um nitrate concentration levels. BBD and response surface methodology were performed to design the experiments and optimize the cultivation process for *C. vulgaris*, *A. convolutus*, *T. striata* and *D. salina*. As seen from Table 2, carotenoid concentration (mg/g dried biomass) of the cells were obtained in run 7 (25.1138 mg/g biomass) and set 10 (24.8029 mg/g biomass) for *A. convutulus* and *C. vulgaris*, respectively.

Different mixing rates A – rpm (100, 175, 250), 3 different light intensities; B – μE/m^{2}s (100, 250, 400) and 3 different sodium nitrate concentrations; C – mM (0, 8.82, 17.65) were tested (Table 2). A t otal of 15 experimental runs were performed to optimize the range and levels of selected variables. Also, the order of treatments was regulated incidentally.

The Analysis of Variance (ANOVA) for the model Equivalent (1) of four green microalgae strains observed a good fit between the models and the experimental values.

The experimental results of *C. vulgaris* (Eq 1.1), *A. convotulus* (Eq 1.2), *T. striata* (Eq 1.3) and *D. salina* (Eq. 1.4) the following response surface model, was defined in the symbol factors (A; B; C):

$$M_{Chlorella} = 19.10117 - 0.21897 * A + 0.016477 * B + 2.63118 * C + 3.13471 * 10^{-3} * A * B - 4.44918 * 10^{-3} * A * C - 4.51924 * 10^{-4} * B * C + 7.31595 * 10^{-4} * A^2 - 5.33986 * 10^{-7} * B^2 - 0.092517 * C^2 \quad (1.1)$$

$$M_{Ankistrodesmus} = 56.94909 - 0.42121 * A - 0.014548 * B - 0.34569 * C + 1.27455 * 10^{-4} * A * B + 2.02616 * 10^{-3} * A * C + 2.45561 * 10^{-5} * B * C + 1.07836 * 10^{-3} * A^2 - 4.37888 * 10^{-5} * B^2 - 7.49953 * 10^{-3} * C^2 \quad (1.2)$$

$$M_{Tetraselmis} = -11.14775 + 0.096766 * A + 0.046668 * B + 0.69212 * C - 6.19064 * 10^{-5} * A * B + 2.82325 * 10^{-5} * A * C - 1.23110 * 10^{-4} * B * C - 2.01400 * 10^{-4} * A^2 - 6.87095 * 10^{-5} * B^2 - 0.035297 * C^2 \quad (1.3)$$

$$M_{Dunaliella} = 4.28276 + 0.070757 * A + +9.44808 * 10^{-3} * B + 0.17251 * C - 6.13871 * 10^{-5} * A * B + 1.96848 * 10^{-4} * A * C - 1.79202 * 10^{-4} * B * C - 1.46438 * 10^{-4} * A^2 + 1.16302 * 10^{-5} * B^2 - 9.65502 * 10^{-3} * C^2 \quad (1.4)$$

The regression balance that reflects the model for carotenoid concentration of *C. vulgaris*, *A. convotulus*, *T. striata* and *D. salina* are seen in Eq 1.1, 1.2, 1.3, 1.4 and R^2 adj = 0.92, 0.91, 0.93, 0.94, respectively. This result of adjusted R^2 demonstrates that the model is highly explainable by analyzed variables. When, adjusted R^2 value is close to forecast 1, the more powerful the design is and the stronger it estimates the response (Demirel et. al., 2018). The regression coefficient (R^2> 0.90) was found to be within acceptable limits in all models. Variables were evaluated as remarkable when all model p values of the variable were less than 0.03 (Table 3).

ANOVA was used to peruse the statistical significance of regression coefficients via performing the F-test, t he most accurate model formed and exhibited in graphical representations with contour plots of factors that represent their relative influence and optimal parameter values. A quadratic polynomial empirical model was used in stated optimum conditions for carotenoid mg/g biomass *C. vulgaris*, *A. convolutus*, *T. striata* and *D. salina*.

Optimum stress parameters of *C. vulgaris* were determined as mixing speed of 100 rpm, light intensity of 400 μE/m^{2}s and nitrogen concentration of 10.85 mM when maximum carotenoid amount was predicted in the experimental design (Table 4). Three-dimensional graphs obtained according to statistical analysis results are shown in Fig. 2. When these graphs were analyzed, it was found that both the rise of the shaking rate and light intensity increased the amount of carotenoid despite the fact that the increase in nitrogen concentration had a negative effect.

The optimization data for the maximum carotenoid value for *A. convolutus* was defined at mixing speed of 250 rpm, under light intensity of 200.94 μE/m^{2}s, using nitrogen concentration of 11.06 mM. The three-dimensional graphs obtained according to the statistical analysis results for this microalgae species are shown in Fig. 3. When the graphs were examined, it was found that the carotenoid yield decreased at the highest values of light intensity and nitrogen scarcity was a significant effect on stress parameters for this species.

The optimum parameters of *T. striata* were determined by targeting the maximum mg carotenoid amount per g of dried biomass and the optimum parameters found through 220.17 rpm mixing speed, 212.78 μE/m^{2}s light intensity and 9.15 mM nitrogen con-

Table 2. Experimental data and levels in the response surface design.

Run	Shaking rate-A (rpm)	Light intensity-B (μE/m^{2}s)	Nitrogen concentration-C (mM)	*C. vulgaris* mg/g biomass	*A. convolutus* mg/g biomass	*T. striata* mg/g biomass	*D. salina* mg/g biomass
1	100	400	8.82	21.4375	14.958	4.11924	4.50883
2	250	100	8.82	19.6464	22.3609	6.74322	5.14317
3	250	250	17.65	10.1538	21.3272	4.01902	3.65652
4	175	100	0.00	5.8838	14.7345	2.53533	3.53524
5	175	100	17.65	9.09091	13.217	3.80448	3.57954
6	175	400	0.00	11.2538	13.605	2.75881	5.30923
7	100	100	8.82	15.6916	25.1138	2.38812	2.66377
8	100	250	17.65	19.0038	16.9722	3.62919	2.72584
9	175	250	8.82	16.7914	15.0128	7.41362	4.69743
10	250	400	8.82	24.8029	17.9407	5.68854	4.2258
11	175	250	8.82	16.7914	15.0128	7.41362	4.69743
12	250	250	0.00	14.2885	21.3348	3.39698	3.25713
13	175	250	8.82	16.7914	15.0128	7.41362	4.69743
14	100	250	0.00	11.3593	22.344	3.08189	2.8476
15	175	400	17.65	12.068	12.2175	3.3761	4.40466

Table 3. Analysis of variance (ANOVA) for response surface quadratic model.

Source	*C. vulgaris*					*A. convolutus*					*T. striata*					*D. salina*				
	SS	DF	MS	F value	p value	SS	DF	MS	F value	p value	SS	DF	MS	F value	p value	SS	DF	MS	F value	p value
Model	374.43	9	41.60	6.57	0.02	207.51	9	23.06	5.40	0.03	45.80	9	5.09	7.13	0.02	10.15	9	1.13	9.36	0.01
A	1.44	1	1.44	0.23	0.65	1.60	1	1.60	0.37	0.56	5.49	1	5.49	7.70	0.03	1.56	1	1.56	12.97	0.01
B	56.44	1	56.44	8.91	0.03	34.88	1	34.88	8.17	0.03	0.02	1	0.02	0.03	0.85	1.55	1	1.55	12.90	0.01
C	7.09	1	7.09	1.12	0.33	8.58	1	8.58	2.01	0.21	1.17	1	1.17	1.64	0.25	0.04	1	0.04	0.35	0.57
AB	0.50	1	0.50	0.07	0.79	8.22	1	8.22	1.93	0.22	1.94	1	1.94	2.72	0.16	1.91	1	1.91	15.83	0.01
AC	34.69	1	34.69	5.48	0.06	7.19	1	7.19	1.69	0.25	$1.39*10^{-3}$	1	$1.39*10^{-3}$	$1.95*10^{-3}$	0.96	0.06	1	0.06	0.56	0.48
BC	1.43	1	1.43	0.23	0.65	$4.22*10^{-3}$	1	$4.22*10^{-3}$	$9.90*10^{-4}$	0.97	0.11	1	0.11	0.15	0.71	0.23	1	0.23	1.87	0.23
A^2	62.53	1	62.53	9.87	0.02	135.85	1	135.85	31.82	0.01	4.74	1	4.74	6.64	0.04	2.51	1	2.51	20.78	0.00
B^2	$5.33*10^{-4}$	1	$5.33*10^{-4}$	$8.41*10^{-5}$	0.99	3.58	1	3.58	0.84	0.40	8.82	1	8.82	12.37	0.01	0.25	1	0.25	2.10	0.20
C^2	191.69	1	191.69	30.27	0.01	1.26	1	1.26	0.30	0.61	27.90	1	27.90	39.11	0.00	2.09	1	2.09	17.32	0.01
Residual	31.66	5	6.33			21.35	5	4.27			3.57	5	0.71			0.60	5	0.12		
Lack of fit	31.66	3	10.55			21.35	3	7.12			3.57	3	1.19			0.60	3	0.20		
Pure error	0.00	2	0.00			0.00	2	0.00			0.00	2	0.00			0.00	2	0.00		
Cor Total	406.09	14				228.86	14				49.37	14				10.75	14	1.13		

*SS, sum of squares; DF, degrees of freedom; MS, mean square; A, shaking rate; B, light intensity; C, nitrogen concentration

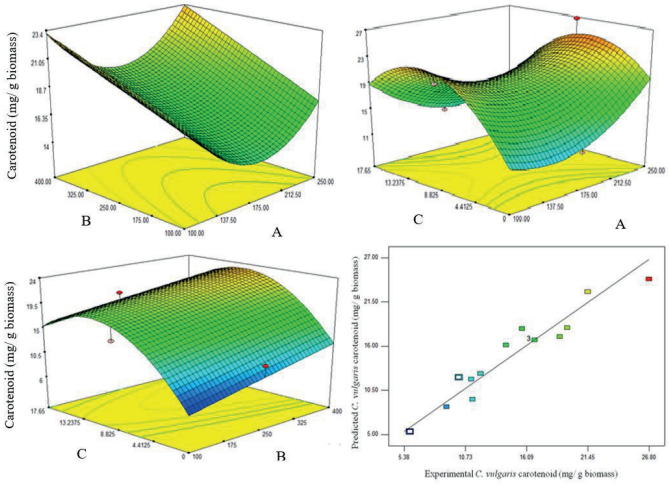

Figure 2. 3D response surface plot of BBD showing the mutual effects A: Shaking of rate (rpm), B: light intensity (µE/ m^2s) and C: nitrogen concentration (mM) on carotenoid concentration of *Chlorella vulgaris*.

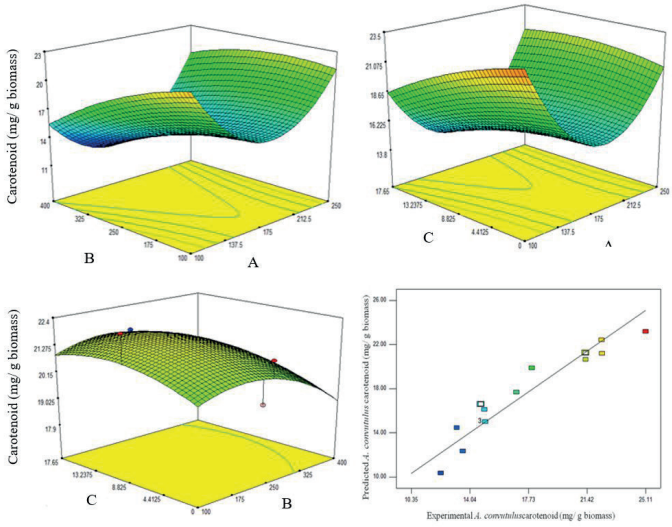

Figure 3. 3D response surface plot of BBD showing the mutual effects of A: Shaking rate (rpm), B: light intensity (µE/ m^2s) and C: nitrogen concentration (mM) on carotenoid concentration of *Ankistrodesmus convotulus*.

centration. The three-dimensional graphs obtained from the statistical analysis of *T. striata* are shown in Fig. 4. Nitrogen concentration was detected to be a sensitive parameter for *T. striata* while the carotenoid yield decreased considerably under low nitrogen concentration. For *D. salina*, nitrogen scarcity was found to be less effective than other stress parameters. As shown in Fig. 5, increasing light intensity appeared to be effective for carotenoid yield.

Wang et al. 2019 reported that optimized medium composition by Central Composite Design (CCD) for freshwater alga *Desmodesmus armatus* was stated indicated the nitrate concentration of 0.93 g L^{-1} under the light intensity of 108 µmol/m^2 s, at 27 °C, at pH 7.00 and air flow rate of 0.50 L/min.

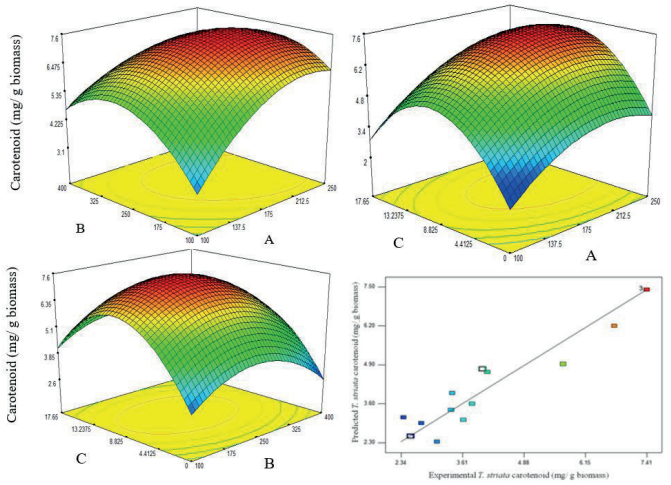

Figure 4. 3D response surface plot of BBD showing the mutual effects of A: Shaking rate (rpm), B: light intensity (µE/ m^2s) and C: nitrogen concentration (mM) on carotenoid concentration of *Tetraselmis striata*.

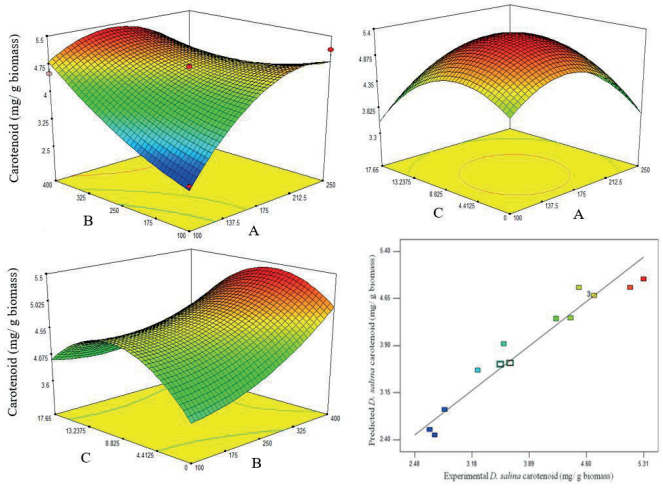

Figure 5. 3D response surface plot of BBD showing the mutual effects of A: Shaking rate (rpm), B: light intensity (µE/ m^2s) and C: nitrogen concentration (mM) on carotenoid concentration of *Dunaliella salina*.

Chlorella zofingiensis optimized the Basal medium using a Plackett-Burman design to peruse seven different factors in which glucose, sodium nitrate and magnesium sulfate heptahydrate were identified to maximize both cell growth and astaxanthin production (Chen &Wang, 2013).

Results of validation studies for experimental and actual data

In order to validate findings, microalgae species were grown for 11 days at the values given in table 4 and spectrophotometric carotenoid analyzes were performed. These results ratified the validness of the model, and the trial values are in accord with the predicted values. Carotenoid concentrations increased when the light intensities were raised for *C. vulgaris* and *D. salina* cultures while maximum carotenoid concentra-

tion for *A. convolutus*, *T. striata* was found under the mild light intensities. This outcome asserts that carotenoids damage is correlate with both the photoprotective role and light-harvesting complexes of carotenoids in the cell compartments, decreasing the singlet oxygen production and scavenging free radicals stimulated by high light and nutrient deficiency (Gonçalves et al. 2019; Faraloni and Torzillo, 2017). Increasing the light intensity resulted in an enhanced ö-carotene productivity in *Dunaliella salina* (Sun et al. 2018).

Production of microalgae in photobioreactor (PBR)

At the end of the whole optimization process, the highest amount of microalgae species carotenoid content compared to the total biomass was found in *A. convolutus*. In view of the results, it was decided to produce a photobioreactor (PBR) using *A. convolutus*.A Controlled panel photoreceptor was used in Flat-plate PBR for the monitoring production of microalgae (Fig. 6). During the 11 days of production,a pH of 8.36, dissolved oxygen and temperature of 23.04 °C were set and regulated by the control panel.

After the production in Flat-plate PBR, freeze-dried biomass was found to be 4.55 g and the total carotenoid was 35.72 mg/g biomass.

Carotenoid analysis

Wang et al. (2018) emphasized that the spectrophotometric method used for the content of fucoxanthin in *Phaeodactylum tricornutum* was easier and faster than high performance liquid chromatography (HPLC). Senge and Senger (1990) reported that neoxanthin, violaxanthin, lutein, α-carotene, *β* -carotene was detected from *Ankistrodesmus braunii*. All sets, validation and Flat-Plate photobioreactor biomass of *A. convotulus* tested, reported the carotenoid profiles and data in Fig. 7, Table 5. Chlorophyta constitutively includes the carotenoids, such as β-carotene, violaxanthin, neoxanthin and lutein, besides chlorophyll a and b (Takaichi 2011). *Asterarcys quadricellulare* PUMCC 5.1.1 produced 47.0, 28.7, 15.5 and 14.0 µg β-carotene, lutein, astaxanthin and canthaxanthin mg^{-1} dry biomass, respectively (Singh et al., 2019). Lutein is a xanthophylls found in some green microalgae species such as *Scenedesmus almeriensis*, *Muriellopsis* sp. and *Chlorella zofingiensis* (Saha et al., 2020). Violaxanthin, β-carotene were the major carotenoids identified in the different microalgae strains. Violaxanthin and astaxanthin were presented in all experiments , while β-carotene was defined in some runs. Astaxanthin, valued as secondary carotenoids under abiotic/biotic stress conditions, was discovered in all of the trial runs in biomass.

Table 4. Results of validation data predicted and experimental carotenoid values.

Microalgae	Shaking rate (rpm)	Light intensity (µE/m²s)	Nitrogen concentration (mM)	Predicted carotenoid values (mg/g biomass)	Experimental carotenoid values (mg/g biomass)
C. vulgaris	100	400	10.85	23.15	23.78
A. convolutus	250	200.94	11.06	21.67	24.13
T. striata	220.17	212.78	9.15	7.51	8.59
D. salina	179.18	390.17	8.84	5.32	5.51

Table 5. The results of carotenoid composition obtained by HPLC-DAD analysis*.

	Violaxanthin (mg/g)	Astaxanthin (mg/g)	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	β-carotene (mg/g)
Run 1	0.13	0.02	5.72	5.03	-
Run 2	0.47	0.04	17.94	15.39	-
Run 3	0.10	0.01	8.21	7.47	-
Run 4	0.23	0.02	1.70	2.70	0.52
Run 5	0.18	0.09	0.52	0.80	-
Run 6	0.08	0.04	19.66	13.90	-
Run 7	0.04	0.01	3.53	3.31	-
Run 8	0.29	0.05	16.72	18.16	0.99
Run 9	0.13	0.02	6.96	7.27	-
Run 10	0.30	0.03	9.28	9.20	-
Run 11	0.13	0.02	5.72	5.03	-
Run 12	0.13	0.02	5.72	5.03	-
Run 13	0.09	0.05	0.21	7.43	0.41
Run 14	0.05	0.03	0.39	11.50	0.75
Run 15	0.21	0.02	0.28	6.31	0.36
Validation	0.21	0.03	8.22	8.62	0.45
Flat- plate photobioreactor	0.84	0.10	34.75	17.03	-

*Results are the data procured from single sample (n=1).

CONCLUSION

In conclusion, growing the green microalgae for the carotenoid production under different light intensity, mixing rate and nitrogen concentration were screened by a Box-Behnken design using spectrophotometric method of total carotenoid. The design emergent in this study through RSM was sufficient for all the essential values it utilized in the optimization. This paper ensures an elaborative study that used both statistical and mathematical methods to detect the optimum levels and interactions among the aforesaid factors in carotenoids production from *C. vulgaris*, *A. convotulus, D. salina, T. striata*. The article stated that carotenoid production by microalgae may be increased by optimized culture conditions. *Ankistrodesmus convotulus*, which cultivated the optimized conditions in 6L flat-plate photobioreactor, was given the highest carotenoid concentration. The carotenoid profile was detected by high-performance liquid chromatography (HPLC) with a diode-array detector (DAD) because of separating and identifying photosynthetic pigments.

Microalgal carotenoid production has recently gained much more research interest due to the possibility of commercial applications especially in food, pharmaceutical, and cosmetic industries. Several research groups have recorded their studies on microalgal carotenoid production . On the other hand, to our knowledge, this is the first report showing the study of comprehensive optimization of culture conditions for total carotenoid in local microalgal strains isolated from Turkish fresh and marine water using Response Surface Methodology. There are still challenges related to the microalgal cultivations and extractions of microalgal carotenoids. For the production of high quality microalgal carotenoids, further studies should focus on the cost-effective microalgal carotenoid processes.

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Conflict of interest: The authors declare that they have no conflict of interest.

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Original Article

Morphological Characterization of *Lithognathus mormyrus* (Linnaeus, 1758) Populations in the Southern Black Sea (Turkey)

Uğur Karadurmuş[1], Mehmet Aydın[2]

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ABSTRACT

Morphological characteristics such as meristic counts and body shape have long been used in stock identification. Fifty striped seabream, *Lithognathus mormyrus*, individuals were sampled from the south Black Sea between January 2020 and November 2020. Morphometric characteristics were given as the percentage of total length (TL%). Species identification and shape differences between sexes of striped seabream were analyzed by different statistical methods. The biggest maximum length and weight values (TL=310 mm for TW=399.73 g) were found for the southern Black Sea in this study. The mean TL of female individuals ($\overline{X}$=205.26 mm) was found higher than male individuals ($\overline{X}$=199.10 mm). The TL% ratio of head length was found as 24.45% for all individuals. In this study, isometric growth (t=2.009, p>0.05) was determined for all individuals and the length-weight relationship (LWR) was found as $W=0.013TL^{3.003}$ (r^2=0.983). This study revealed that the morphological characteristics of *L. mormyrus* differ significantly between female and male individuals and the species may be a good example of sexual dimorphism.

Keywords: Black Sea, fish morphology, LWR, Sparid, Striped seabream

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INTRODUCTION

Sparids are spread in coastal waters worldwide and sustain commercial and recreational fishing (Fischer, Schneider, & Bauchot, 1987). The striped seabream *Lithognathus mormyrus* (Linnaeus, 1758) (Perciformes: Sparidae) is naturally found in the Eastern Atlantic, South Africa coastal waters, and the Western Indian Ocean. It is a demersal species living in groups over various sea bottoms, mainly sandy, seagrass beds and rocky beds to a maximum depth of eighteen meters (Bauchot & Hureau, 1986). It feeds on crustaceans, small teleosts, and molluscs (Chessa et al., 2005). They can reach up to 55 cm in total length, and the highest estimated age is twelve years old (Kraljević, Dulčić, Cetinić, & Pallaoro, 1996). Striped sea breams are hermaphrodites where juveniles are male. After 14 cm total length, the female character is dominant. The striped sea bream is one of the representative species of Sparid in the Black Sea. It is known that the adaptation of species inhabiting the Mediterranean Sea (Monteiro et al., 2010) occurred by passing the Turkish Straits System to the Black Sea (Aydın, 2017). This species is also thought to have adapted to the Black Sea ecosystem in this way. The first record of this species on the Black Sea coast of Turkey was reported by Satılmış, Sümer, Aksu, and Çelik (2014) in the province of Sinop, and the second occurrence was recorded by Engin, Keskin, Akdemir, and Seyhan (2015) in the provinces of Istanbul, Trabzon, Rize and Artvin. Next, it was recorded by Aydın (2017) in the province of Ordu. It is noted, lastly, that Kasapoğlu, Çankırılıgil, Düzgüneş, Çakmak, and Eroğlu (2020), who studied biological features of the species in the Black Sea, gave important information on the bio-ecological and genetic aspects.

Morphometric studies are based on a set of measurements that are continuous data, re-

vealing the shape variation and size (Turan, 1999). Morphological characters, such as meristic counts and body shape, have long been used in stock identification (Silva, 2003). Because it can yield information complementary, it has provided valuable results for assessing the stock structure of several species of marine fish (Schaefer, 1989). Geographic and different environmental components can vary in single morphological characteristics, growth patterns, and body shape within and among populations (Firmat, Schliewen, Losseau, & Alibert, 2012). For fish, sexual dimorphism, generally, has been recorded in body size, fin size and shape, color pattern, and head morphology (Morbey, 2018). Females are usually larger than males of the same age, while males are larger than females for some species (Mann, 1980). Dentition, such as number, shape, and arrangement, may also be sexually dimorphic between males and females (de Santana & Vari, 2010).

According to the FAO catch statistics, the population of *L. mormyrus* has been affected by overfishing and may also indicate a future decline. In addition, this species has a Least Concern (LC) status in the Red List of Threatened Species (Russel, Carpenter, Pollard, Mann, & Buxton, 2014). This decline is also seen in Turkey. The total amount of striped seabream fishing in Turkey was reported as 390 tons in 2009 while it was reported as 73.9 tons in 2019 (Turkish Statistical Institute, 2019). Some studies were carried out on the distribution, biology, growth, and genetic and morphological characterization of *L. mormyrus* in Turkey (Türkmen & Akyurt, 2003; Akyol, Kınacıgil, & Şevik, 2007; Emre, Balık, Sümer, Oskay, & Yeşilçimen, 2010; Sümer, Özdemir, & Ertekin, 2014; Altın, Ayyıldız, Kale, & Alver, 2015; Aydın, 2017; Kasapoğlu et al., 2020; Reis & Ateş, 2020). In this study, we investigate the hypothesis that male and female individuals differ significantly from each other morphologically. In addition to the existing literature, we studied samples with a wider size range in *L. mormyrus* populations in the Black Sea, determined morphological differences according to gender, and evaluated the relationship of morphometric characteristics with each other. Considering the commercial importance of the species and the current critical situation, it is essential to follow and manage the population of the species. Any information about this species will shed light on the future in terms of scientific and fishery management.

MATERIALS AND METHODS

Fifty striped seabream individuals (female=27, male=23) were sampled along the Turkish coast of the southern Black Sea (41°40′24″-41°26′10″N & 35°32′09″-41°22′00″E) between January 2020 and November 2020 by using a trammel net (300 m long with 44 mm mesh size) set at 5-20 m depths. The widest possible size range of individuals was collected from both genders to make the study representative of the entire population. Gonads were removed and identified as ovaries or testes from examinations of the gonadal tissue macroscopically. Morphological characters were measured in the laboratory with an electronic caliper to the nearest 0.01 mm. The weight of samples was weighed to the nearest 0.01 g. Fifteen morphometric characteristics were measured on each sample, and those are shown in Fig. 1 (Gharaei, 2012).

Body length is usually expressed as a proportion of standard (SL), fork (FL), and total length (TL). For instance, in species with rigid caudal fins, such as tuna and billfish, FL is frequently used. Indeed, SL is preferred in fish collections as the caudal fin becomes brittle over time and may break off. Gaygusuz et al. (2006) suggest that the use of TL had significantly lower errors compared to FL and SL for all species. For this reason, the TL was used on the undamaged samples for a standard body shape in this study. Different morphometric characteristics were calculated as the percentage of total length (TL%), and the variance coefficient was determined by methods of statistical analysis by sexes. The value for the coefficient of variation (Avşar, 1998) was calculated using this formula:

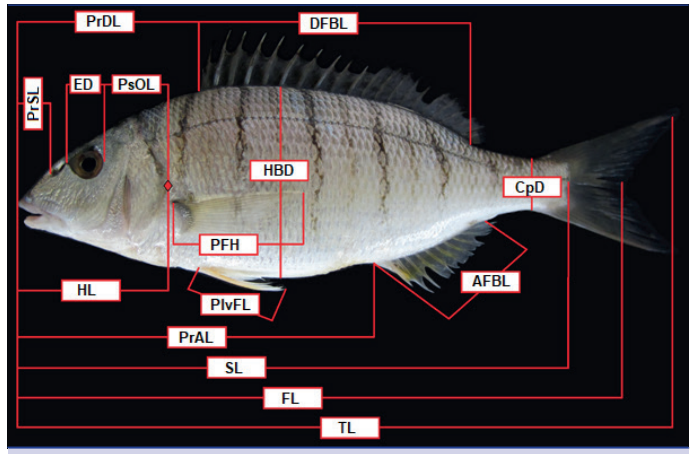

Figure 1. Morphological measurements of *L. mormyrus*. Abbreviations are presented in Table S1.

$$CV\ \% = \frac{Standard\ deviation}{Mean} \times 100$$

Relationships between total length and weight were determined for all individuals using the equation $W = aTL^b$, where W is the fish weight (g), TL is total length (mm), and a and b are the coefficients of the functional regression between W and TL (Ricker, 1975). The linear relationships between TL and the other morphological measurements were estimated with the power equation Y = a+bTL., where Y is the morphological measurements (mm), TL is the total length measurement (mm), and a and b are the regression parameters as the intercept and the slope, respectively (Froese, 2006). The t-test was performed to evaluate whether the estimated b values are significantly different ($p<0.05$) from the isometric value 3 (Pauly, 1983). The 95% confidence limits (Cl) of parameters a and b were estimated, and the coefficient of determination (r^2) was used to evaluate the correlation between TL and morphological measurements (Ricker, 1975). The distribution normality of the morphometric characteristics was analyzed using the Kolmogorov-Smirnov test, and the homogeneity was analyzed using the ANOVA (Analysis of Variance) test. The results were considered statistically significant at $p<0.05$. Thus, non-parametric analyses (Mann-Whitney U) were used for statistical tests. A hierarchical Ward cluster analysis was used to determine closely related variables after Z score correction (Lopez et al., 2004). The data were analyzed by using the statistics software SPSS 26.0 and tabulated with Microsoft Excel 2019.

RESULTS AND DISCUSSION

The TL of females ranged between 160 and 310 mm and males between 142 and 300 mm. The mean TL and standard error (±SE) were calculated as 202.43±5.88 mm while the mean W was 119.47±12.53 g for all samples. The most considerable maximum length and weight values (TL=310 mm for W=399.73 g) were found for the southern Black Sea. The morphological measurements and statistical differences for males, females, and all individuals are presented in Table 1. The mean TL of female individuals ($\bar{X}$=205.26 mm) was found higher than male individuals ($\bar{X}$ =199.10 mm). It was determined that there was no statistically significant difference between the TL of female and male individuals regarding morphometric characteristics (U=272.00; z=-0.750; p>0.05). Similar statistical results (Mann-Whitney U, p>0.05) were found as the same for the other morphological characters and W (Table 1). All morphometric measurements of females were found higher than those of males. Fischer et al.

Table 1. Summary of morphometric measurements (mean±standard error, minimum and maximum) in mm, and statistics of the Mann-Whitney U rank sum for females (♀) and males (♂) of *L. mormyrus*.

Characters (mm)	♀ (N=27)			♂ (N=23)			♀+♂ (N=50)			Statistical Analyze		
	$\bar{X}$±SE	Min	Max	$\bar{X}$±SE	Min	Max	$\bar{X}$±SE	Min	Max	U	z	p-value
TL	205.26±8.19	160.00	310.00	199.10±8.55	142.00	300.00	202.43±5.88	142.00	310.00	272.00	-0.750	0.453 ns
FL	184.12±7.14	146.00	280.00	177.98±7.25	130.00	267.00	181.29±5.07	130.00	280.00	283.00	-0.536	0.592 ns
SL	168.07±6.56	134.00	255.00	160.87±7.04	105.00	247.00	164.76±4.78	105.00	255.00	274.50	-0.701	0.483 ns
HL	50.14±2.23	40.00	81.40	48.84±2.06	38.00	75.00	49.54±1.52	38.00	81.40	299.00	-0.224	0.823 ns
ED	10.44±0.35	8.00	14.80	9.86±0.26	8.00	12.50	10.17±0.22	8.00	14.80	266.50	-0.861	0.389 ns
PrSL	16.63±0.96	12.00	30.20	15.63±0.99	10.00	29.80	16.17±0.69	10.00	30.20	247.00	-1.238	0.216 ns
PsOL	19.49±0.79	15.00	30.50	19.47±0.78	15.00	29.00	19.48±0.55	15.00	30.50	307.50	-0.058	0.953 ns
PrDL	64.02±2.63	50.00	102.90	60.87±2.57	45.20	96.00	62.57±1.84	45.20	102.90	266.00	-0.867	0.386 ns
DFBL	80.45±3.37	62.50	124.28	78.36±3.59	60.20	126.00	79.49±2.44	60.20	126.00	282.00	-0.555	0.579 ns
PrAL	108.92±4.64	83.80	170.00	105.13±4.47	81.10	157.00	107.18±3.22	81.10	170.00	275.00	-0.692	0.489 ns
AFBL	29.26±1.25	23.00	46.40	28.68±1.29	21.40	44.00	28.99±0.89	21.40	46.40	295.00	-0.302	0.762 ns
PFH	34.97±1.59	26.10	54.56	33.50±1.49	25.80	52.00	34.29±1.10	25.80	54.56	272.50	-0.740	0.459 ns
PlvFL	25.52±0.89	20.00	38.40	24.75±0.86	19.10	34.00	25.17±0.62	19.10	38.40	273.00	-0.730	0.465 ns
HBD	55.90±2.21	43.00	86.70	52.50±2.09	40.70	81.00	54.34±1.54	40.70	86.70	246.50	-1.246	0.213 ns
CpD	13.80±0.57	11.00	21.81	13.62±0.66	10.20	23.00	13.72±0.43	10.20	23.00	293.00	-0.342	0.733 ns
W	123.75±18.62	59.67	399.73	114.44±16.63	38.40	336.39	119.47±12.53	38.40	399.73	273.00	-0.730	0.465 ns

*For all tests, df=1. Values followed by ns indicate non-significant differences between genders.

Table 2. Summary of morphometric measurements (mean±standard error) in TL%, and statistics of the rank sum of *L. mormyrus*.

Characters (mm)	♀ (N=27)		♂ (N=23)		♀+♂ (N=50)		Statistical Analyze		
	$\bar{X}$±SE (min–max)	Cv%	$\bar{X}$±SE (min–max)	Cv%	$\bar{X}$±SE (min–max)	Cv%	U	z	p-value
FL (TL%)	89.79±0.23 (88.01-92.91)	1.35	89.56±0.34 (85.37-92.63)	1.83	89.69±0.20 (85.37-92.91)	1.58	301.50	-0.175	0.861 ns
SL (TL%)	81.95±0.16 (79.71-83.75)	1.04	80.76±0.52 (73.94-83.50)	3.11	81.40±0.27 (73.94-83.75)	2.32	249.00	-0.197	0.231 ns
HL (TL%)	24.36±0.23 (19.80-26.26)	4.81	24.56±0.15 (23.31-26.76)	3.01	24.45±0.14 (19.80-26.76)	4.05	293.00	-0.341	0.733 ns
ED (TL%)	5.13±0.09 (4.16-5.94)	8.79	5.02±0.09 (4.14-5.64)	8.23	5.08±0.06 (4.14-5.94)	8.52	275.50	-0.681	0.496 ns
PrSL (TL%)	7.99±0.12 (7.34-9.74)	7.92	7.75±0.14 (6.96-9.93)	8.60	7.88±0.09 (6.96-9.93)	8.29	199.00	-2.171	0.030 *
PsOL (TL%)	9.50±0.10 (8.76-11.17)	5.29	9.82±0.14 (9.11-11.64)	6.76	9.65±0.08 (8.76-11.64)	6.20	219.50	-1.772	0.076 ns
PrDL (TL%)	31.18±0.17 (29.19-33.19)	2.90	30.65±0.35 (25.26-32.92)	5.52	30.94±0.19 (25.26-33.19)	4.32	264.00	-0.905	0.365 ns
DFBL (TL%)	39.13±0.18 (37.80-41.99)	2.35	39.30±0.26 (37.04-42.39)	3.20	39.21±0.15 (37.04-42.39)	2.75	285.00	-0.496	0.620 ns
PrAL (TL%)	52.95±0.17 (51.51-54.84)	1.66	52.84±0.26 (50.86-57.11)	2.40	52.90±0.15 (50.86-57.11)	2.02	275.00	-0.691	0.489 ns
AFBL (TL%)	14.22±0.12 (13.34-15.87)	4.45	14.39±0.15 (13.51-16.50)	4.99	14.30±0.10 (13.34-16.50)	4.70	265.50	-0.973	0.330 ns
PFH (TL%)	16.96±0.14 (14.71-18.65)	4.28	16.82±0.12 (16.00-18.17)	3.47	16.90±0.09 (14.71-18.65)	3.92	240.50	-1.363	0.173 ns
PlvFL (TL%)	12.49±0.07 (11.53-13.30)	3.07	12.52±0.10 (11.33-13.45)	3.87	12.50±0.06 (11.33-13.45)	3.43	291.00	-0.380	0.704 ns
HBD (TL%)	27.28±0.29 (25.38-32.18)	5.47	26.46±0.23 (24.39-28.72)	4.23	26.90±0.20 (24.39-32.18)	5.14	205.00	-2.054	0.040 *
CpD (TL%)	6.72±0.04 (6.35-7.13)	2.71	6.82±0.06 (6.32-7.67)	4.14	6.77±0.03 (6.32-7.67)	3.51	233.00	-1.509	0.131 ns

* For all tests, df=1. Values followed by * indicate significant differences (p<0.05) by gender, Cv, coefficient of variance

(1987) reported that striped seabream generally grow up to 30 cm but can reach a length (TL) of 55 cm. Satılmış et al. (2014), Engin et al. (2015), Aydın (2018), and Kasapoğlu et al. (2020), in their studies in the Black Sea, reported that the TL of individuals was 25.4, 22.6, 30.0, and 23.5 cm, respectively. In the Atlantic Ocean, Dorel (1986) observed the biggest individuals, with a TL of 52.5 cm, in the Bay of Biscay. Kraljević et al. (1996) observed that individual striped seabream from the northern Adriatic Sea reached a length of 38 cm. Monteiro et al. (2010) observed the heaviest individual with a weight of 895.4 g and a TL of 42.7 cm in their study in Portugal. We found the mean TL and the mean W as 202.43±5.88 mm and 119.47±12.53 g for all samples, respectively. In general, it can be argued that the growth of *L. mormyrus* in the Mediterranean and the Atlantic Ocean is faster than that in the Black Sea. Kasapoğlu et al. (2020) determined a significant differentiation between three populations (Black Sea, Mediterranean and the Aegean Sea) of *L. mormyrus*. All morphometric measurements of females were found higher than males in this study. Females are usually larger than males of the same age (Mann, 1980). Kasapoğlu et al. (2020) determined an opposite situation in their studies and found that all morphological measurements of males were higher than females in the same species.

The original TL was considered in the morphometric evaluations, and the other characteristics are presented as a percentage of total length (TL%) as shown in Table 2. According to the values, the most variable features were ED and PrSL in females and males while the least variable features were the SL in females and the FL in males. The TL% ratio of HL was found as 24.45% for all individuals. Although TL% ratios of males were found than those of females, HL, PsOL, DFBL, AFBL, PlvFL, and CpD have a higher rate in females. When looking in more detail, it is seen that fin lengths mostly caused these differences for females. It was determined that there was no statistically significant difference between the TL% of female and male individuals regarding the morphometric characteristics, excluding PrSL and HBD. The TL% ratio of head length was found as 24.45% for all individuals. It was observed that the head makes up almost a quarter of the body. Sparids, such as striped seabream, possess a longer body, a larger mouth gap, a relatively larger head region, and a caudal peduncle which is longer and narrower (Costa & Cataulle, 2007). The pelvic fin was found as approximately 13% of the TL for the combined samples. Pectoral fins are used for balance, deceleration, and partial displacement. In addition, they act as a brake in forward movement, rotation, and sudden stops. Sparids have a long dorsal fin, and it is about 40% of its full neck. The dorsal fin is a median fin located on the dorsal side of the fish. It starts at the level of the pectoral fin base and ends at the level where the pelvic fin ends towards the tail. This fin helps the fish swim in a balanced way and is also used in sudden changes of direction. These features are seen in all members of the Sparidae family (Pervin, 2007). It was determined that there was a statistically significant difference between the TL% of PrSL and HBD for both sexes. The morphological discrepancy in the head region was highlighted in several marine fish such as carangids (Turan, 2004), gobies (Mejri, Lo Brutto, Hassine, Arculeo, & Ben Hassine, 2012), and Sparids (Palma & Andrade, 2002). However, the p values were found high for FL, HL, PlvFL. and DFBL, and this situation showed that head and fin length might differ by sex. Similarly, Morbey (2018) reported that sexual differences were generally seen in fin size and head morphology. It may occur because aquatic environments present different characteristics, habitats, or diets (Delariva & Agostinho, 2001).

Table S1. Description of measured morphological characters of *L. mormyrus*.

Abbreviations	Character	Description
TL	Total length	The longest distance between the tip of the snout and the end of the tail
FL	Fork length	Distance from the tip of the nose to the middle part of the caudal fin
SL	Standard length	Distance from the tip of the nose to the middle part of the end of the vertebral column
HL	Head length	Distance from the tip of snout to the upper posterior to gill cover
ED	Eye diameter	The maximum diameter of the eye with parallel to the longitudinal axis of the body
PrSL	Pre-snout length	Distance from the tip of the nose to the front nostril
PsOL	Post-orbital length	Distance from the end of the orbit to the posterior margin of the operculum
PrDL	Pre-dorsal length	Distance from tip of snout to anterior margin of the dorsal fin base
DFBL	Dorsal fin base length	The longest horizontal distance of the dorsal fin base
PrAL	Pre-anal length	Distance from tip of snout to anterior margin of the anal fin base
AFBL	Anal fin base length	The longest horizontal distance of the anal fin base
PFH	Pectoral fin height	Distance from the lower end to the upper end of the pectoral fin
PlvFL	Pelvic fin length	Distance from the lower end to the upper end of the pelvic fin
HBD	Highest body depth	The deepest distance from the lower end to the upper end of the body
CpD	Caudal peduncle depth	The minimum depth of the caudal peduncle

The statistical data relevant for the evaluation of the linear relationships of each characteristic with TL are included in Table 3, showing the estimated regression parameters along with their

Table 3. Length-weight relationship (LWR) and relationship of morphological characters with total length (TL) of *L. mormyrus*.

	Sex	r^2	a	95% CI (a)		b	95% CI (a)		p
				min	max		min	max	
	♀	0.996	5.500	0.776	10.223	0.870	0.848	0.893	
TL-FL	♂	0.993	9.728	3.110	16.346	0.845	0.812	0.878	p<0.05
	♀+♂	0.994	7.225	3.347	11.104	0.860	0.841	0.879	
	♀	0.997	3.868	0.397	7.340	0.800	0.783	0.817	
TL-SL	♂	0.980	-1.301	-12.073	9.471	0.815	0.761	0.868	p<0.05
	♀+♂	0.988	1.117	-4.131	6.365	0.808	0.783	0.834	
	♀	0.959	-4.492	-9.236	0.253	0.266	0.243	0.289	
TL-HL	♂	0.984	1.221	-1.631	4.074	0.239	0.225	0.253	p<0.05
	♀+♂	0.966	-1.883	-4.752	0.987	0.254	0.240	0.268	
	♀	0.799	2.669	1.032	4.307	0.038	0.030	0.046	
TL-ED	♂	0.879	4.208	3.237	5.178	0.028	0.024	0.033	p<0.05
	♀+♂	0.799	3.286	2.264	4.308	0.034	0.029	0.039	
	♀	0.967	-7.100	-8.934	-5.265	0.116	0.107	0.124	
TL-PrSL	♂	0.945	-6.688	-9.183	-4.193	0.112	0.100	0.124	p<0.05
	♀+♂	0.957	-6.973	-8.426	-5.519	0.114	0.107	0.121	
	♀	0.929	0.420	-1.794	2.634	0.093	0.082	0.104	
TL-PsOL	♂	0.900	2.344	-0.299	4.988	0.086	0.073	0.099	p<0.05
	♀+♂	0.911	1.383	-0.289	3.056	0.089	0.081	0.098	
	♀	0.976	-0.958	-5.283	3.367	0.317	0.296	0.337	
TL–PrDL	♂	0.921	3.464	-4.301	11.229	0.288	0.250	0.327	p<0.05
	♀+♂	0.950	0.772	-3.440	4.984	0.305	0.285	0.326	
	♀	0.989	-3.466	-7.174	0.242	0.409	0.391	0.427	
TL-DFBL	♂	0.979	-4.416	-10.058	1.227	0.416	0.388	0.444	p<0.05
	♀+♂	0.984	-3.805	-6.925	-0.685	0.411	0.396	0.427	
	♀	0.996	-7.266	-10.281	-4.251	0.566	0.552	0.580	
TL-PrAL	♂	0.990	1.515	-3.402	6.432	0.520	0.496	0.545	p<0.05
	♀+♂	0.992	-3.418	-6.392	-0.445	0.546	0.532	0.561	
	♀	0.959	-1.542	-4.210	1.127	0.150	0.137	0.163	
TL-AFBL	♂	0.949	-0.593	-3.728	2.543	0.147	0.132	0.162	p<0.05
	♀+♂	0.954	-1.051	-3.001	0.899	0.148	0.139	0.158	
	♀	0.970	-4.391	-7.295	-1.487	0.192	0.178	0.206	
TL-PFH	♂	0.974	-0.829	-3.431	1.772	0.172	0.160	0.185	p<0.05
	♀+♂	0.969	-2.862	-4.839	-0.886	0.184	0.174	0.193	
	♀	0.982	3.371	2.097	4.645	0.108	0.102	0.114	
TL-PlvFL	♂	0.986	4.966	3.872	6.060	0.099	0.094	0.105	p<0.05
	♀+♂	0.982	4.060	3.205	4.915	0.104	0.100	0.108	
	♀	0.924	2.681	-3.749	9.112	0.259	0.229	0.290	
TL-HBD	♂	0.957	4.815	0.137	9.494	0.239	0.216	0.263	p<0.05
	♀+♂	0.930	3.272	-0.893	7.436	0.252	0.232	0.272	
	♀	0.981	-0.423	-1.250	0.405	0.069	0.065	0.073	
TL-CpD	♂	0.964	-1.416	-2.761	-0.072	0.076	0.069	0.082	p<0.05
	♀+♂	0.969	-0.815	-1.584	-0.046	0.072	0.068	0.076	
	♀	0.983	0.013	0.008	0.021	2.986	8.824	3.147	
TL-W	♂	0.984	0.012	0.007	0.020	3.032	2.857	3.207	p<0.05
	♀+♂	0.983	0.013	0.009	0.018	3.003	2.889	3.116	

*♀, female (N=27); ♂, male (N=23); ♀+♂ combined (N=50); r^2, coefficient of determination; a, intercept of relationship; b, slope of relationship; CI, confidence interval 95%

Table 4. Proximity matrix of morphometric variables for *L. mormyrus*.

Proximity Matrix															
	TL	FL	SL	HL	ED	PrSL	PsOL	PrDL	DFBL	PrAL	AFBL	PFH	PlvFL	HBD	CpD
TL	0.000														
FL	0.008	0.000													
SL	0.013	0.008	0.000												
HL	0.129	0.107	0.116	0.000											
ED	0.244	0.233	0.258	0.526	0.000										
PrSL	0.771	0.716	0.692	0.373	1.506	0.000									
PsOL	0.088	0.063	0.077	0.157	0.316	0.698	0.000								
PrDL	0.146	0.113	0.111	0.055	0.481	0.356	0.146	0.000							
DFBL	0.039	0.031	0.036	0.070	0.359	0.527	0.086	0.085	0.000						
PrAL	0.033	0.024	0.032	0.051	0.332	0.536	0.088	0.073	0.018	0.000					
AFBL	0.081	0.069	0.077	0.099	0.432	0.522	0.090	0.109	0.038	0.046	0.000				
PFH	0.062	0.057	0.059	0.087	0.408	0.528	0.132	0.097	0.043	0.032	0.083	0.000			
PlvFL	0.034	0.028	0.046	0.157	0.177	0.886	0.107	0.163	0.083	0.065	0.121	0.125	0.000		
HBD	0.097	0.069	0.072	0.117	0.343	0.550	0.095	0.053	0.064	0.067	0.085	0.093	0.110	0.000	
CpD	0.188	0.155	0.155	0.056	0.641	0.276	0.157	0.056	0.081	0.095	0.091	0.116	0.233	0.102	0.000

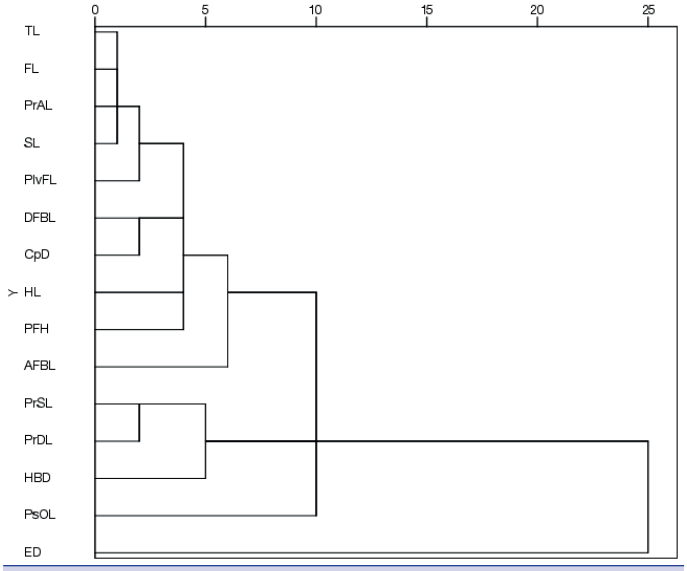

Figure 2. Dendrogram topology of the morphometric variables of *L. mormyrus*.

95% confidence interval and the coefficient of correlation. The linear regression equations are found highly significant with r^2 values ranged from 0.799 to 0.997 for females while r^2 values ranged from 0.879 to 0.993 for males. The interrelationships among the length parameters with TL are also found at high values with all r^2 values being >0.90, excluding ED. Among the linear regression values, the morphometric characteristic most closely related to TL was determined as FL (r^2=0.994), and the weakest correlation was defined as the ED (r^2=0.799). It was determined that the correlation between morphometric characteristics and TL was statistically similar in female and male individuals ($p<0.05$). As a result of the proximity matrix and cluster analysis, it was determined that there were very close relationships between TL and FL (0.008), TL and SL (0.013), and FL and SL (0.008). However, there appeared to be weak relationships of ED and PrSL with other variables, a tendency very similar to that presented by the correlation analysis (Fig. 2; Table 4). These findings were similar to that shown by the correlation analysis (Table 3).

The expected range for the a value was reported between 0.001 and 0.05 for the natural fish populations by Froese (2006). In this study, the value was calculated as 0.013 and 0.012 for females and males, respectively. According to the b value obtained from the length-weight relationship (LWR) equations, Pauly's t-test result showed that *L. mormyrus* (t=2.009, p>0.05) exhibits isometric growth (b=3). The LWR was found as $W=0.013TL^{3.003}$ (r^2=0.983). The high value of the coefficient of determination (r^2>0.95) was determined for *L. mormyrus*. The b value, one of the parameters of the LWR in fish, provides information about the environmental conditions and the body shape of the fish (Avşar, 1998). It is reported that this value varies depending on many conditions such as the number of samples, sampling season, characteristics of the aquatic ecosystem, gonadosomatic index value, and nutrition (Bagenal & Tesch, 1978). In this study, the b value was calculated as 3.003 for all individuals, and it being over three indicates that the sampled fish have sufficiently good environmental conditions and that total growth was achieved as required. In Turkey, most of the researchers (Türkmen & Akyurt, 2003; Akyol et al., 2007; Gökçe, Aydın, & Metin, 2007; İlkyaz, Metin, Soykan, & Kınacıgil, 2008; Emre et al., 2010; Acarlı, Kara, & Bayhan, 2014) have reported positive allometry growth for *L. mormyrus* whereas some of the researchers (Bilge, Yapıcı, Filiz, & Cerim, 2014; Sümer et al., 2014; Altın et al., 2015; Aydın, 2017; Aydın & Sözer, 2019; Reis & Ateş, 2020) have reported negative allometry growth. The relationship between a specimen length and its weight varies over time and between locations, depending on the abundance of food, temperature, salinity, or reproductive activity (Yankova, 2016). It is thought that conditions such as physical and chemical characteristics of water systems, the genetic structure of populations, competition between species, and pressure from fisheries may affect regional differences. The LWR of fish may be influenced by geographical location, environmental conditions, sex, and matu-

rity (Bagenal & Tesch, 1978). The high value of the coefficient of determination (r^2>0.95) was determined for *L. mormyrus*. The r^2 value was reported as 0.817 for the same species by Aydın (2017) and reported relatively lower values. According to the literature, the r^2 values in the present study were similar, which may be based on many factors such as season, region, length range, fish physiology, sampling size, and habitat (Froese, 2006).

CONCLUSION

This paper represents a study to determine morphological characterization and to investigate shape differences by sex in the southern Black Sea coast populations of *L. mormyrus*, which cover all months, comparatively. As such, the values represent reference data for the Sparid species. Furthermore, the study can serve as a reference for future investigation in terms of scientific studies and fishery management. Although this is a local study, our results can be generalized to understand the biological and ecological aspects of *L. mormyrus*.

Conflict of interests: The authors declare that for this article, they have no actual, potential, or perceived conflict of interests.

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Original Article

Effects of sodium nitrate on the growth and proximate composition of the indigenous marine microalgae *Tetraselmis chuii* (Butcher, 1959)

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ABSTRACT

Nitrogen is one of the fundamental nutrients for algal growth, underpinning the microalgal biochemical composition. Therefore, this study compared the growth and proximate compositions of *Tetraselmis chuii* (Butcher, 1959), cultured in different nitrate ($NaNO_3$) concentrations (25, 50, 100, 200 and 500 mg L^{-1}). Thus, the cell density, optical density, specific growth rate and division rate of T. chuii were measured daily. Furthermore, protein and carbohydrate contents were also determined in the stationary phase. The results showed that *T. chuii* cultivated in a $NaNO_3$ concentration of 500 mg L^{-1} had significantly ($p<0.05$) higher growth in terms of cell density, biomass, optical density, specific growth rate and division rates compared with other concentrations. Likewise, protein content was also significantly higher under the $NaNO_3$ concentration of 500 mg L^{-1}, whereas significantly ($p<0.05$) higher carbohydrate content was found at 25 mg L^{-1} $NaNO_3$ compared with the other concentrations, showing a contrary trend between protein and carbohydrate concentrations, respectively. Since the primary focus has been on improving the quality of microalgal biomass in order to develop novel processes and products, this is the first study to use higher concentrations of modified nitrate on *T. chuii* isolated from the coastal area of Bangladesh. Thus the indigenous marine *T. chuii* had significantly utilised $NaNO_3$ concentrations with higher growth and proximate contents in this study. However, further study is needed on microalgal genetics and metabolic engineering to create a new molecular era of the indigenous marine microalgae isolated from the coastal water of Bengal.

Keywords: Nitrogen concentrations, marine microalgae, growth, proximate composition

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INTRODUCTION

Microalgae are vital components in the food chain of aquatic ecosystems (Sathasivam et al., 2019). Their fast growth rates and high value of photosynthetic efficacies (Mostafa, 2012) mean that they potentially have better capacities for CO_2 moderation than terrestrial plants do (Nigam & Singh, 2011). Microalgae are widely used for larval feeding (Khatoon et al., 2013) because of their positive effects on the growth, survival and hatching of aquatic animals. They are also considered potential probiotics in aquaculture because of their antibacterial properties and effective antioxidant system. With the acceleration of climate change, there is a great demand for the development of alternative energy sources with a reduced environmental impact. Microalgae as a source of biofuel and renewable feedstocks could be part of the solution (Ramanna, Rawat, & Bux, 2017).

Nitrogen is fundamental for all functional and structural proteins, including chlorophylls in microalgae. The availability of nitrogen is crucial for microalgal growth (Cai, Park, & Li, 2013). Studies have shown that nitrogen limitation in the culture medium increases lipid or carbohy-

drate content, but it reduces protein synthesis of microalgal biomass, thus reducing microalgae's cell growth rate (Ho et al., 2014). Thus, the physicochemical structure of marine microalgae depends not only on the species but also on the growing conditions; for instance, temperature, salinity, pH, lighting conditions (intensity and photoperiod), nutrients and medium agitation all play an important role (Khatoon et al., 2014; Bartley et al., 2016).

Tetraselmis is a genus of rapidly growing flagellated marine chlorophytes that can tolerate a wide variety of physical conditions (Khatoon et al., 2014). *Tetraselmis chuii,* the species of interest in this study, has recently been approved as a novel food in Europe and may play an important role in supplying essential nutrients because of its high protein, lipid, essential fatty acid and sterol contents (Ghezelbash et al., 2008). Thus, *T. chuii* can be economically important, and there is a need to optimise the growth rate for the production of this microalga. At the same time, the availability of nitrogen needs to be assured to accomplish optimum carbohydrate production and a rational growth rate (Razaghi, Godhe, & Albers, 2014). Although growth, biochemical composition and different nutritional aspects of *T. chuii* have been studied (Khatoon et al., 2018), there is little information available on the effects of different nitrogen concentrations on the growth and proximate composition of indigenous marine *Tetraselmis* species isolated from the Bay of Bengal coast of Bangladesh. Some previous studies have considered the effects of different nitrogen concentrations on *T. chuii*, but the present study was the first to be conducted in a tropical environment, so its region is geographically isolated from the regions described in other studies. Most previous studies have been conducted using very low concentrations of nitrogen, but in this study, a higher concentration of nitrogen was used to evaluate the growth and proximate composition of this indigenous species for the first time. This will help to optimise the nitrogen concentration for higher growth of *T. chuii*. In this study, *T. chuii* was considered because it has high nutritive value, enabling its high potential as feed in aquaculture (Kim, Mujtaba, & Lee, 2016). The main interest was to enhance the microalgal biomass quality so that innovative processes and products can be established. The biochemical composition of microalgae can be modified and improved by manipulating nutrient concentrations. Therefore, the objective of this study was to determine the effects of different nitrogen concentrations on the growth and proximate composition of indigenous *T. chuii*.

MATERIALS AND METHODS

Sample collection, culture and maintenance

The indigenous marine microalgae *T. chuii* strain (CVASUAQ02) was obtained from the pure stock culture of the Department of Aquaculture, Faculty of Fisheries, Chattogram Veterinary and Animal Sciences University, Bangladesh. Filtered natural seawater was used as the seawater base, and the salinity was set at 26 ppt confirmed by a hand refractometer (Refractometer: Atago ATC- S/ Mill- E Salinity 0 - 100%, Japan). The seawater was autoclaved at 121 °C for 15 minutes (DAC 60 Autoclave). A healthy and good stock of microalgae was maintained by observing its morphological features under the microscope. The health status of the cells was considered based on the three following criteria: the cells had to be oval in shape and green in colour, and they could not be found settled at the bottom of the flask. To maintain a healthy stock, *T. chuii* was cultured in an Erlenmeyer flask containing Conway medium at 24 ± 1 °C and a light intensity of 150 µE m^{-2} s^{-1}, maintained using cool fluorescent light. Sub-culturing was done every 2 weeks to prepare the required stock for the main experiment.

Modified minerals stock solution preparation

A stock solution modified in terms of its main mineral contents with different N concentrations was prepared, with sodium nitrate ($NaNO_3$) used as the main source of nitrogen. The procedure was adapted from James (1996). Hence, the stock solution was prepared with five different treatments, where 100 mg L^{-1} of $NaNO_3$ was used as the control treatment (see Table 1). Each solution was tightly capped in a Schott Duran® bottle and stored in a refrigerator (Samsung SilverNano) until further use.

Determination of growth curve

Table 1. Different concentrations of nitrate ($NaNO_3$) with the corresponding N concentrations of each treatment for the study on growth of *Tetraselmis chuii*.

Treatments	$NaNO_3$ concentration (mg L^{-1})	Corresponding N concentration (mg L^{-1})
Treatment 1 (T_1)	25	4.125
Treatment 2 (T_2)	50	8.25
Control 3 (T_3)	100	16.5
Treatment 4 (T_4)	200	33.0
Treatment 5 (T_5)	500	82.5

The growth curve experiment was conducted in sterile 500 mL borosilicate Erlenmeyer flasks, where the *T. chuii* culture volume was 300 mL (270 mL of Conway medium and 30 mL of stock of *T. chuii*). This was done in triplicate. The cultures were maintained at 24 ± 1 °C at a light intensity of 150 µE m^{-2} s^{-1} using cool fluorescent light for 24 h of continuous lighting and aerated continuously with natural sterile air using an air pump. Growth curves based on cell density (cells mL^{-1}) and optical density (450 nm) measurement were constructed. The highest cell density (6.86×10^6 cells mL^{-1}) and optical density (0.327) was found at Day 8, indicating the stationary phase of the growth curve and the beginning of the death phase (Fig. 1).

Experimental design

Fifteen autoclaved 500 mL borosilicate Erlenmeyer flasks were used and filled with approximately 100 mL of culture media, 30 mL of *T. chuii* stock culture and 170 mL of Conway medium. The cultures were maintained at 24 ± 1 °C at a light intensity of 150 µE m^{-2} s^{-1} using cool fluorescent light for 24 h. The cultures were aerated with natural sterile air using an air pump. The flask openings were closed with autoclaved cotton, each with a sterile pipette aeration tube inserted through the cotton into the flask. The growth of the cultures was monitored daily throughout the experiment. Finally, *T. chuii* cultures were centrifuged (Hitachi® High- Speed Refrigerated Centrifuge, himac CR 21G-II) 2 days

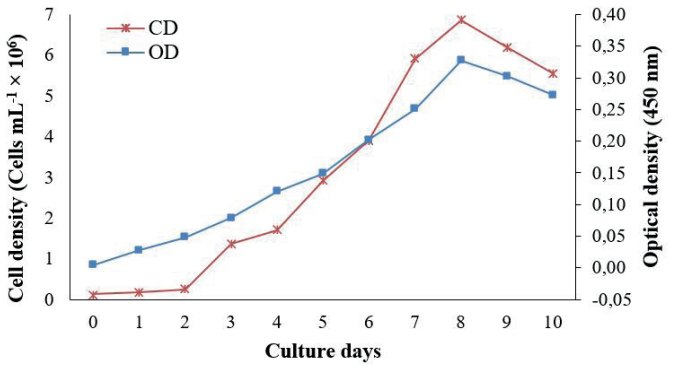

Figure 1. Growth curve of *Tetraselmis chuii* in terms of cell density (CD) and optical density (OD), cultured in Conway medium.

before reaching the stationary phase (based on the growth curve experiment) to obtain pellets for the main experiment. The pellets were rinsed twice with sterilised distilled water before being transferred randomly into fresh medium. Microalgal pellets were randomly transferred into fifteen flasks with a 2 L capacity, containing 1.5 L of culture medium with different $NaNO_3$ concentrations. The growth of the culture was monitored in terms of cell density, biomass, optical density and specific growth rate. The water salinity and pH were maintained at 26 ppt and 7.3–7.5, respectively; nevertheless, the CO_2 concentration was adjusted through aeration during the experimental period. The experiment was conducted in a controlled condition in the laboratory.

Collection of freeze-dried sample

At the end of the experiment, the matured *T. chuii* cells were harvested in the stationary phase (depending on different N concentrations of five treatments) by centrifugation at 7000 rpm for 3 minutes, followed by rinsing twice with sterilised distilled water. Since the highest productivity (cell density and biomass) and matured cells (due to higher nutrition level) were found at the end of the exponential phase, i.e. indicating the stationary phase of *T. chuii* growth (according to the growth curve), microalgae were harvested at this phase (Araujo et al., 2020). The cells were then dried (overnight at 60 °C) and the dried biomass was at -20 °C for proximate composition analysis.

Determination of growth parameters

Cell density, biomass and optical density were measured to determine microalgal growth. Cell density was determined using a haemocytometer (Hawksley AC1000, UK) according to the method reported by Lavens & Sorgeloos (1996). For determination of biomass, 1 mL of culture aliquoted from each of the flasks was filtered onto the pre-weighed GF/C (Whatman glass microfiber filters combine) glass fibre filters using a vacuum pump filtration unit (Millipore). Then, the filters were dried at 100 °C for 4 h and subsequently cooled in the desiccator (Nalgene) for 15 min. Next, the filter papers were individually weighed (AND, GR-200), and the biomass (dry weight basis) was determined for each sample. Optical density was determined using a spectrophotometer (UV-1601 UV Visible Spectrophotometer, Shimadzu). The absorbance readings were taken at a wavelength of 450 nm, considering the culture medium for *T. chuii* as the blanks (Lavens & Sorgeloos, 1996).

Determination of specific growth rate (SGR) and division rate

The specific growth rate (SGR, μ day^{-1}) of *T. chuii* of different treatment was calculated using the following equation, developed by Clesceri, Greenberg, & Trussel (1989):

$$SGR = \ln (X_2 - X_1) / t_2\text{-}t_1,$$

where X_1 represents the biomass concentration at the beginning of the selected time interval, X_2 represents the biomass (dry weight basis) concentration at the end of the selected time interval and t_2-t_1 is the selected time (in days) for the determination of biomass (dry weight) of *T. chuii*.

The division rate (day^{-1}) of the indigenous *T. chuii* was calculated using the following equation (Teo et al., 2014):

$$\text{Division rate} = SGR/ \ln 2$$

Determination of proximate compositions

Protein determination

For every sample, 5 mg of freeze-dried microalgae was taken to make a 25 mL solution by mixing homogenously with distilled water. From this 25 mL microalgal solution, 0.5 mL was taken for protein analysis. Prior to the start of the analysis, Reactive 1 (1% NP tartrate) and Reactive 2 (2 g of $NaCO_3$ in 100 mL of 0.1 NaOH) were prepared. For protein analysis, 50 mL of Reactive 2 and 1 mL of Reactive 1 were mixed. Then, 0.5 mL of microalgal solution was added with 0.5 mL of 1N NaOH and kept in a 100 °C water bath for 5 minutes. Afterwards, it was cooled in a water bath, and 2.5 mL of the prepared mixed reagent was added 10 minutes after cooling. The mixed solution was added with 0.5 mL of Folin - Ciocalteau reagent and then kept in the dark for 30 minutes. Standard protein concentrations were prepared using bovine serum albumin. The absorbance was measured using a spectrophotometer (Shimadzu UV-1601, Japan) at a wavelength of 750 nm (Lowry et al., 1951).

Carbohydrate determination

Freeze-dried microalgae (5 mg) were used to make a 25 mL solution by mixing homogenously with distilled water. Prior to the start of the analysis, 5% phenol solution and concentrated sulfuric acid were prepared. For carbohydrate analysis, 1 mL of microalgal solution was taken and a 5% phenol solution was added, followed by 5 mL of sulfuric acid. The standard was prepared using glucose. The optical density was measured at 488 nm in a spectrophotometer (Shimadzu UV-1601, Japan; Dubois et al., 1956).

Statistical analysis

The results of the growth and proximate compositions were analysed by one-way analysis of variance (ANOVA) considering the nitrate ($NaNO_3$) concentrations with a significance level of 95% ($p<0.05$) as well as Tukey multiple comparisons test (where applicable). The graphical presentation of the growth parameters and proximate compositions (% dry weight) with different treatments were analysed by SPSS software and Microsoft Excel. All results were presented as mean ± standard deviation.

RESULTS AND DISCUSSION

Microalgal growth under different nitrate ($NaNO_3$) concentrations

The cell density, biomass and optical density of the indigenous marine microalga *T. chuii* cultured in controlled conditions under five different concentrations of $NaNO_3$ are shown in Fig. 2. The results showed that *T. chuii* cultured at different $NaNO_3$ concentrations stretched their stationary phase at different days, although no significant difference was found between the T_4 (200 mg L^{-1}) and T_5 (500 mg L^{-1}) treatments. The comparison between T_4 and T_5 was based on the culture days in the stationary phase, in which both treatments indicated the same stationary phase at day 9. In sum, there were no significant differences for biomass. While this finding doesn't support most other studies in which the increasing $NaNO_3$ concentrations indicate the increment of biomass production, the biomass productivity of microalgae is highly dependent on the culture media as well as the species and strain of microalgae (Araujo et al., 2020), which might be consistent with the present findings as this new strain is isolated from the Bay of Bengal. Furthermore, the corresponding N concentrations in the growth medium for each treatment were significantly different with respect to the nitrate ($NaNO_3$) concentrations (Table 1).

Nutrient convenience has a significant impact on the growth and biochemical composition of marine microalgae (Xia et al., 2013). Moreover, the plentiful supply of nutrients (nitrogen, phosphorus, potassium nitrate, etc.) is the principle of attaining high growth rates in microalgal cells (Xia et al., 2013). The growth rate declines and ceases only when the metabolic requirements do not match the supplied nutrients. There are also other important factors (temperature, light, salinity, pH, etc.) that might distress the growth and biochemical compositions of microalgae (Yeh & Chang, 2012). Microalgae growth and biomass are influenced by light and photoperiod during culture (Ahmad et al., 2020; Yusof et al., 2021). Furthermore, the length of light and dark (24:0h) exposure until the saturation point, at which the maximum photosynthetic rate is reached, may influence cellular contents such as chlorophyll and antioxidants (Darvehei, Bahri, & Moheimani, 2018; Yusof et al., 2021). However, in this study, sodium nitrate, a source of nitrogen, was considered one of the most crucial nutrients for microalgal cell growth because its concentrations in the growth medium significantly affected the growth rate of algal cells (Wu & Miao, 2014), as well as the biochemical compositions of microalgae (Kim, Mujtaba, & Lee, 2016).

Zarrinmehr et al. (2020) studied the effects of different nitrogen concentrations (0, 36, 72, 144 and 288 mg L^{-1}) on the growth and biochemical composition of *Isochrysis galbana*. Their study revealed that diminishing concentrations of nitrogen reduce the cell growth and biomass production of *I. galbana*. In the present study, the lowest concentration of $NaNO_3$ (25 mg L^{-1}) showed comparatively very low cell growth; however, the highest cell density (3.00×10^6 cells mL^{-1}) was recorded with the maximum concentration of $NaNO_3$ (500 mg L^{-1}). These results are consistent with Huang et al. (2013), who also reported that the increment of nitrogen supply significantly increased the growth rates of three microalgae species, namely, *Tetraselmis subcordiformis* (Wille) Butcher, 1959; *Nannochloropsis oculata* (Droop) Hibberd, 1981; and *Pavlova viridis* Tseng, Jiaofu, & Zhefu, 1992. These results were also consistent with the optical density measurements of the present study. Studies also reported that some species- *Scenedesmus acutus, Chlorella vulgaris, Nannochloropsis* sp. and *Nannochloropsis oleoabundans*- grow well in nitrogen-deficient environments by exploiting their intracellular nitrogen reserves, such as pigment-protein molecules (Gu et al., 2015); this result also supports the present findings (i.e. growing cell concentration under low concentrations of nitrogen). However, the studies of Araujo et al. (2020) have found the highest productivity at low $NaNO_3$ concentrations for the sole microalgae *T. chuii*, which doesn't support the present study findings. This is because of very low concentrations (25 to 75 mg L^{-1}) of $NaNO_3$ have been conducted in their study, but in this study, a higher concentration of nitrogen was used to evaluate the growth and proximate composition of this indigenous species for the first time. Thus, the aim of this study will help to optimise the nitrogen concentration for higher growth of *T. chuii*.

The marine microalgae *T. chuii* efficiently utilised nitrate ($NaNO_3$) for growth and the present results showed that the increased N concentrations in the culture media significantly enhanced the growth performance. However, the maximum density was found in T_5 (500 mg L^{-1}) treatment (Fig. 2a) with a respective cell density of 3.00×10^6 cells mL^{-1}; lower cell density was observed in T_1 (25 mg L^{-1}) at 1.05×10^6 cells mL^{-1}. There were significant differences among all the treatments of $NaNO_3$ concentrations when compared with the control treatment T_3 (100 mg L^{-1}; Fig. 2a). The increase of nitrate ($NaNO_3$) concentrations in the culture media, ranging from 4.125 to 82.5 mg L^{-1} of N (N concentrations ranges indicated the same as nitrate concentrations based on different $NaNO_3$ concentrations in the medium), significantly induced the increment of biomass production. The biomass concentration of *T. chuii* in the stationary phase under 4.1 mg L^{-1} N (T_1) was only 0.0094 g L^{-1} (23% increase of biomass), whereas the maximum biomass concentration (in dry weight) was 0.0140 g L^{-1} in the stationary phase (81% increase of biomass) under 82.5 mg L^{-1} N (T_5), representing a significant difference between the two results (Fig. 2b). Moreover, there was a significant difference in biomass production among all the treatments compared with the control treatment T_3 (0.0123 g L^{-1} of dry biomass), which exhibited about a 58% increase (comprising 16.5 mg L^{-1} N) of dried biomass. A similar trend was noted for optical density (OD), for which higher absorbance was recorded with increasing N concentrations for nitrate ($NaNO_3$; Fig. 2c).

Marine microalgae can utilise inorganic nitrogen for their growth and metabolic activities and increase their biomass concentrations in conjunction with the nitrogen-enriched condition (Rizwan et al., 2017). The present study findings also revealed that the increment of nitrogen ($NaNO_3$) supply in the medium significantly increased biomass production of *T. chuii*, but the contrary result was found for decreased concentrations of nitrogen. This finding was subsequently supported by Zarrinmehr et al. (2020), who also reported that the diminishing concentrations of nitrogen decreased the biomass production of *I. galbana*, whereas opposite results were found for increased nitrogen concentra-

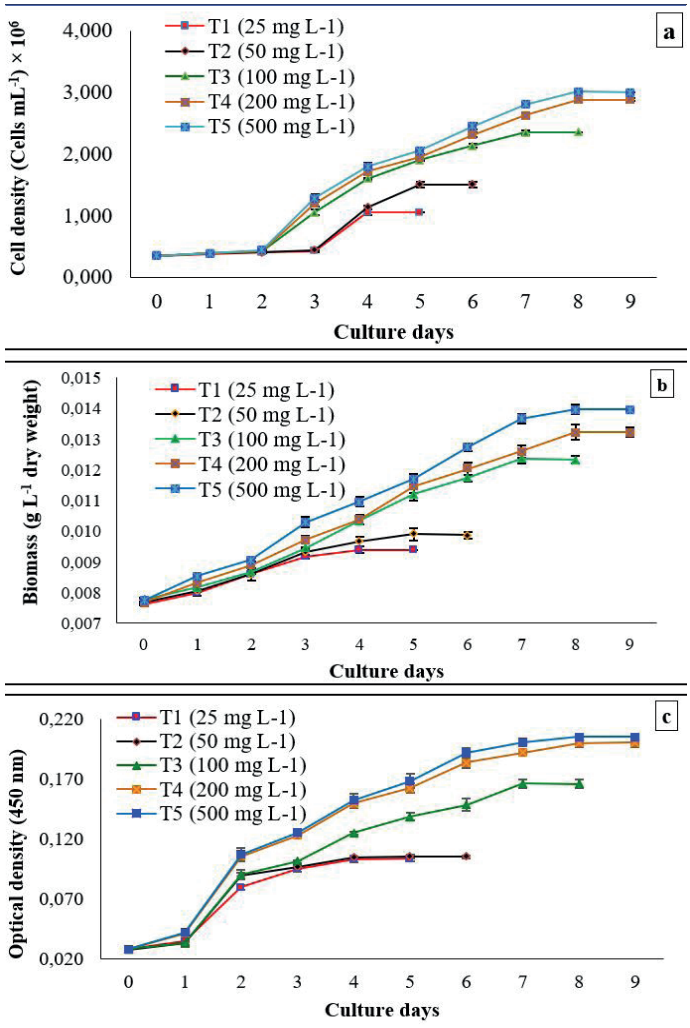

Figure 2. Effects of different $NaNO_3$ concentrations on the growth of *Tetraselmis chuii* in terms of (a) cell density (cells mL^{-1}), (b) biomass (g L^{-1}) and (c) optical density (450 nm; values are mean ± standard deviation).

tions in the medium. Those findings were also consistent with the results reported by El-Kassas (2013), who stated that the cell density and biomass production of *Picochlorum* sp. decreased under the deficient condition of the major macro-nutrients, such as nitrogen or phosphorus, compared with the control treatments. Another important factor is mixing of water, which can also significantly affect the biomass production of microalgal cells. It is important to obtain the proper light absorptions in the culture medium to achieve appropriate growth and biomass production.

It is noteworthy that the culture volume of the present study was 1.5 L in each 2 L of the conical flask supplied with aeration of pure air (comprising 0.03% CO_2), resulting in a low volume of surface area. Thus, the small surface area was supposed to bring about the poor mixing rate and low light penetration in the medium, resulting in low biomass production. Mohsenpour & Willoughby (2016) also reported that increased concentrations of CO_2 in the air stream enhanced the CO_2 fixation rate in *Chlorella vulgaris,* and the biomass concentrations increased with 5% CO_2 aeration compared with the pure air (comprising 0.03% CO_2) concentration, which varied in the present study findings because of the small volume of surface area and low percentage of CO_2 concentrations through aeration. The research by Uggetti et al. (2018) also proved that the addition of CO_2 through aeration induced the rise of biomass concentrations by between 66% and 100%, in which proper mixing was achieved.

The present study findings showed a comparable trend for the SGR (μ day^{-1}) and division rate of *T. chuii,* which increased with the increasing nitrogen concentrations (Table 2). The highest SGR (0.0664 μ day^{-1}) and division rate (0.0958 day^{-1}) were measured for treatment T_5 (500 mg L^{-1}) with a $NaNO_3$ concentration of 82.5 mg L^{-1} N.

Table 2. Specific growth rate (SGR) and division rate of *Tetraselmis chuii* under different concentrations of $NaNO_3$. Values are Mean ± SD (standard deviation).

Treatment	SGR μ day^{-1}	Division rate day^{-1}
T_1	0.0425±0.0002	0.0613±0.0001
T_2	0.0419±0.0001	0.0604±0.0003
T_3	0.0569±0.0003	0.0821±0.0002
T_4	0.0599±0.0002	0.0864±0.0001
T_5	0.0664±0.0001	0.0958±0.0001

Zhu et al. (2014) demonstrated that the specific growth rate of *Chlorella zofingiensis* was 0.48 μ day^{-1} under low concentrations of nitrogen in the medium, and this condition increased the SGR to 1.02 μ day^{-1} during nitrogen repletion. The present study findings were also consistent with Zarrinmehr et al. (2020), who reported that *I. galbana* can grow well in a nitrogen-enriched medium rather than a nitrogen-deficient condition.

Effects of different nitrate (NaNO3) concentrations on protein and carbohydrate content

The different nitrate ($NaNO_3$) concentrations in the growth medium of *T. chuii* showed significant effects on protein and carbohydrate content (Fig. 3) in some treatments. The protein and carbohydrate contents exhibited significant changes of contrary trends in relation to their sufficient and deficient N concentrations for $NaNO_3$, respectively (Fig. 3). The increased N concentrations in the culture medium significantly induced the increment of protein content (% dry weight). The highest protein content of 37.57% dry weight (comprising 82.5 mg L^{-1} N) was recorded from T_5 (500 mg L^{-1}), and this result was significantly different from that of the control treatment T_3. In contrast, the carbohydrate content (% dry weight) revealed the highest concentration, comprising 29.76% dry weight (corresponding concentration of 4.1 mg L^{-1} N) in T_1 (25 mg L^{-1}) for nitrate ($NaNO_3$), which was also significantly different with T_4 and T_5, and the control treatment T_3 (Fig. 3).

The availability of major nutrients has a significant effect on the proximate composition of microalgal cells (Xia et al., 2013). Microalgae generate both lipid and proteins when the carbon:nitrogen ratio is balanced, i.e. when there is a lot of nitrogen, that increases the net nitrogen consumption. But, when nitrogen is

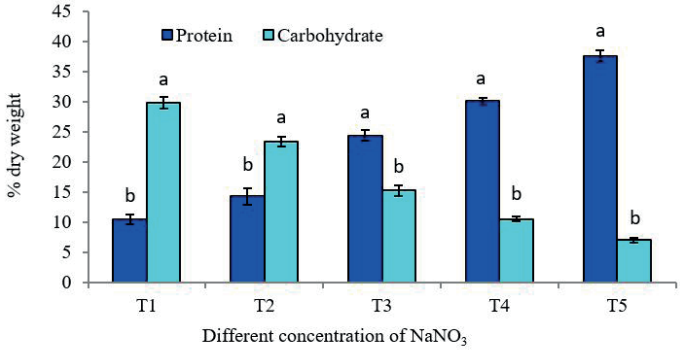

Figure 3. Proximate composition (% dry weight) of *T. chuii* under different sodium nitrate ($NaNO_3$) concentrations (values are mean ± standard deviation). Letters indicate the significant levels of protein and carbohydrate among the different treatments.

scarce, microalgae produce fewer nitrogen-containing molecules and store lipids, resulting in a reduction in nitrogen consumption (Araujo et al., 2020). However, the suitable N concentrations for microalgal growth and proximate composition differ from species to species (Kim, Mujtaba, & Lee, 2016). The present study findings revealed that the increased nitrogen concentrations in the growth medium significantly induced the protein content up to 37.57% (dry weight), while the opposite trend was found for carbohydrate concentrations. This finding was consistent with Kim, Mujtaba, & Lee (2016), who also reported this trend for marine chlorophyte *Tetraselmis* sp., in which carbohydrate production increased up to 55% and protein concentrations gradually decreased under nitrogen-deficient conditions. The findings of this study proved that microalgae (*T. chuii*) cause protein accumulation in N-sufficient culture conditions, while carbohydrate accumulation occurs in N-deficient conditions, which was also described by Kim, Mujtaba, & Lee (2016). Guihéneuf & Stengel (2015) also stated that the cellular carbohydrate concentrations of *Porphyridium purpireum* increased up to 40% during the N-deficient logarithmic phase. A similar study conducted by Pancha et al. (2014) demonstrated that different nitrate concentrations, ranging from 247 to 0 mg L^{-1}, decreased the protein percentage of *Scenedesmus* sp. from 47.75% to 16.87%. Since nitrogen is the most important pioneer for protein synthesis, nitrogen deficiency is a common physiological response in microalgae to decreasing protein content (Zarrinmehr et al., 2020; Araujo et al., 2020). Although nitrogen deficiency is effective, easy and cost effective for biofuel production, it has some effects on cell physiology, such as decreases in proteins and chlorophyll, and it influences photosynthesis ability, resulting in the decreased growth rates (Ördög et al., 2012).

CONCLUSION

The indigenous (isolated from the Bay of Bengal) marine microalgae *T. chuii* was found to utilise sodium nitrate efficiently. Though most previous studies have been conducted using very low concentrations of nitrogen, the current study was conducted with higher concentrations of nitrogen to evaluate the growth and proximate composition of this indigenous species for the first time. It is noteworthy that the present study findings will help to enhance the nitrogen concentration for higher growth of indigenous *T. chuii* as well as to enhance both the protein and lipid content, directing possible future research efforts. In addition, future studies can include experiments on ways to improve the quality and quantity of protein and lipids in microalgae, especially those with commercial advantages. Furthermore, extensive research in the field of microalga genetics and metabolic engineering are necessary to determine methods of producing biofuel in continuous, economic and sustainable ways.

Conflict of interest: The author has declared no conflict of interest.

Ethics committee approval: The research has complied with all regional, national, and institutional ethical clearance and been approved.

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Short Communication

New Additions to the Jellyfish Fauna of the Sea of Marmara

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ABSTRACT

This manuscript reports on four new jellyfish species in the Sea of Marmara (Turkey) (the scyphozoans *Mawia benovici* and *Drymonema dalmatinum*, hydrozoan *Aequorea forskalea*, and thaliacean *Salpa maxima*) based on plankton collections made in the years 2019–2021. This is the first record of *Mawia benovici* in both Turkish coastal areas and the Sea of Marmara. The jellyfish that was previously recorded as *Drymonema* sp. in the repetition Sea of Marmara was identified at the species level as *D. dalmatinum*. Furthermore, their possible introduction pathways are briefly discussed here.

Keywords: *Aequorea forskalea*, *Drymonema dalmatinum*, *Mawia benovici*, *Salpa maxima*, the Sea of Marmara

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INTRODUCTION

The Sea of Marmara is an inland sea that connects the Mediterranean and the Black Seas via the Çanakkale and Istanbul Straits, respectively, and acts as a biogeographical crossroad that includes elements of both Black Sea and Mediterranean Sea biodiversity. The Sea of Marmara is characterized by a permanent, two-layered water system because of the distinct characteristics of two adjacent basins. The upper layer (on average, <25 m depth) consists of brackish Black Sea water (~18 psu) while the lower layer (below the halocline) consists of saline Mediterranean water (~38 psu) (Beşiktepe, Sur, Özsoy, Latif, & Oğuz, 1994). As a consequence of its geographical and hydrographical properties, it forms a barrier and/or a corridor depending on species' potential to acclimatize (Beşiktepe et al., 1994; Ozturk & Ozturk, 1996).

Recent studies of new species demonstrated that the number of jellyfish in the Sea of Marmara has been rising (Isinibilir, Yilmaz, & Demirel, 2015; Isinibilir, Yüksel, & Dalyan, 2020; Yilmaz, Isinibilir, Vardar, & Dursun, 2017). *Mnemiopsis leidyi* caused major harm to the ecosystem after it expanded its niche to the Sea of Marmara following its introduction to the Black Sea at the end of 1980 (Isinibilir, Tarkan, & Kideys, 2004; Shiganova, 1998). Ever since, non-indigenous jellyfish species such as *Chrysaora hysoscella, Liriope tetraphylla, Aequorea vitrina, Cotylorhiza tuberculata*, and others have been introduced to the basin (İnanmaz, Bekbolet, & Kıdeyş, 2002; Isinibilir, Yilmaz, & Piraino, 2010; İşinibilir et al., 2020; Yilmaz et al., 2017). In general, jellyfish are important consumers of zooplankton in pelagic ecosystems, although their ecological role is still poorly understood (Lucas, Gelcich, & Uye, 2014). This report aims to contribute to the knowledge of the changing diversity of jellyfish in the Sea of Marmara by presenting the first records of four gelatinous taxa belonging to the classes Hydrozoa, Scyphozoa, and Thaliacea.

MATERIALS AND METHODS

The samples of *Aequorea forskalea* and *Mawia benovici* were collected through deep trawling

during cruises by R/V Yunus-S in the southern and northeastern part of the Sea of Marmara during July 2019 and June 2020. The samples of *Salpa maxima* were collected through scuba diving, with the collaboration of local divers, in the vicinity of the Princes' Islands in the Sea of Marmara in November 2020. The sample of *Drymonema dalmatinum* from the Maltepe coast was caught in large plastic buckets with the help of local fishermen and transported to the faculty laboratory in January 2021. The location of the sampling stations is shown in Figure 1. Specimens were fixed in 96% ethanol solution for the subsequent analysis. Identification of specimens was carried out under stereomicroscope and light microscope in the laboratory. Samples were taken from all species for molecular analysis and stored in the faculty laboratory for further analysis. In this report, DNA barcoding from a single scyphozoan species, namely *Mawia benovici*, was carried out.

The species identity of specimens of *Mawia benovici* was also confirmed by a DNA-barcoding approach using the cytochrome oxidase subunit I (COI) mitochondrial marker. DNA was extracted using Qiagen DNeasy Blood & Tissue Kits, according to the manufacturer's handbook. Partial COI sequences were amplified by polymerase chain reaction (PCR), setting the parameters as in Furfaro, Oliverio, and Mariottini (2017) (PCR profile: 5 min denaturation step at 94°C; 35 cycles of 94°C/30 s, 48°C/60 s, 72°C/60 s; 7 min. final extension at 72°C), using the universal primers LCO1490 and HCO2198 (Folmer, Black, Hoeh, Lutz, & Vrijenhoek, 1994). Amplicons were sequenced by the European Division of Macrogen Inc. (Amsterdam, The Netherlands). A BLASTN (Altschul, Gish, Miller, Myers, & Lipman, 1990) search was conducted to rule out sample contamination. Sequence homology was investigated by comparing newly produced sequences with the ones already available in GenBank.

RESULTS AND DISCUSSION

In the present study, three jellyfish species (one scyphozoan, one hydrozoan, and one thaliacean) were reported for the first time and the genus *Drymonema* was identified at the species level in the Sea of Marmara (Figure 1). Bouillon et al. (2004), Van Soest (1974), and Piraino et al. (2014) have been used as the primary sources for taxonomic identification and description of characteristics of the species. Notes related to their spatial distribution are reported below.

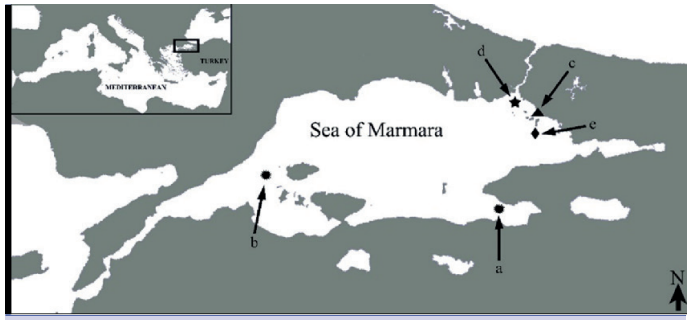

Figure 1. Map of the Sea of Marmara and sampling locations of the specimens (a, b: *Mawia benovici;* c: *Drymonema dalmatinum*; d: *Aequorea forskalea*; e: *Salpa maxima*).

Class: Scyphozoa

Subclass: Discomedusae

Order: Semaeostomeae

Family: Pelagiidae

Genus: *Mawia*

***Mawia benovici* (Piraino, Aglieri, Scorrano & Boero, 2014)**

Mawia benovici specimens were collected as bycatch during trawl surveys from 71 m (st. MD22, Figure 1, Station a) and 108 m (st. MD13A, Figure 1, Station b). In the survey, four living specimens were collected. The first specimen was observed on November 2, 2019 at the MD22 station (Figure 1, Station a) and the other three specimens on November 3, 2019 at the MD13A station (Figure 1, Station b). The umbrella diameters ranged from 60 to 70 mm and were hemispherical to somewhat flattened with a thin transparent mesogleal layer. The exumbrella was yellow ochre in color and was covered by conspicuous cnidocyst warts; eight marginal tentacles; 16 marginal lappets; 16 unbranched, simple radial septa terminating between sense organs and tentacles, as in the genus *Pelagia*; and 8 tentacular and 8 rhopalial separate pouches. The manubrium had a whitish, transparent, very short mouth tube and sharp, long, delicate mouth arms. The gonads were milky white in color, horseshoe shaped, and developed on the subumbrellar surface (Figure 2A, B).

Mawia benovici (formerly described *Pelagia benovici*) was recorded in several locations, such as the northern Adriatic (Trieste to Chioggia (Venice)), the Central Adriatic (near Numana, Ancona), and the Ionian Sea (Igoumenitsa Bay) (Avian, Ramšak, Tirelli, D'ambra, & Malej, 2016; Chartosia et al., 2018; Piraino et al., 2014) in the Mediterranean. However, it has not been recorded in the eastern part of the Mediterranean so far. This species, which

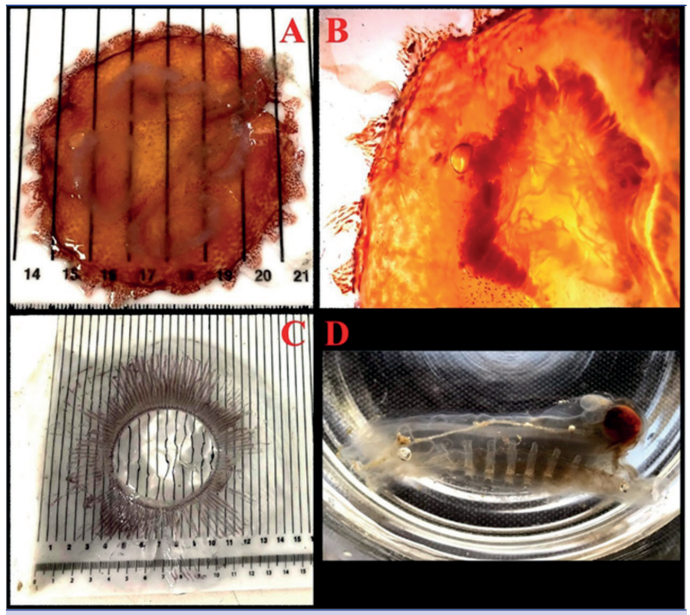

Figure 2. Photographs of the new jellyfish records in the Sea of Marmara. (A) and (B) *Mawia benovici*; (C) *Aequorea forskalea*; (D) *Salpa maxima*.

is assumed to be non-indigenous to the Mediterranean, has also been recorded on the coast of Senegal (Bayha, Collins, & Gaffney, 2017). According to Bayha et al. 2017, the species which was found in the Mediterranean might have originated from the western coast of Africa and been introduced as a result of commercial shipping or fishing. As suggested by Piraino et al. (2014), this species was probably introduced into the Adriatic Sea by ship translocation through ballast waters.

Little information is still available concerning the establishment of *M. benovici* in the Adriatic Sea and, more generally, in the Mediterranean basin. DNA sequences (GenBank COI accession numbers OU193096 and OU193097) were obtained from two specimens collected at the MD13A station (Figure1, Station b). DNA barcoding analysis revealed 100% of homology with COI sequences already present in GenBank and ascribed to *Mawia benovici* (in GenBank as *Pelagia benovici*), definitively confirming that the two collected specimens belong to this rare species. As a result, this is the first record of the species in both Turkish coastal areas and the Sea of Marmara.

Class: Scyphozoa

Subclass: Discomedusae

Order: Semaeostomeae

Family: Cyaneidae

Genus: *Drymonema*

***Drymonema dalmatinum* Haeckel, 1880**

The large-sized semaeostome jellyfish *Drymonema dalmatinum* has recently been reported in various regions of the Mediterranean, especially the Adriatic Sea. Earlier records of *Drymonema dalmatinum* (the only *Drymonema* species in the Mediterranean Sea) go back to 1892 in the Gulf of Izmir (Antipa, 1892). This genus was recorded without species identification for the first time in the Sea of Marmara as *Drymonema sp.* in 2020 (Öztürk, 2020). One year later, the occurrence of *D. dalmatinum* was reported in a fishermen's observation on January 18, 2021 on the Maltepe coast in the northeastern Sea of Marmara (Figure 1, Station c). The jellyfish was found near the sea surface, with a bell diameter of approximate 120 cm. The orientation of the umbrella was upwards; the bell was milkfish white, shield-shaped, and flatly rounded with a thicker central part (Figure 3). The numerous tentacles were longer than the diameter of the bell and of unequal lengths and thickness, and were not grouped in separated clusters.

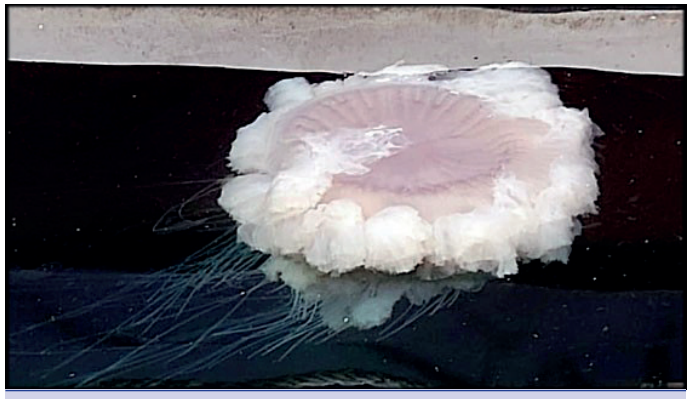

Figure 3. *Drymonema dalmatinum* in the Sea of Marmara.

Class: Hydrozoa

Subclass: Hydroidolina

Order: Leptothecata

Family: Aequoreidae

Genus: *Aequorea*

***Aequorea forskalea* Péron & Lesueur, 1810**

Although *Aequorea forskalea* is a common and abundant hydromedusan species in the Mediterranean (Bouillon et al., 2004) and has been recorded in Turkish coastal areas (Gürlek, Yağlıoğlu, Ergüden, & Turan, 2013; Topcu, Martell, & Isinibilir, 2017), it was not encountered in zooplankton samples from the Sea of Marmara before the present study. Two specimens were first sampled in June 2020 from the lower layer of the northeastern Sea of Marmara (off the coast of Tuzla; sampling depth 80m) (Figure 1, Station d). The umbrella was large, up to 140 mm wide, saucer shaped, and thick in the center. It had 120 simple and dark colored radial canals and 120 tentacles. The medusa had tube-shaped tentacle bulbs (Figure 2C). In the coastal waters of Turkey, four species of *Aequorea* have been reported: *Aequorea forskalea* (Péron & Lesueur, 1810), *Aequorea globosa* (Eschscholtz, 1829), *Aequorea pensilis*, and *Aequorea vitrina* (Gosse, 1853) (Gürlek et al., 2013; Onmuş, Bakir, & Katağan, 2016; Topcu et al., 2017; Turan, Gürlek, Yağlıoğlu, & Seyhan, 2011; Yilmaz et al., 2017). In the Mediterranean, *A. forskalea* is distributed in the eastern and western Mediterranean, including the Adriatic and Aegean Seas (Bouillon et al., 2004; Topcu et al., 2017; Yilmaz & Isinibilir, 2016). So far, no records of *A. forskalea* were available from the Sea of Marmara (Yilmaz et al., 2017).

Class: Thaliacea

Order: Salpida

Family: Salpidae

Subfamily: Salpinae

Genus: *Salpa*

***Salpa maxima* Forskål, 1775**

Although *Salpa maxima* is known as a common species in the Mediterranean Sea (Durgham & Ikhtiyar, 2013; Mutlu, 2005; Peinert & Miquel, 1994; Topcu et al., 2017), it had not been recorded in the Sea of Marmara before. Many colonies of *S. maxima* have been observed and one of them was sampled through scuba diving, with the collaboration of local divers in the vicinity of the Princes' Islands in the Sea of Marmara (Figure 1, Station e). The solitary forms have an 8 cm length with a smooth body with shallow longitudinal depressions. Because of its extreme thickness, the appearance is not completely transparent. There are a total of nine muscles along the dorsal side of the body. The colonies were impressively long (Figure 4), reaching up to 2 m in length, and were seen by fishermen and scuba divers.

Because of its geographical and hydrological characteristics, the Sea of Marmara is a vulnerable ecosystem. This eutrophic sea is

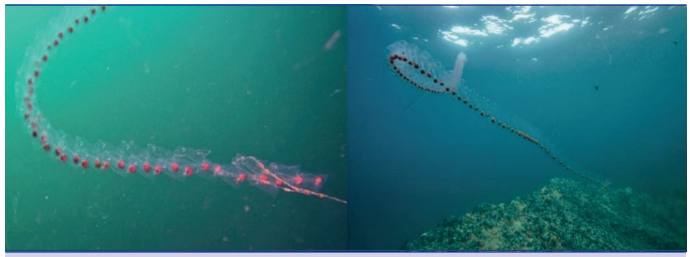

Figure 4. Chains of *Salpa maxima* in the waters of the Princes' Islands (Photograph: İsmail Cem Odabaşı).

Turkey's second commercially important fishing ground (Yılmaz, Akay, & Gümüş, 2008). As a result, any increase in jellyfish populations could have negative consequences for fisheries (Isinibilir & Yilmaz, 2016). With its unique properties, the Sea of Marmara also serves as a barrier and/or transitional acclimatization zone for biological organisms. Shipping and currents are two potential routes for jellyfish introduction in this Sea. Salps can feed on all sizes of phytoplankton, from viruses to protists, thus being in direct competition with crustaceans (Bone, 1998). Therefore, bloom of *Salpa maxima* may cause depletion of phytoplankton and adversely impact the food web (Boero, 2013). The presence of *Aequorea forskalea* in Turkish coastal waters may be related to its migration from the western Mediterranean, where it is common. *Aequorea forskalea* prey mainly on copepods and amphipods (Zavolokin, Glebov, & Kosenok, 2008), hence the bloom of this jellyfish may threaten commercially important fish stocks through food competition.

Mawia benovici could have been transported via ballast waters from the Mediterranean Sea or introduced through the lower layer flow of the Çanakkale Strait, because it already existed in the Mediterranean Sea. The life cycle of this species is still unknown. However, the medusa can reproduce by fission, a common asexual reproduction method in cnidarians (Chartosia et al., 2018). For this reason, the possible effect of its invasion is still unclear, and monitoring is necessary to understand the ecological consequences.

On the other hand, the Sea of Marmara, besides acting as a transition zone between the Black Sea and the Mediterranean, has important ports and most of them are located in the northeastern part. All species except *M. benovici* were recorded for the first time in the vicinity of Istanbul, thus suggesting the arrival of these species through ships' ballast waters. However, higher prey availability in the upper layer of the northeastern part of the Sea of Marmara may also have been an important factor in the availability of this species.

CONCLUSION

Recent studies have demonstrated that the environmental changes caused by intensive anthropogenic activity (e.g. eutrophication, overfishing, translocations, habitat modification, coastal development, etc.) and climate change due to global warming increase jellyfish blooms all over the world (Dong, Liu, & Keesing, 2010; Purcell, Uye, & Lo, 2007; Richardson, Bakun, Hays, & Gibbons, 2009). Overfishing may also result in increased jellyfish populations due to the removal of jellyfish predators and competitors.

As the Sea of Marmara has a long history of overfishing (Zengin & Mutlu, 2000), the potential effects on jellyfish populations cannot be ignored. Other human activities can also influence jellyfish populations. Similar to the effects of eutrophication, increased coastal urban development may ultimately lead to more jellyfish due to an increase in prey and substrate for the establishment of their polyps (Brotz & Pauly, 2012). Warning signs of ecological deterioration such as algae blooms, marine mucilage events, and jellyfish blooms have increased significantly in the Sea of Marmara in recent years. (Isinibilir, 2012; İşinibilir-Okyar, Üstün, & Orun, 2015; Turkoglu, 2013; Yılmaz, 2014). In addition, the number of jellyfish species recorded from the Sea of Marmara increased in the last decades and is still increasing (İnanmaz et al., 2002; Isinibilir et al., 2010; Isinibilir et al., 2020; Yilmaz et al., 2017). The rise of new jellyfish species might represent a future threat for the region by switching available trophic resources from zooplankton to unfavorable jellyfish biomass, thus eventually affecting fisheries and triggering changes in the functioning of the pelagic food web. Intensive and continuous monitoring of the Sea of Marmara is recommended in order to obtain updated information and predict the ecological effects of jellyfish on marine coastal ecosystems.

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

Ethics committee approval: The authors declare that this study does not include any experiments with human or animal subjects.

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