

Growth responses of a *Cylindrotheca closterium* strain from the Golden Horn Estuary to temperature, salinity and irradiance

Haliç'ten izole edilen diyatom *Cylindrotheca closterium* suşunun sıcaklık, tuzluluk ve ışınım karşısındaki büyüme tepkileri

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Abstract: The growth response of a diatom *Cylindrotheca closterium* strain (DEB132303) isolated from the Golden Horn Estuary (Sea of Marmara) was investigated under different environmental conditions. *C. closterium* cells were grown in a silica-enriched K-medium under various temperatures (11, 18, 25 and 32°C), salinities (6, 12, 18, 24, 30 and 36 psu) and irradiances (10, 20, 50 and 65 µmol photons m⁻² s⁻¹). By the end of the acclimatization period, no viable *C. closterium* cells were present at temperatures of 4°C and 32°C and salinities of 6 and 36 psu, which marked the upper and lower boundaries of its environmental tolerance. The maximum specific growth rates (µ_{max}) were 1.45 d⁻¹ at temperature of 18°C (18 psu, 50 µmol photons m⁻² s⁻¹), 1.08 d⁻¹ at salinity of 24 psu (18°C, 50 µmol photons m⁻² s⁻¹) and 1.80 d⁻¹ at irradiance of 65 µmol photons m⁻² s⁻¹ (18°C, 18 psu), in temperature, salinity and light experiments, respectively. The maximum cell density (D_{max}) was 5×10⁵ cells mL⁻¹ at temperature of 25°C, salinity of 18 psu and irradiance of 50 µmol photons m⁻² s⁻¹. Growth rate varied significantly among different temperature, salinity and irradiance treatments (ANOVA, p<0.001) and there was a positive correlation between growth rate and irradiance (r= 0.98, p<0.01). These results demonstrate that these environmental parameters affect significantly the growth of this *C. closterium* strain.

Keywords: *Cylindrotheca closterium*, diatom, growth rate, Golden Horn Estuary, Sea of Marmara

Öz: Haliç'ten (Marmara Denizi) izole edilen diyatom *Cylindrotheca closterium* suşunun (DEB132303) farklı çevresel koşullar altında büyüme tepkileri araştırıldı. *C. closterium* hücreleri, çeşitli sıcaklık (4, 11, 18, 25 ve 32°C), tuzluluk (6, 12, 18, 24, 30 ve 36 psu) ve ışık seviyelerinde (10, 20, 50 ve 65 µmol foton m⁻² s⁻¹) silikatca zenginleştirilmiş K-kültür ortamında yetiştirildi. İklimlendirme döneminin sonunda, 4°C ve 32°C sıcaklık ile 6 ve 36 psu tuzluluk seviyelerinde canlı *C. closterium* hücreleri bulunamadı ve bu değerler suşun çevresel toleransının üst ve alt sınırları olarak belirlendi. Maksimum spesifik büyüme hızı (µ_{max}), sıcaklık, tuzluluk ve ışık deneylerinde sırasıyla 18°C sıcaklıkta (18 psu, 50 µmol foton m⁻² s⁻¹) 1.45 d⁻¹, 24 psu tuzlulukta (18°C, 50 µmol foton m⁻² s⁻¹) 1.08 d⁻¹ ve 65 µmol foton m⁻² s⁻¹ ışık şiddetinde (18°C, 18 psu) 1.80 d⁻¹ olarak bulundu. Maksimum hücre yoğunluğu (D_{max}) ise 25°C sıcaklık, 18 psu tuzluluk ve 50 µmol foton m⁻² s⁻¹ ışık şiddetinde 5×10⁵ hücre mL⁻¹ olarak tespit edildi. Büyüme hızı, farklı sıcaklık, tuzluluk ve ışık şiddeti uygulamaları arasında önemli ölçüde farklılık gösterdi (ANOVA, p<0.001) ve ışık şiddeti ile arasında pozitif ilişki görüldü (r= 0.98, p<0.01). Bu sonuçlar, sıcaklık, tuzluluk ve ışığın bu *C. closterium* suşunun büyümesini önemli ölçüde etkilediğini göstermiştir.

Anahtar kelimeler: *Cylindrotheca closterium*, diyatom, büyüme hızı, Haliç, Marmara Denizi

INTRODUCTION

Diatoms are one of the major components of marine phytoplankton and form the basis of the food chain for many other marine organisms (Hoppenrath et al., 2009). They have benthic and pelagic forms and are the most diverse group of marine phytoplankton (Armbrust, 2009). The cosmopolitan pennate diatom *Cylindrotheca closterium* (Ehrenberg) Reimann & J.C.Lewin, 1964 can be found in planktonic and benthic forms on substrates such as seaweeds and polar ice. It has been suggested that slime production during *C. closterium* blooms is thought to harm fisheries (Kraberg et al., 2010). Some previous studies have suggested the role of this diatom species in mucilage events via the production of extracellular polysaccharides (EPS) under culture conditions (de Brouwer and

Stal, 2002; Najdek et al., 2005; Pistocchi et al., 2005; Totti et al., 2005; Urbani et al., 2005). *Cylindrotheca closterium* has also been identified as one of the most abundant species in mucilage events in the Sea of Marmara (SoM) in recent years (Aktan et al., 2008; Tufekci et al., 2010; Balkis et al., 2011; Balkis-Ozdelice et al., 2021). In some previous studies were reported the frequent occurrence and bloom of *C. closterium* in the Golden Horn Estuary (GHE) (Tas, 2019; Semin et al., 2023).

The Golden Horn Estuary (GHE) is located in the northwestern Sea of Marmara (SoM). It is approximately 7.5 km long and 0.7 km wide and covers an area of 2.5 km². Two small streams in the north flow into the estuary: The GHE has

a two-layered system similar to that of the neighboring Strait of Istanbul (Figure 1). The upper layer has low salinity (~18 psu) originating from the Black Sea and it extends to a depth of approximately 25 m. The lower layer has higher salinity (~38 psu) originating from the Mediterranean Sea (Özsoy, 1988).

Although the responses of *C. closterium* to temperature,

salinity, and light have been previously studied, the effects on cell length, width, and length-to-width ratio during exponential and stationary phases remain poorly understood. In the present study, the effects of temperature, salinity, and light were evaluated in a locally isolated *C. closterium* strain, with a particular focus on differences between exponential and stationary growth phases.

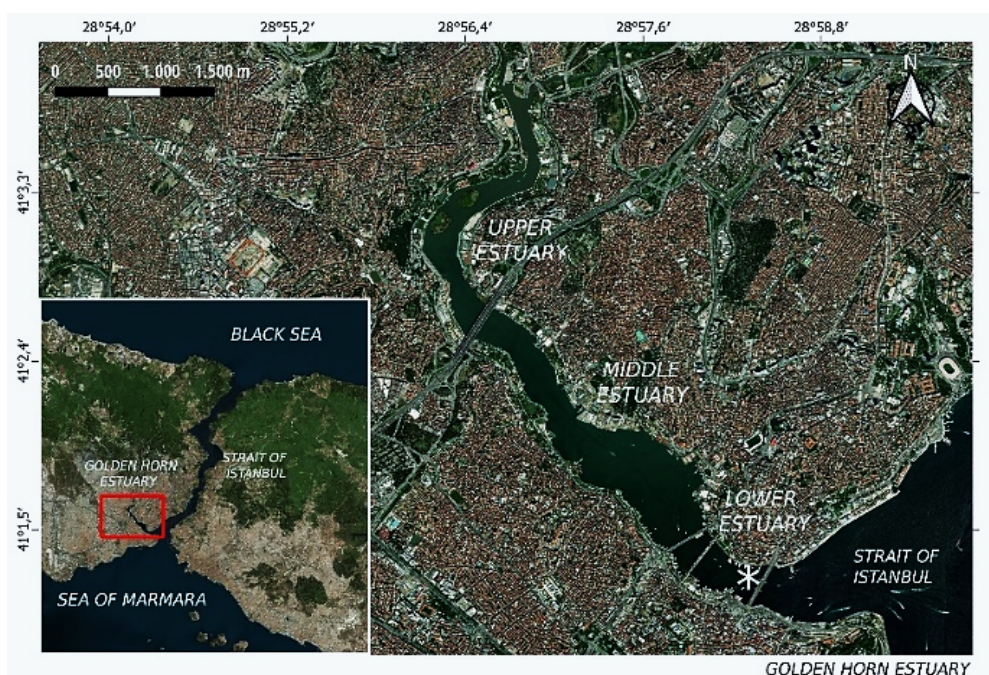


Figure 1. The study area and the sampling point

Understanding how environmental factors affect estuarine diatoms is crucial for predicting the responses of primary producers in changing coastal ecosystems. Temperature, salinity and light intensity play an important role in diatom cell division (Smayda, 1969). Affan et al. (2009) estimated the optimal growth conditions for *C. closterium* under various temperature, salinity and nutrient combinations. These previous studies emphasized the importance of revealing the growth characteristics and responses of *C. closterium* to environmental factors. Consequently, these variables were selected as environmental factors, which affect the growth of this *C. closterium* strain isolated from the GHE.

This study aims to characterize the ecophysiology of the *C. closterium* strain isolated from the GHE under non-axenic culture conditions, and to evaluate its growth performance under different temperatures, salinities and irradiances.

MATERIALS AND METHODS

Sampling strategy

Sea water samples for the isolation of single cells of the *Cylindrotheca closterium* strain were collected using a 40 cm diameter Apstein plankton net with a 20 μm mesh size by vertical tows (from 10 m to the surface) at the sampling point in

the lower estuary (41°01'14" N, 28°58'23" E) at a single sampling time in March 2023 (Figure 1, Google Maps). The net samples were then passed through 200 μm gauze to remove mesozooplankton. The remaining sample was kept alive and subsamples were transferred to petri dishes for cell isolation studies.

Morphological identification

Light micrographs were captured using a camera system (Leica DFC295 LAS V4.8 version) for morphometric measurements (length and width) of 30 cells in the exponential growth phase (EGP) and stationary phase (SP) under a light microscope (Leica DM 2500) at a magnification of 200 \times . The following references were used for the morphological identification of *C. closterium*: Hasle and Syversten (1997); Hoppenrath et al. (2009); and Kraberg et al. (2010).

Single-cell isolation and culture conditions

An inverted light microscope (Leica DMIL 3000 LED) was used to isolate single cell and establish monoclonal cultures of *C. closterium* strain. The subsamples in glass petri dishes were kept under cool-white fluorescent lights (50 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$) in a 12:12 h light:dark (L:D) photocycle in a temperature-controlled room at 18°C (Guillard and Ryther, 1962). Single *C.*

closterium cells were isolated using a mouth-sucking micropipette technique (a capillary tube). The micropipette needle was placed close to the target cell under the inverted light microscope, after which gentle suction was applied. Then, the cells were transferred into individual wells of a 96-well plate that had been pre-filled with filtered seawater from the study area that had been enriched with K-medium (Keller et al., 1987). The clonal cultures were then transferred to 70 mL plastic culture flasks and the stock cultures were routinely grown under the aforementioned conditions. K-medium was prepared using sterile filtered (0.1 µm VacuCap filters, Pall Life Sciences, Dreieich, Germany) GHE water (salinity ~18–19 psu) with slight modification involving the replacement of organic phosphorus with inorganic phosphate at the same molar concentration as in the original recipe. Additionally, inorganic silicate was added to the K-medium. The pH of the culture medium was adjusted to 8.1–8.2 (WTW Inolab pH 720, Germany) by adding 1 M HCl.

Molecular and phylogenetic analysis

The dense cell culture, which was determined to be in the late exponential phase based on its growth characteristics, was harvested by centrifugation (3500 g for 15 minutes) for DNA extraction. Genomic DNA was isolated using the High Purity PCR Template Preparation Kit (Roche) following the manufacturer's guidelines. Molecular identification was carried out by amplifying the large subunit ribosomal RNA (LSU rDNA) gene using the D1R primer (Scholin et al., 1994) together with the D3B primer (Nunn et al., 1996). PCR amplification reactions were prepared in a total volume consisting of 25 µl of 2× MyTaq Red Mix, 14 µl of distilled deionised water (ddH₂O), 2 µl of bovine serum albumin (BSA), 2 µl of each primer, and 5 µl of template DNA. Thermal cycling was initiated with an initial denaturation step at 95 °C for 4 min, followed by 35 cycles comprising denaturation at 95 °C for 30 s and annealing–extension at 60 °C for 2 min, and concluded with a final extension at 72 °C for 10 min for 28S rDNA amplification (Borchhardt et al., 2021). PCR products were visualized on a 1.5% agarose gel to confirm successful amplification. Sanger sequencing of the amplified fragments was performed by BM Yazılım Danış. ve Lab. Sis. Ltd. Şti. (Ankara, Türkiye). The resulting sequences were edited and aligned using BioEdit software and compared with reference sequences retrieved from the NCBI BLAST database (Hall, 1999). The LSU rDNA sequence obtained from strain DEB132303 has been deposited in the GenBank database under the accession number PQ220126. Phylogenetic analysis was conducted using MEGA 11 software (Tamura et al., 2021) based on multiple sequence alignments, and the phylogenetic tree was reconstructed using the maximum likelihood (ML) method with 1000 bootstrap replicates.

Growth experiments

Growth experiments were conducted in triplicate to examine the effects of temperature, salinity, and light on the *Cylindrotheca closterium* strain. *C. closterium* cultures were

stepwise acclimated to temperatures of 4, 11, 18, 25, and 32 °C, salinities of 6, 12, 18, 24, 30, and 36 psu, and irradiances of 10, 20, 50, and 65 µmol photons m⁻² s⁻¹, with at least three cell divisions at each acclimation step. Stock cultures were acclimated for 1–2 weeks at each treatment to minimize environmental shock. During acclimatization, cells from the stock culture were inoculated at approximately 1000 cells mL⁻¹. Cell densities were monitored daily for temperature and light experiments, and every two days for salinity experiments. Cultures were diluted back to 1000 cells mL⁻¹ when the stock reached ~30×10³ cells mL⁻¹ before being transferred to the next target temperature, salinity, or light level. By the end of the acclimation period, no live *C. closterium* cells remained at temperatures of 4 and 32 °C and at salinities of 6 and 36 psu which may be considered extreme conditions for this marine diatom species, so experiments could not be conducted at these values. All experiments were then initiated with approximately 500 cells mL⁻¹ at temperature, salinity and light conditions where cultures can survive.

Temperature: Experiments were conducted at 11, 18, and 25°C with ~500 cells mL⁻¹, at a salinity of 18 psu and a light intensity of 50 µmol photons m⁻² s⁻¹. Temperature controlled incubators (Nüve cooled incubator ES 120) were used for the temperature experiments.

Salinity: Experiments were conducted at salinities of 12, 18, 24, and 30 psu with ~500 cells mL⁻¹ at 11±1°C and an irradiance of 50 µmol photons m⁻² s⁻¹. Salinity levels were measured using a Portable Refractometer LYK-206.

Light: Experiments were conducted at irradiances of 10, 20, 50, and 65 µmol photons m⁻² s⁻¹, at 18±1°C and a salinity of 18 psu, with ~500 cells mL⁻¹. Light intensity was measured using Digital Lux Meter AS803.

Growth rate and cell size

The growth response of *C. closterium* cells to changes in three variables (temperature, salinity and irradiance) was measured during the experiments. After acclimatization, three replicates (n=3) were conducted for each variable treatment at the ecophysiological experiment stage. Thus, *C. closterium* was tested in triplicate under 11 different treatment conditions for the three parameters: temperature (11, 18 and 25°C), salinity (12, 18, 24 and 30 psu) and irradiance (10, 20, 50 and 65 µmol photons m⁻² s⁻¹).

To calculate cell density, 0.5 mL samples were taken daily or every two days from each culture flask and placed in a 12 mm diameter sedimentation chamber and fixed with acidic Lugol's iodine solution (final concentration 2%). After waiting approximately 30 minutes, the chamber was placed on a counting slide consisting of 1000 squares, each with an area of 1 mm². Cell enumeration was performed using an inverted light microscope (Leica DMIL 3000 model) with a magnification of 200× and in each chamber was counted at least 300 cells. Growth was monitored until the cells reached stationary phase. The growth curves plotted for all treatments were based on the

mean cell density of the three replicates. The specific growth rate (μ) was defined as the increase in cell density per unit time (Pirt, 1975) and it was calculated as the exponent of the exponential equation for a defined period of exponential growth using Microsoft Excel.

Morphometric measurements of the cell dimensions (cell length, L; cell width, W and length/width ratio, L/W) of at least 30 cells in the EGP and SP were taken during the experiments using a Leica DFC295 camera with LAS V4.8 software under a light microscope (Leica DM 2500) equipped with phase-contrast optics.

Statistical analysis

All experiments were conducted in triplicate ($n=3$) to determine growth characteristics. One-way analysis of variance (ANOVA) was applied to test for statistically significant differences between the means of independent variables, followed by Tukey's test. The data obtained from the environmental parameters were compared with the growth rate and cell dimensions of the *C. closterium* strain. Prior to ANOVA, each parameter was normalized by logarithmic transformation. It was hypothesized that there would be differences and/or relationships between the independent groups and each independent variable. Pearson correlation was used to analyze the relationships between the environmental data and the average values of growth rate and cell dimensions. IBM SPSS Statistics software (version 29.0) was used in all statistical analyses.

RESULTS

Morphological and phylogenetic analyses

Cells of the *Cylindrotheca closterium* strain, which were isolated from the Golden Horn Estuary exhibit the typical morphological features described in the literature, namely a lanceolate shape with needle-like ends and two chloroplasts in the centre. The cell sizes are as follows: Total length (apical axis): 155–160 μm ; length of cell body: 35–40 μm ; width (transapical axis): 4.5–5.5 μm (Figure 2).



Figure 2. Light microscope image of the *Cylindrotheca closterium* strain

Despite the use of bidirectional primers, only the D1R primer produced a sequence, yielding a 795 bp LSU fragment. Phylogenetic analysis placed the DEB132303 strain within the *C. closterium* clade with 100% bootstrap support (GenBank no. PQ220126; Figure 3), clustering closely with the AF417666 strain from Kattegat in Denmark.

Growth characteristics under different temperatures

The maximum specific growth rate (μ_{max}) at 11, 18 and 25°C was 0.86, 1.45 and 0.78 d^{-1} , respectively. The maximum cell density (D_{max}) was 2.26×10^5 cells mL^{-1} at 11°C on day 5, 1.93×10^5 cells mL^{-1} at 18°C on day 5 and 5×10^5 cells mL^{-1} at 25°C on day 7 (Figure 4). Growth rates varied significantly ($p < 0.001$) across the different temperatures (Table 1).

During the EGP, the mean cell lengths (L) were 38.3, 38.9, and 32.1 μm , the mean cell widths (W) were 5.8, 6.0 and 5.35 μm and the mean length/width ratio (L/W) 6.6, 6.47 and 6.0 at 11, 18 and 25°C, respectively. During the SP, the L were 40.0, 38.8 and 34.5 μm , the W were 7.0, 6.7 and 6.7 μm and the L/W were 5.7, 5.8 and 5.1 at 11, 18 and 25°C, respectively (Figure 5). The L varied significantly ($p < 0.001$) among the different temperature treatments (Table 1). There was a significant negative correlation between temperature and the L ($r = -0.80$, $p < 0.05$) and between temperature and the L/W ($r = -0.67$, $p < 0.05$) (Table 2).

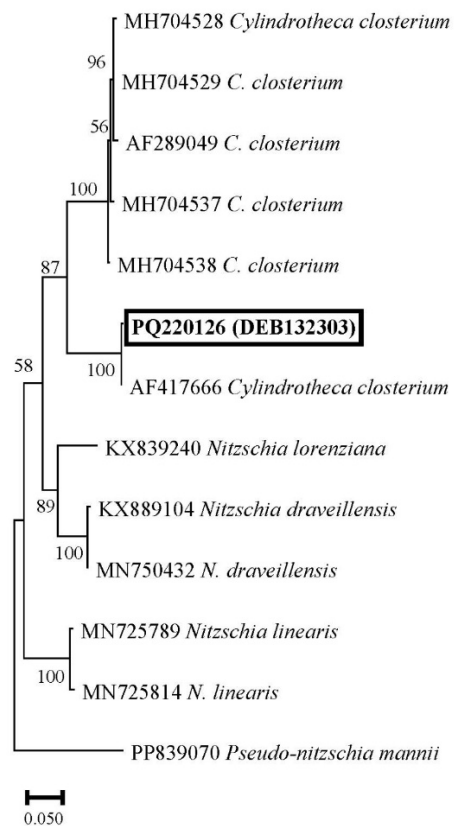


Figure 3. Molecular phylogenetic tree of *Cylindrotheca closterium* strain based on the LSU rDNA sequences using ML method

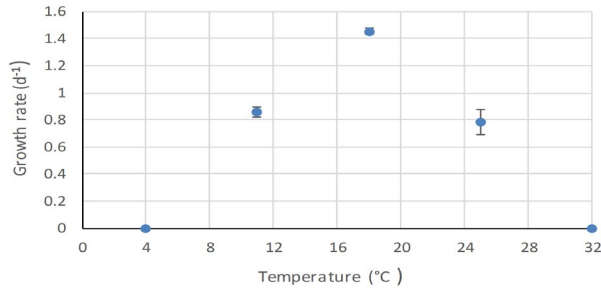


Figure 4. Maximum growth rates (blue circles) with standard deviation (n=3) of the *Cylindrotheca closterium* strain under different temperatures at salinity of 18 psu and a constant irradiance during the EGP

Table 1. Significant ANOVA results of variations in the growth rate and cell dimensions according to environmental parameters. GR: Growth rate, L: Cell length, W: Cell width, L/W: Length/Width

Parameters	Response variable	ANOVA result
Temperature (°C)	GR	$F_{2,8}=119.160, p<0.001$
	L	$F_{2,8}=52.019, p<0.001$
Salinity (psu)	GR	$F_{3,11}=125.643, p<0.001$
Irradiance ($\mu\text{mol photons m}^{-2} \text{s}^{-1}$)	GR	$F_{3,11}=145.263, p<0.001$
	L/W	$F_{3,11}=27.090, p<0.001$

*P-value of <0.05 was considered significant

Table 2. Pearson correlation coefficients (r) between environmental parameters and the growth characteristics of the *Cylindrotheca closterium* strain. GR: Growth rate, L: Cell length, W: Cell width, L/W: Length/Width

Parameters	GR	L	W	L/W
Temperature (°C)	-	-.796*	-	-.667*
Salinity (psu)	.619*	-	-.594*	.618*
Irradiance ($\mu\text{mol photons m}^{-2} \text{s}^{-1}$)	.984**	.677*	-.588*	.772**

*Correlation is significant at the 0.05 level. **Correlation is significant at the 0.01 level

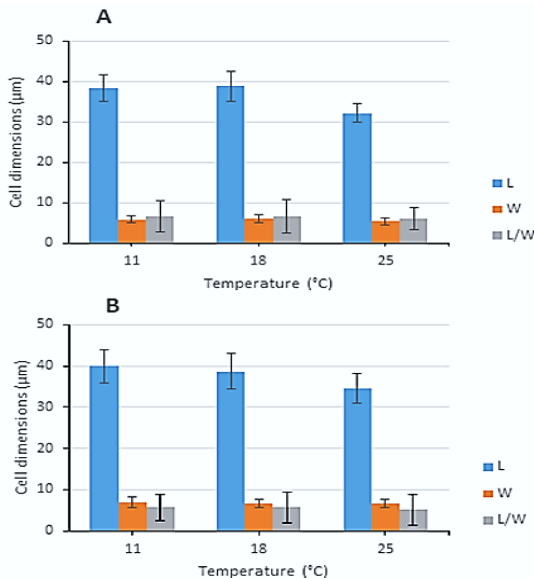


Figure 5. Cell dimensions (n=30) of the *Cylindrotheca closterium* strain under different temperatures at salinity of 18 psu and a constant irradiance during the EGP (A) and SP (B)

Growth characteristics under different salinities

The μ_{max} at salinities of 12, 18, 24 and 30 psu were 0.62, 0.89, 1.08 and 0.86 d^{-1} , respectively (Figure 6). The D_{max} was

1.45×10^5 cells mL^{-1} at salinity 12 psu on day 11, 2.67×10^5 cells mL^{-1} at salinity 18 psu on day 9, 2.58×10^5 cells mL^{-1} at salinity of 24 psu on day 9 and 4.5×10^5 cells mL^{-1} at salinity 30 psu on day 9. The growth rate varied significantly ($p<0.001$) among the different salinities and it was positively correlated with salinity ($r=0.62, p<0.05$) (Tables 1 and 2).

At the EGP, the L were 37.6, 36.4, 35.4 and 38.2 μm , the W were 7.1, 5.5, 5.6 and 5.8 μm and the L/W were 5.31, 6.65, 6.39 and 6.65 at salinities of 12, 18, 24 and 30 psu, respectively. At the SP, the L of cells were 43.1, 42.9, 38.8 and 39.5 μm , and the W were 8.2, 7.3, 6.7 and 6.3 μm and the L/W were 5.2, 5.9, 5.8 and 6.4 at salinities of 12, 18, 24 and 30 psu respectively (Figure 7). The W was negatively correlated with salinity ($r=-0.59, p<0.05$), while L/W was positively correlated with salinity ($r=0.62, p<0.05$) (Table 2).

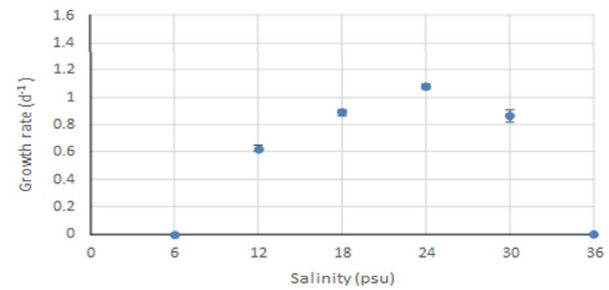


Figure 6. Maximum growth rates (blue circles) with standard deviation (n=3) of the *Cylindrotheca closterium* strain under different salinity conditions at temperature 11°C and a constant irradiance during the EGP

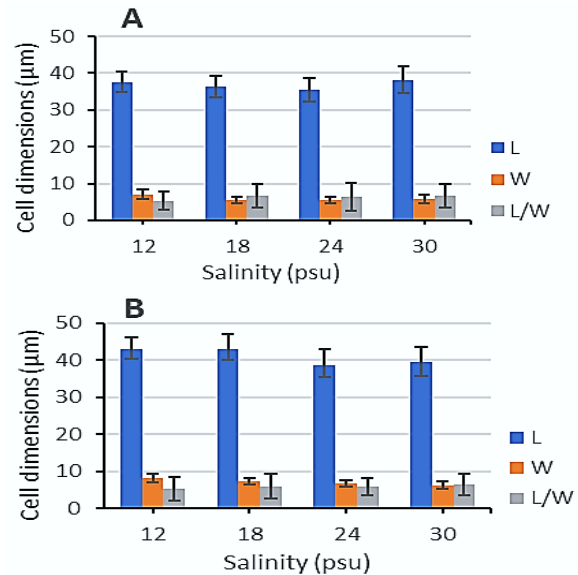


Figure 7. Cell dimensions (n=30) of the *Cylindrotheca closterium* strain under different salinity at temperature of 11°C and a constant irradiance during the EGP (A) and SP (B).

Growth characteristics under different irradiances

The μ_{max} at irradiances of 10, 20, 50 and 65 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ were 0.62, 0.75, 1.44 and 1.80 d^{-1} , respectively (Figure 8). The D_{max} was 1.11×10^5 cells mL^{-1} at 10 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ on day 9, 2.4×10^5 cells mL^{-1} at 20 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ on day

7, 1.53×10^5 cells mL⁻¹ at 50 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ on day 5 and 1.86×10^5 cells mL⁻¹ at 65 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ on day 4. The growth rate varied significantly ($p < 0.001$) among the different irradiances (Table 1) and there was a strong positive correlation between the irradiance and the growth rate ($r = 0.98$, $p < 0.01$) (Table 2).

During the EGP, the L were 38.1, 36.1, 35.9 and 35.07 μm , the W were 5.1, 5.8, 5.7 and 5.72 μm and the L/W were 7.5, 6.3, 6.3 and 6.1 at irradiances of 10, 20, 50 and 65 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, respectively (Figure 9). During the SP, the L were 39.2, 46.7, 40.3, 39.4 μm , the W were 6.9, 7.2, 6.6 and 6.2 μm , the L/W were 5.7, 6.4, 6.1 and 6.4 at irradiances of 10, 20, 50 and 65 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ respectively (Figure 9). The L/W differed significantly ($p < 0.001$) among the different irradiances (Table 1). There was a significant positive correlation between the L/W and irradiance ($r = 0.77$, $p < 0.01$) (Table 2). The L was positively correlated with irradiance ($r = 0.68$, $p < 0.05$) while the W was negatively correlated with irradiance ($r = -0.59$, $p < 0.05$). (Table 2).

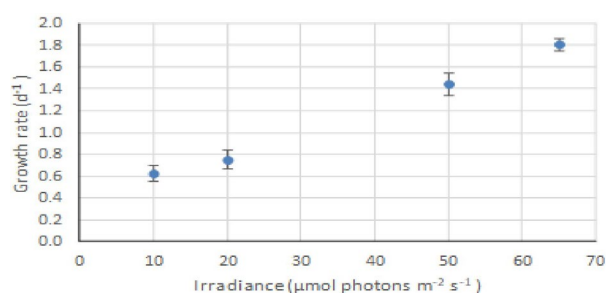


Figure 8. Maximum growth rates (blue circles) with standard deviation (n=3) of the *Cylandrotheca closterium* strain under different irradiances at temperature 18°C and salinity of 18 psu during the EGP

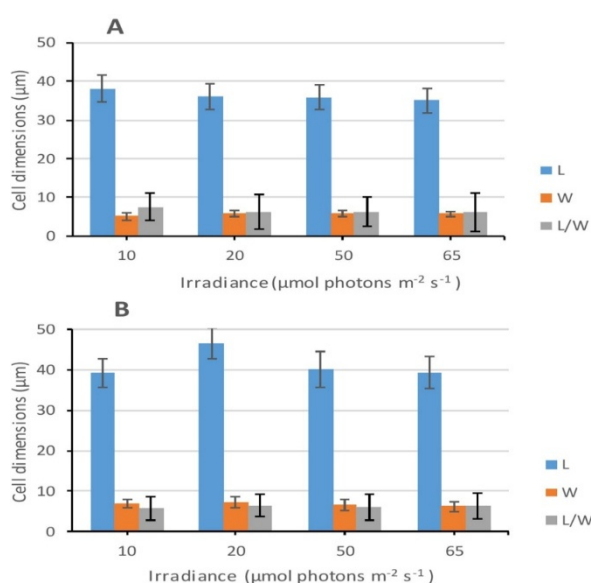


Figure 9. Cell dimensions (n=30) of the *Cylandrotheca closterium* strain under different irradiances at a temperature of 18°C and salinity of 18 psu during the EGP (A) and SP (B)

DISCUSSION

The findings of this study indicated that specific temperature, salinity, and light conditions significantly influence the growth characteristics of this *C. closterium* strain. In particular, its sensitivity to extreme temperatures and salinities suggests that its natural distribution and abundance may be constrained by environmental variability.

Phylogenetic analysis

Despite their geographical separation, the clustering of DEB132303 with AF417666, suggests a close evolutionary relationship, which is potentially driven by similar salinity regimes in the Golden Horn Estuary and Kattegat (Danielsson et al., 2004; Koyuncu, 2018; Tas, 2020). This supports the hypothesis that environmental factors such as salinity can shape the phylogenetic structure in populations of *Cylandrotheca closterium* (Rynearson et al., 2006, 2009; Lebrét et al., 2012). Such clustering aligns with the concept of niche conservatism (NC), where ecologically similar conditions across regions promote the persistence of comparable traits and genetic structures (Harvey and Pagel, 1991; Ackerly, 2003; Wiens et al., 2010). Our results support previous findings indicating that *C. closterium* forms genetically distinct clades that correspond to ecological niches (Audoor et al., 2024), despite its cosmopolitan morphology (Kooistra et al., 2008; Degerlund et al., 2012). This phylogenetic proximity suggests that these strains from different geographic regions may share similar ecological adaptations and evolutionary history. Rather than providing direct evidence of recent gene flow, the observed clustering may suggest a common adaptation to similar local environmental conditions.

By contrast, other strains in the phylogenetic tree (e.g., those from Australia and Antarctica) form distinct clades, which is likely due to their adaptation to different salinity levels, illustrate how environmental divergence may lead to genetic differentiation (Stock et al., 2019). These findings emphasize the role of both gene flow and local selective pressures in shaping the evolutionary trajectory of *C. closterium* (Reynolds et al., 2017; Hughes et al., 2018).

Temperature experiments

The μ_{max} of *C. closterium* was found to be 1.45 d⁻¹ at 18°C, with the D_{max} (5×10^5 cells mL⁻¹) occurring at 25°C in the present study. Affan et al. (2009) reported that the μ_{max} of *C. closterium* was 0.97 d⁻¹ at 25°C with salinity of 25 psu under high irradiance (180 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) and D_{max} was 7.2×10^4 cells mL⁻¹ at 20°C with a salinity of 30 psu, while the μ_{max} was 0.82 d⁻¹. Therefore, the present findings are consistent with those reported by Affan et al. (2009). It is difficult to explain this situation, but it is thought that it may be a species-specific behavior. In addition, Kingston (2009) reported that an average growth rate of over 2.5 d⁻¹ at 27°C with a salinity of 31 psu and an irradiance of 240 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ in a culture chamber with a 24:0 h L:D cycle. Urbani et al. (2005) suggested that the D_{max} was

$1342 \pm 55.5 \times 10^3$ cells mL⁻¹ when cultured at 20°C, with a 16:8 h L:D photoperiod and 112 μmol photons m⁻² s⁻¹. In this study, the water temperature and salinity were measured as 11°C and 18, respectively, at the location where the *C. closterium* strain was isolated. A moderate temperature may favor suitable conditions for this *C. closterium* strain.

Salinity experiments

The D_{max} (4.5×10^5 cells mL⁻¹) of *C. closterium* occurred at a salinity of 30 psu, while μ_{max} (1.08 d⁻¹) occurred at a salinity of 24 psu in this study. The *C. closterium* strain tested in this study exhibited a lower growth rate at low salinity (12 psu) than at higher salinities, as well as a narrower tolerance range along the salinity gradient (12 to 30 psu), compared to previous studies. Glaser and Karsten (2020) suggested the strong genotypic differentiation among six *C. closterium* strains of different biogeographic origin. Van Bergeijk et al. (2003) found that a strain showed similar growth rates across a salinity gradient of 11–55 psu, whereas Scholz and Liebezeit (2012) reported much lower growth rates between salinities of 10–35 psu. All these findings highlight the strong biogeographic differentiation between strains. The optimum salinity and salinity tolerance range are strain-specific (Glaser and Karsten, 2020). Salinity tolerance may vary among various *C. closterium* strains. A positive correlation was found between growth rate and salinity ($r = 0.62$, $p < 0.05$), indicating a significant influence of salinity on *C. closterium* growth. Higher growth rates and cell densities were found at salinities of 24 and 30 psu, revealing that an increase in salinity affect the growth of this *C. closterium* strain positively. Had salinity experiments been performed on different strains isolated from the same region, the salinity tolerance of each strain might have differed.

Light experiments

An increase in irradiance significantly accelerated the growth of *C. closterium* ($r = 0.98$, $p < 0.01$), as indicated by the strong positive correlation between growth rate and irradiance. A study by Bialevich et al. (2022) involving cultures of three microalgal species grown under three irradiance levels (100, 250 and 500 μmol photons m⁻² s⁻¹) suggested that increasing irradiance leads to an increase in maximum cell size. Their results also showed that microalgae respond differently to irradiance depending on the species. Previous studies in different regions have applied various irradiances, such as 10, 60, 112, and 240 μmol photons m⁻² s⁻¹, for ecophysiological experiments (de Brouwer and Stal, 2002; Urbani et al., 2005; Kingston, 2009; Glaser and Karsten, 2020). These studies have shown that the growth conditions necessary for *C. closterium* vary according to its origin. de Brouwer and Stal (2002) reported that soluble EPS in the benthic diatoms *C. closterium* and *Nitzschia* sp was produced continuously when the cultures reached the SP, however bound EPS production occurred only in the light, reaching its peak during the EGP. Statistical analyses have proven that the growth rate, cell density and cell dimensions of this *C. closterium* strain are positively influenced by increased irradiance. These positive relationships also prove that irradiance is a determining factor in cell growth, as

mentioned in the above studies. This may provide ecological evidence that irradiance accelerates photosynthetic activity, thereby promoting cell growth. Glaser and Karsten (2020) highlighted strong genotypic differentiation among six strains of *C. closterium*. However, it should also be noted that higher light conditions may lead to higher growth rates. The differences between the results of this and previous studies are thought to be related to differences in the applied cultural conditions.

CONCLUSION

The cultures of this *C. closterium* strain were maintained under non-axenic conditions. Although routine microscopic examinations did not reveal visible bacterial contamination, this does not indicate the absence of bacterial influence. Therefore, potential microbiome effects should be taken into account when interpreting the present results. The results of this study indicate that temperature, salinity, and irradiance play a significant role in the growth of the *Cylindrotheca closterium* strain isolated from the Golden Horn Estuary. Based on growth rate responses, the most favorable conditions for this strain were observed at 18 °C, a salinity of 24 psu, and an irradiance of 65 μmol photons m⁻² s⁻¹. These findings suggest that the prevailing environmental conditions in the Golden Horn Estuary are within the tolerance range of *C. closterium*, supporting its persistence and growth in this estuarine system.

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AUTHORSHIP CONTRIBUTIONS

Sebahat Şemin, Seyfettin Taş and Fuat Dursun: Conceiving the study, conducting experimental analyses, writing-review & editing. Rabia Sezgin, Turgay Durmuş and Muharrem Balcı: Performing the phylogenetic analysis and interpretation the data, writing-review & editing. Halim Aytekin Ergül: Writing-review & editing. The final version of manuscript was read and reviewed by all co-authors.

CONFLICT OF INTEREST STATEMENT

The authors have no competing interests to declare that are relevant to the content of this article.

ETHICAL APPROVAL

No specific ethical approval was required for this study.

DECLARATION OF AI USE

We have not used AI-assisted technologies in creating this article.

DATA AVAILABILITY

The data that support the results of the present research study are available when solicited to the corresponding author.

REFERENCES

- Ackerly, D.D. (2003). Community assembly, niche conservatism, and adaptive evolution in changing environments. *International Journal of Plant Sciences*, 164(S5), S165–S184. <https://doi.org/10.1086/368401>
- Affan, A., Heo, S.J., Jeon, Y.J., & Lee, J.B. (2009). Optimal growth conditions and antioxidative activities of *Cylindrotheca closterium* (Bacillariophyceae). *Journal of Phycology*, 45, 1405–1415. <https://doi.org/10.1111/j.1529-8817.2009.00763.x>
- Aktan, Y., Dede, A., & Ciftci, P.S. (2008). Mucilage event associated with diatom and dinoflagellates in the Sea of Marmara, Turkey. *Harmful Algae News*, No. 36, 1–3.
- Arnbrust, E.V. (2009). The life of diatoms in the world's oceans. *Nature*, 459, 185–192. <https://doi.org/10.1038/nature08057>
- Audoor, S., Bilcke, G., Pargana, K., Belišová, D., Thierens, S., Van Bel, M., Sterck, L., Rijsdijk, N., Annunziata, R., Immacolata Ferrante, M., Vandepoole, K., & Vyverman, W. (2024). Transcriptional chronology reveals conserved genes involved in pennate diatom sexual reproduction. *Molecular Ecology*, 33(8), e17320. <https://doi.org/10.1111/mec.17320>
- Balkis-Ozdelice, N., Durmus, T., & Balci, M. (2021). A preliminary study on the intense pelagic and benthic mucilage phenomenon observed in the Sea of Marmara. *International Journal of Environment and Geoinformatics*, 8, 414–422. <https://doi.org/10.30897/ijegeo.954787>
- Balkis, N., Atabay, H., Turetgen, I., Albayrak, S., Balkis, H., & Tufekci, V. (2011). Role of single-celled organisms in mucilage formation on the shores of Büyükkada Island, the Marmara Sea. *Journal of the Marine Biological Association of the United Kingdom*, 91, 771–781. <https://doi.org/10.1017/S0025315410000081>
- Bialevich, V., Zachleder, V., & Bišová, K. (2022). The effect of variable light source and light intensity on the growth of three algal species. *Cells*, 11(8), 1293. <https://doi.org/10.3390/cells11081293>
- Borchhardt, N., Chomérat, N., Bilien, G., Zentz, F., Rhodes, L., Murray, S.A., & Hoppenrath, M. (2021). Morphology and molecular phylogeny of *Bindiferia* gen. nov. (Dinophyceae), a new marine, sand-dwelling dinoflagellate genus formerly classified within *Amphidinium*. *Phycologia*, 60(6), 631–643. <https://doi.org/10.1080/00318884.2021.1978040>
- Danielsson, Å., Rahm, L., Conley, D.J., & Carstensen, J. (2004). Identification of characteristic regions and representative stations: A study of water quality variables in the Kattegat. *Environmental Monitoring and Assessment*, 90, 203–224. <https://doi.org/10.1023/B:EMAS.0000003590.58753.0e>
- de Brouwer, J.F.F., & Stal, L.J. (2002). Daily fluctuations of exopolymers in cultures of the benthic diatoms *Cylindrotheca closterium* and *Nitzschia* sp. (Bacillariophyceae). *Journal of Phycology*, 38, 464–472. <https://doi.org/10.1046/j.1529-8817.2002.01164.x>
- Degerlund, M., Huseby, S., Zingone, A., Sarno, D., & Landfald, B. (2012). Functional diversity in cryptic species of *Chaetoceros socialis* Lauder (Bacillariophyceae). *Journal of Plankton Research*, 34, 416–431. <https://doi.org/10.1093/plankt/fbs004>
- Glaser, K., & Karsten, U. (2020). Salinity tolerance in biogeographically different strains of the marine benthic diatom *Cylindrotheca closterium* (Bacillariophyceae). *Journal of Applied Phycology*, 32, 3809–3816. <https://doi.org/10.1007/s10811-020-02238-6>
- Guillard, R.R.L., & Ryther, J. H. (1962). Studies of marine planktonic diatoms: I. *Cyclotella nana* Husted and *Detonula confervacea* (Cleve). *Canadian Journal of Microbiology*, 8, 229–239. <https://doi.org/10.1139/m62-029>
- Hall, T.A. (1999). BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, 41, 95–98.
- Harvey, P. H., & Pagel, M. D. (1991). *The comparative method in evolutionary biology*. Oxford University Press. <https://doi.org/10.1093/oso/9780198546412.001.0001>
- Hasle, G.R., & Syvertsen, E.E. (1997). Marine diatoms. In C. R. Tomas (Ed.), *Identifying marine phytoplankton* (pp. 292–294). Academic Press. <https://doi.org/10.1016/B978-012693018-4/50004-5>
- Hoppenrath, M., Elbrächter, M., & Drebes, G. (2009). *Marine phytoplankton: Selected microphytoplankton species from the North Sea around Helgoland and Sylt*. E. Schweizerbart'sche Verlagsbuchhandlung.
- Hughes, A.R., Cebrian, J., Heck, K., Goff, J., Hanley, T.C., Scheffel, W., & Zerebecki, R.A. (2018). Effects of oil exposure, plant species composition, and plant genotypic diversity on salt marsh and mangrove assemblages. *Ecosphere*, 9, e02207. <https://doi.org/10.1002/ecs2.2207>
- Keller, M.D., Selvin, R.C., Claus, W., & Guillard, R.R.L. (1987). Media for the culture of oceanic ultraphytoplankton. *Journal of Phycology*, 23, 633–638. <https://doi.org/10.1111/j.1529-8817.1987.tb04217.x>
- Kingston, M.B. (2009). Growth and motility of the diatom *Cylindrotheca closterium*: Implications for commercial applications. *Journal of the North Carolina Academy of Science*, 125, 138–142.
- Kooistra, W.H.C.F., Sarno, D., Balzano, S., Gu, H., Andersen, R.A., & Zingone, A. (2008). Global diversity and biogeography of *Skeletonema* species (Bacillariophyta). *Protist*, 159, 177–193. <https://doi.org/10.1016/j.protis.2007.09.004>
- Koyuncu, A. (2018). *The seawater transfer from the Bosphorus to the Golden Horn*. In *Third Marmara Sea Symposium* (pp. 155). Istanbul, Turkey.
- Kraberg, A., Baumann, M., & Dürselen, C. (2010). *Coastal phytoplankton: Photo guide for Northern European seas*. Alfred Wegener Institute.
- Lebret, K., Kritzberg, E.S., Figueroa, R., & Rengefors, K. (2012). Genetic diversity within and genetic differentiation between blooms of a microalgal species. *Environmental Microbiology*, 14(9), 2395–2404. <https://doi.org/10.1111/j.1462-2920.2012.02769.x>
- Najdek, M., Blažina, M., Djakovac, T., & Kraus, R. (2005). The role of the diatom *Cylindrotheca closterium* in a mucilage event in the northern Adriatic Sea. *Journal of Plankton Research*, 27, 851–862. <https://doi.org/10.1093/plankt/fbi057>
- Nunn, G.B., Theisen, B.F., Christensen, B., & Arctander, P. (1996). Simplicity-correlated size growth of the nuclear 28S ribosomal RNA D3 expansion segment in the crustacean order Isopoda. *Journal of Molecular Evolution*, 42, 211–223. <https://doi.org/10.1007/BF02198847>
- Özsoy, E., Oguz, T., Latif, M.A., Unluata, U., Sur, H.I., & Besiktepe, S. (1988). *Oceanography of Turkish straits: Second annual report*. Middle East Technical University.
- Pirt, S.J. (1975). *Principles of microbe and cell cultivation*. Blackwell Scientific Publications.
- Pistocchi, R., Cangini, M., Totti, C., Urbani, R., Guerrini, F., Romagnoli, T., Sist, P., Palamidesi, S., Boni, L., & Pompei, M. (2005). Relevance of the dinoflagellate *Gonyaulax fragilis* in mucilage formations of the Adriatic Sea. *Science of the Total Environment*, 353, 307–316. <https://doi.org/10.1016/j.scitotenv.2005.09.087>
- Reynolds, L.K., Stachowicz, J.J., Hughes, A.R., Kamel, S.J., Ort, B.S., & Grosberg, R. K. (2017). Temporal stability in patterns of genetic diversity of *Zostera marina*. *Heredity*, 118, 404–412. <https://doi.org/10.1038/hdy.2016.114>
- Ryneron, T.A., Newton, J.A., & Arnbrust, E.V. (2006). Spring bloom development and population succession in *Ditylum brightwellii*. *Limnology and Oceanography*, 51, 1249–1261. <https://doi.org/10.4319/lo.2006.51.3.1249>
- Ryneron, T.A., Lin, E.O., & Arnbrust, E.V. (2009). Metapopulation structure in *Ditylum brightwellii*. *Protist*, 160, 111–121. <https://doi.org/10.1016/j.protis.2008.10.003>
- Scholin, C.A., Herzog, M., Sogin, M.L., & Anderson, D.M. (1994). Identification of genetic markers for *Alexandrium*. *Journal of Phycology*, 30, 999–1011. <https://doi.org/10.1111/j.0022-3646.1994.00999.x>
- Scholz, B., & Liebezeit, G. (2012). Growth responses of benthic marine diatoms under varying culture conditions. *Diatom Research*, 27, 65–73. <https://doi.org/10.1080/0269249X.2012.660875>

- Semin, S., Tas, S., & Dursun, F. (2023). Spatial-temporal variability of phytoplankton in the Golden Horn Estuary. *Journal of the Marine Biological Association of the United Kingdom*, 103, e56. <https://doi.org/10.1017/S0025315423000449>
- Smayda, T.J. (1969). Experimental observations on *Detonula confervacea*. *Journal of Phycology*, 5, 150–157. <https://doi.org/10.1111/j.1529-8817.1969.tb02596.x>
- Stock, W., Vanelander, B., Rüdiger, F., Sabbe, K., Vyverman, W., & Karsten, U. (2019). Thermal niche differentiation in *Cylindrotheca closterium*. *Frontiers in Microbiology*, 10, 1395. <https://doi.org/10.3389/fmicb.2019.01395>
- Tamura, K., Stecher, G., & Kumar, S. (2021). MEGA11. *Molecular Biology and Evolution*, 38(7), 3022–3027. <https://doi.org/10.1093/molbev/msab120>
- Tas, S. (2019). Microalgal blooms in a eutrophic estuary (Golden Horn, Sea of Marmara) following a remediation effort. *Botanica Marina*, 62, 537–547. <https://doi.org/10.1515/bot-2019-0035>
- Tas, S. (2020). Changes in phytoplankton composition following remediation. *Journal of the Marine Biological Association of the United Kingdom*, 100(7), 1053–1062. <https://doi.org/10.1017/S0025315420001058>
- Totti, C., Cangini, M., Ferrari, C., Kraus, R., Pompei, M., Pugnetti, A., Romagnoli, T., Vanucci, S., & Socal, G. (2005). Phytoplankton size distribution and mucilage occurrence. *Science of the Total Environment*, 353, 204–217. <https://doi.org/10.1016/j.scitotenv.2005.09.028>
- Tufekci, V., Balkis, N., Polat-Beken, C., Ediger, D., & Mantikci, M. (2010). Phytoplankton composition during a mucilage event. *Turkish Journal of Biology*, 34, 199–210.
- Urbani, R., Magaletti, E., Sist, P., & Cicero, A.M. (2005). Extracellular carbohydrates released by marine diatoms. *Science of the Total Environment*, 353, 300–306. <https://doi.org/10.1016/j.scitotenv.2005.09.026>
- Van Bergeijk, S.A., Van der Zee, C., & Stal, L.J. (2003). Uptake and excretion of DMSP driven by salinity changes. *European Journal of Phycology*, 38, 341–349. <https://doi.org/10.1080/09670260310001612600>
- Wiens, J.J., Ackerly, D.D., Allen, A.P., Anacker, B.L., Buckley, L.B., Cornell, H.V., Damschen, E.I., Davies, T.J., Grytnes, J.A., Harrison, S.P., Hawkins, B.A., Holt, R.D., McCain, C.M., & Stephens, P.R. (2010). Niche conservatism as an emerging principle. *Ecology Letters*, 13, 1310–1324. <https://doi.org/10.1111/j.1461-0248.2010.01515.x>