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Araştırma Makalesi / Research Article

An Effective Parameter on the Biochemical and Physiological Properties of *Polysiphonia morrowii* Harvey: Salinity Stress

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Polysiphonia morrowii;
Salinity Stress

Abstract

In recent years, rising temperatures due to global climate change can constitute a decrease in salinity in the oceans by causing the ice to melt early and freeze late in its annual cycle and increased precipitation. On the other hand, high temperatures can increase the salinity of seawater locally by enhancing evaporation. It is expected that the changes in salinity of sea water would affect communities of seaweeds. In this study, *Polysiphonia morrowii* Harvey samples were collected from Altıntaş station in the Gulf of Gemlik in April 2013, and cultured in mediums with four different salt concentrations (10‰, 23‰, 33‰, 42‰). The changes of this species in the amounts of total protein, total phenol, phycocyanin (PC), phycoerythrin (PE), chlorophyll-a (Chl a), water-soluble antioxidant, oil-soluble antioxidant, total solid organic matter and the activity of the carbonic anhydrase were determined. The study clearly showed that studied biochemical and physiological properties of the species were affected by salinity changes. It is also detected that *P. morrowii* is a tolerant species that can adapt to changing salinity conditions with various defense strategies.

Polysiphonia morrowii Harvey'nin Biyokimyasal ve Fizyolojik Özellikleri Üzerinde Etkili Bir Parametre: Tuzluluk Stresi

Anahtar kelimeler
Antioksidan;
Fenolik Bileşikler;
Pigmentler;
Polysiphonia morrowii;
Tuzluluk Stresi

Öz

Son yıllarda küresel iklim değişikliğine bağlı olarak artan sıcaklıklar, yıllık döngüsünde buzların erken eriyip geç donmasına ve yağışların artmasına neden olarak okyanuslarda tuzluluk azalmaları oluşturabilmektedir. Diğer yandan yüksek sıcaklıklar, buharlaşmayı artırarak deniz suyunun tuzluluğunu bölgesel olarak artırabilmektedir. Deniz suyunun tuzluluğundaki değişikliklerin deniz yosunu topluluklarını etkilemesi beklenmektedir. Bu çalışmada, *Polysiphonia morrowii* Harvey örnekleri Nisan 2013'te Gemlik Körfezi'ndeki Altıntaş istasyonundan toplanmış ve dört farklı tuz konsantrasyonuna (10‰, 23‰, 33‰, 42‰) sahip ortamda kültüre alınmıştır. Bu türün toplam protein, toplam fenol, fikosiyanin (PC), fikoeritrin (PE), klorofil-a (Chl a), suda çözünen antioksidan, yağda çözünen antioksidan, toplam katı organik madde ve karbonik anhidraz aktivitesi miktarlarındaki değişimler belirlenmiştir. Bu çalışma, türün incelenen biyokimyasal ve fizyolojik özelliklerinin tuzluluk değişimlerinden etkilendiğini açıkça göstermiştir. *P. morrowii* türünün çeşitli savunma stratejileri ile değişen tuzluluk koşullarına uyum sağlayabilen toleranslı bir tür olduğu da tespit edilmiştir.

1. Introduction

Salinity is a parameter that shows a very stable state in ocean waters, but changes depending on climatic factors such as precipitation, tides, wind, drought periods in coastal waters (Dickson *et al*. 1982, Lartigue *et al*. 2003). Salinity, which is one of the environmental factors, affects the local and regional distribution of seaweeds by changing the ion concentration and osmoregulation (Ramlov *et al.* 2012). Seaweeds respond to the stress caused by salinity changes with various adaptation mechanisms such as changes in cell ion composition, adjustment of intracellular osmotic active solutes, changes in antioxidant molecule amount and activities (Bisson and Kirst 1995, Eggert *et al.* 2007, Karsten *et al.* 1991, Kim *et al.* 2005).

Global warming and climate change cause various changes in marine ecosystems. According to the important scenario of the Intergovernmental Panel on Climate Change (IPCC) report (IPCC 2007), it is estimated that the surface temperatures of North Arctic waters will increase between 2.4-6.4°C until 2100 annually. On a global scale, it is estimated that the increasing temperature of the Arctic Ocean will cause ice to melt early and freeze late in its annual cycle, increased precipitation, and decreased ocean salinity in the first 500 meters downwards from the upper part (Symon 2005, IPCC 2007). On the other hand, the Marmara Sea is located in the Turkish Straits System (TSS) and is exposed to regional salinity and temperature changes (Oğuz and Öztürk 2011). The Sea of Marmara shows unique hydrological conditions with the stratification of Black Sea waters with low salinity in the upper layer and Mediterranean waters with high salinity at the bottom. In addition, the warming rate in the Marmara Sea is quite high due to its location in the Mediterranean basin (Turan *et al.* 2016). It is thought that the high warming rates in the Marmara Sea (Bengil and Mavruk 2018) as a result of its location in the Mediterranean basin will cause an increase in seawater salinity with evaporation. Due to the increasing water temperature, especially changes in salinity, oxygen level and water circulation are expected to cause changes in the ecological cycle and food chain by affecting the seaweed, plankton, fish and zooplankton communities (Scherner *et al.* 2013). Resistant genotypes can tolerate salinity with adaptation mechanisms such as avoiding salt, increasing osmotic potential, etc., while sensitive genotypes might fail to adapt salinity and are affected by salt (Doğan *et al.* 2008). Depending on the increasing surface water temperatures in the Sea of Marmara, the spread of Mediterranean species in the Marmara and Black Seas emerges as "Mediterraneanization process" (Turan *et al.* 2016). Seaweeds are important organisms in marine ecosystems in terms of being in the first step of the food chain providing food and habitat for other living beings. They contain high amounts of protein, fatty acids, vitamins and minerals, as well as a wide range of primary and secondary metabolites, and contribute to national income in many different industrial sectors in various countries (e.g. China, Indonesia, Chile, Norway, France, Ireland) (Camarena-Gómez *et al.* 2022, Deepika *et al.* 2022). They are also bioindicator organisms that can respond quickly to environmental changes in coastal ecosystems (Tribollet and Vroom 2007).

Polysiphonia morrowii is a filamentous red algae with a wide distribution in rocky areas within water. It is located at the depth of 0.1 m more densely than in the deeper parts of the water. The algae with blackish or red thallus form dense clusters on rocks. The thallus, which is usually 3-25 cm in length, has a delicate structure (Erduğan *et al.* 2009). It was determined in the carried out studies that *P. morrowii* Harvey (Harvey 1856), which was recorded among the specimens collected in Hakodate region of Japan for the first time, entered the Mediterranean from the Northwest Pacific Ocean (Kim *et al.* 2004). After being reported in the Mediterranean by Marzocchi *et al.* (2001) and Curiel *et al.* (2002), Erduğan *et al.* (2009) reported this taxon on the Turkish coasts of the Mediterranean for the first time.

In this study, it was aimed to determine the possible effects of salinity changes that may occur depending on climate change on *P. morrowii*. It is thought that the results of this study, which are

unique in terms of the fact that no studies have been carried out with regard to salinity in the Marmara Region and on the *P. morrowii* before, will contribute to the literature. It will also provide a foresight about how the ever changing climate system can change living communities in the coming years.

2. Materials and Methods

2.1 Collection of samples and experimental setup

The Gulf of Gemlik is the southeast branch of the Sea of Marmara extending in the east-west direction. The gulf which has a length of 31 km and a width of 6 km, consists of two different water layers as the Black Sea waters (23-29‰) at the top and the Mediterranean waters (38.5‰) at the bottom (Ünlü and Alpar 2009). In this study, the algae samples were collected from the depth of 1-1.5 m of Altıntaş station (40° 19′ N, 28° 55′ E) in the Gulf of Gemlik, in April 2013. The algal samples were quickly brought to the laboratory in cold transport containers with ambient seawater. After the epiphytes on the samples brought to the laboratory were cleaned, individuals with healthy thallus were cultured. The algae samples were taken into a 4-days acclimation period with a salt concentration of 23‰ (which is the natural salinity for Sea of Marmara and the environment where the samples were collected), at 20°C, under 100 µmol photon $m^{-2}s^{-1}$ light intensity (provided fluorescence tubes Osram L36W/965, Biolux, Germany) and 12:12 h light:dark cycle. Lower light intensity was applied to the species in the culture media to prevent epiphyte growth, photoinhibition, and nutrient depletion (Gao *et al.* 2016, Rautenberger *et al.* 2015). After the acclimation period, the seaweed samples were cultured for 4 weeks in aquariums with a salinity of average 10‰ ± 0.2 (hyposaline stress); 23‰ ± 1 (control); 33‰ ± 1 (hypersaline stress); 42‰ ± 1 (hypersaline stress). The culture medium was refreshed every 3 days. Other variables except salinity in the culture medium were measured daily and kept constant. By applying low and high salt concentrations, the effects of salinity stress on *P. morrowii* were determined and the effects of salinity were investigated. Salinity measurements were carried out by using a Hach Sension5 salinimeter. The culture media were prepared with artificial sea water and filtered (0.45 µ pore size, Whatman polycap GW), then enriched with ¼ strength Provasoli solution (Provasoli 1968). In addition, $CaCl_2.2H_2O$ (Woelkerling *et al.* 1983) was added to the culture medium to provide sufficient calcium as well as vitamins. The pH was adjusted by adding bubbled CO_2 directly into the culture aquariums. Temperature and pH measurements were made with Hanna HI 8314 brand pH meter. Light measurements were carried out with the LI-COR LI-250A light meter which has a LI-192 underwater quantum sensor. All measurements were made in three replicates and mean values and standard deviations were calculated. Species identification of the samples were done under a Zeiss Primo Star light microscope.

2.2 Measurement of chlorophyll-a and phycobiliproteins

The algae samples were extracted with N,N-Dimethylformamide (DMF) at 4°C for 12 hours in the dark for chlorophyll-a (Chl-a) analysis. After measuring the absorbance of the extract spectrophotometrically, Chl-a amounts were calculated according to the method of Inskeep and Bloom (1985). In order to determine the phycocyanin (PC) and phycoerythrin (PE) content of the samples, the algae samples were centrifuged after being extracted with 0.1 M phosphate buffer. PC and PE amounts were determined by measuring the absorbance of the supernatant at different wavelengths (455 nm, 564 nm, 592 nm, 618 nm, 645 nm) and using the formula (Beer and Eshel 1985).

2.3 Determination of total protein and total phenolic content

Total protein content was determined spectrophotometrically at 595 nm absorbance according to the (Bradford 1976). The amount of protein in the samples was calculated considering the bovine serum albumin calibration curve.

Total phenolic contents of crude methanol extracts of seaweed samples were determined according to Folin Ciocalteau's method defined by Taga *et al.* (1984). For each sample, 100 µl of methanol extract was mixed with 2 ml of 2% Na_2CO_3 and incubated for 2 minutes at room temperature. 100 µl of 50% Folin Ciocalteau reagent was added and mixed after incubation and left in the dark at room temperature for 30 minutes. The absorbance of the mixture at 720 nm wavelength was measured spectrophotometrically. Total phenolic content was calculated by gallic acid (GA) standard and expressed as milligram gallic acid equivalent (mg GAE)/g fresh weight (FW) of algae material.

2.4 Determination of total oil- and water-soluble antioxidant capacity

The extracts of algae samples frozen in liquid nitrogen were prepared with hexane in order to determine the oil-soluble antioxidant capacity. The homogenate was centrifuged at 6000 g for 10 minutes. After evaporation of hexane from the extract of the sample that was placed in an Eppendorf tube (200 µl supernatant), the pellet was re-dissolved in the same volume of ethanol. The appropriate amount of phosphomolybdenum reagent (32 mM sodium phosphate, 4 mM ammonium molybdate, 0.6 M sulfuric acid) was added to the mixture and then mixed. After incubation at 95°C for 90 minutes, the absorbance was measured at 695 nm. The oil-soluble antioxidant capacity is expressed as miligram α-tocopherol equivalent per gram fresh weight of algae material (Prieto *et al.* 1999).

Phosphomolybdenum reagent (1 ml) was added to the water-prepared extracts of algae samples frozen in liquid nitrogen (200 µl) for the determination of water-soluble antioxidant capacity. The absorbances of the samples incubated at 95°C for 90 minutes were measured at 695 nm. The water-soluble antioxidant capacity is expressed as milligram L-ascorbic acid equivalent per gram fresh weight of algae material (Prieto *et al.* 1999).

2.5 Carbonic anhydrase activity

In order to determine the carbonic anhydrase activity (CA) (EC 4.2.1.1), samples frozen in liquid nitrogen were ground in the extraction buffer (50 mM Tris, 25 mM Dithiothreitol, 25 mM isoascorbic acid ve 5 mM EDTA, pH 8.5) with the help of a cooled mortar and pestle. The enzymatic reaction was initiated by adding 3 ml of homogenate (algae extract) or buffer (blank) to 2 ml of cold distilled water saturated with CO_2 (substrate). During the measurements made at 0-2°C, the time taken for a pH decrease of 0.4 units within the pH range of 8.1-7.1 was recorded. Carbonic anhydrase enzyme activity was calculated with the help of the formula (to/tc-1)/FW in the Haglund *et al.* (1992) method. The CA activity in the samples is given in Unit (U)/g FW. In the formula, the time taken for a pH decrease of 0.4 units in non-enzymatic and enzymatic reactions is shown as to and tc, respectively, and the fresh weight of algae sample is shown as FW.

2.6 Total solid organic matter

After cleaning the epiphytes of seaweed samples exposed to different salt concentrations, their wet weights were measured. Then, the aluminum foil wrapped samples were dried in an oven at 50°C for 24 hours. Dry weights (DW) of seaweed samples removed from the drying oven were weighed and total solid organic matter amount in g/g was calculated.

2.7 Statistics

The changes in total protein, Chl-a, PE, PC, total phenol, total oil- and water-soluble antioxidant values of algae samples cultured for four weeks at different salt concentrations were evaluated by a two-way ANOVA test. The CA activity and total solid organic matter amounts were statistically analyzed by a one-way ANOVA test. Tukey and Tamhane's T2 tests were used as multiple comparison tests. The homogeneity of normality and variance were determined by Kolmogorov

Smirnov and Levene tests, respectively. All statistical analyzes were tested at a significance level of 0.05 using the commercial SPSS 17.0 (IBM Corporation) software.

3. Results

3.1 Physical and chemical analyzes

The pH, salinity, temperature and light intensity values of culture medium and of the station where the algae samples were collected are shown in the Table 1. The other variables were kept constant in aquariums where the samples were treated with four different salt concentrations.

Table 1. Physical and chemical variables of the environment and medium in which *P. morrowii* was collected and cultured.

Variables	The Sample Collection Station	The Culture Medium of the Specimens
pH	8.32 ± 0.06	8.4 ± 0.15
Salinity (‰)	20.94 ± 0.46	10 ± 0.2, 23 ± 1, 33 ± 1, 42 ± 1
Temperature (˚C)	14.23 ± 0.23	20 ± 1
Light intensity (µmol foton $m^{-2}s^{-1}$)	1029.33 ± 61.65	100

3.2 Chlorophyll-a and phycobiliproteins

Chl-a content of *P. morrowii* varied depending on salinity (F=283.067, p<0.05), time (F=54.605, p<0.05), combined effect of salinity and time (F=13.679, p<0.05). When the Chl-a amounts of *P. morrowii* were examined in a four-week period depending on time and salinity, an increase in the Chl-a values of the samples with 10‰ and 23‰ salt concentrations compared to the Chl-a values with 33‰ salt concentration and a decrease in 42‰ salt concentration was observed in all weeks (Figure 1). In the first week, the highest Chl-a value (0.67 ± 0.24 mg/g FW) was determined in the samples with 10‰ salt concentration, and a decrease in Chl-a values was observed as the salinity concentration increased. The Chl-a values of the samples cultured at different salt concentrations showed a similar change to the 1st week in the 2nd and 3rd weeks. Compared to the 1st week, there was a 1.4-fold increase in the Chl-a values of the samples at 10‰ salt concentration in the 4th week and the highest Chl-a value was determined as 0.94 ± 0.13 mg/g FW among all samples examined during 4 weeks of culture (p<0.05). The Chl-a values of the samples at 23‰ salt concentration in the 4th week were found to be higher than the Chl-a values in the 1st week (0.80 ± 0.33 mg/g FW, p<0.05).

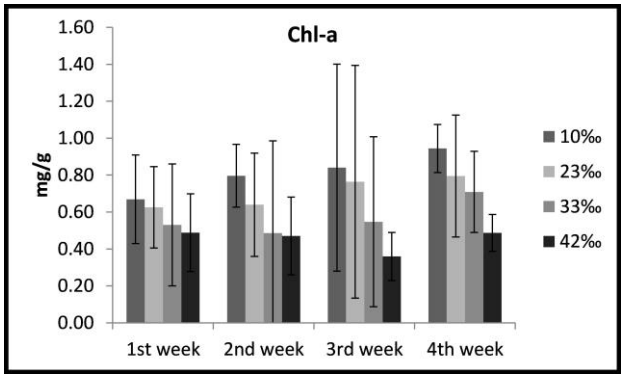

Figure 1. Chl-a amounts of *P. morrowii* depending on time and salinity.

The variation of the PC values of the samples depending on time and salinity is given in Figure 2. The PC values of the samples cultured at 10‰ salt concentration were higher than the values at other salt concentrations in all weeks and it reached the highest value observed throughout the culture period in the 2nd week (0.105 ± 0.01 mg/g FW). The lowest PC values were determined in the samples with 33‰ salt concentration and they decreased to 0.044 ± 0.01 mg/g FW at the 3rd week. On the other hand, while the PC values at 33‰ salt concentration were close to each other in all weeks, the PC values of the samples with 42‰ salt concentration increased by 1.4 times in the 3rd week, compared to the 1st week. The PC content of *P. morrowii* differed between salinity treatments and between 1st and 4th weeks, 1st and 2nd weeks (p<0.05). The PC content of *P. morrowii* varied depending on salinity (F=51.427, p<0.05), time (F=4.801, p<0.05), and the combined effect of salinity and time (F=2.415, p<0.05).

Except for the 4th week, PE values of *P. morrowii* were observed to be higher at low salt concentrations (10‰ and 23‰) than those at high salt concentrations (33‰ and 42‰) (p>0.05)

(Figure 3). In the 2nd week, PE values of the samples with 10‰ and 23‰ salt concentrations increased by 1.5 and 1.3 times, respectively, compared to the first week. The PE value of the samples with 42‰ salt concentration increased by 0.5 times compared to the PE values at 33‰ salt concentration. In all weeks, the values at 33‰ salt concentration detected were lower than those at 23‰ salt concentration.

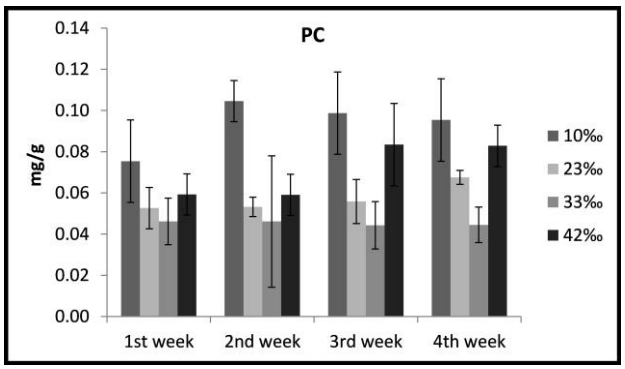

Figure 2. PC amounts of *P. morrowii* depending on time and salinity.

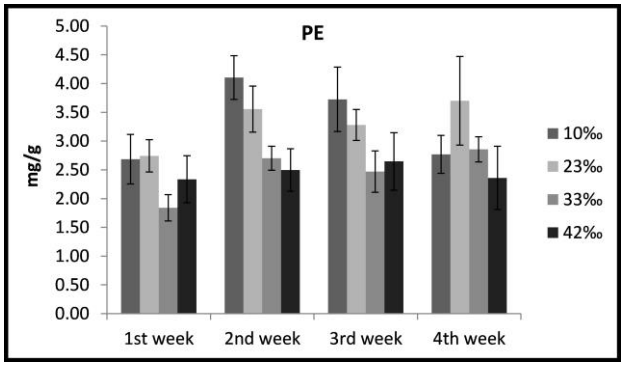

Figure 3. PE amounts of *P. morrowii* depending on time and salinity.

In the samples cultured with different salt concentrations for four weeks, the highest PE value was observed at 10‰ salt concentration in the 2nd week (4.10 ± 0.38 mg/g FW), and the lowest PE value was observed at ‰33 salt concentration in the 1st week (1.84 ± 0.23 mg/g FW). Salinity ($F=35.902$, $p<0.05$), time ($F=17.789$, $p<0.05$) and both factors together ($F=4.978$, $p<0.05$) affected the phycoerythrin values of the samples. The PE values of the samples with 10‰ and 23‰ salt concentrations differed from the PE values of the samples with 33‰ and 42‰ salt concentrations ($p<0.05$).

3.3 Total protein and total phenolic content

The total protein content of *P. morrowii* changed depending on salinity ($F=15.690$, $p<0.05$), time ($F=51.180$, $p<0.05$), and the combined effect of salinity and time ($F=5.649$, $p<0.05$). Considering all weeks, the lowest total protein values were observed in applications with 33‰ salt concentration, and it was determined as 5.54 ± 0.74 mg/g FW in the 1st week. The highest total protein values were found in the samples with 10‰ salt concentration at the 4th week (10.88 ± 1.42 mg/g FW) (Figure 4). When the protein values determined in the samples in the 4th week at 10‰ salt concentration were compared with the 1st week, an increase of approximately 1.6 times was determined. A similar change was also observed in the total protein values of the samples with 23‰ salt concentration. The protein values of the samples with 42‰ salt concentration were observed at higher levels than the protein values at 33‰ salt concentration, and an increase was determined depending on time in the 3rd and 4th weeks (respectively, 9.38 ± 0.91 mg/g FW, 9.04 ± 1.20 mg/g FW). The difference between the protein values of the samples with both 33‰ and 23‰, and 33‰ and 10‰ salt concentrations was significant ($p<0.05$).

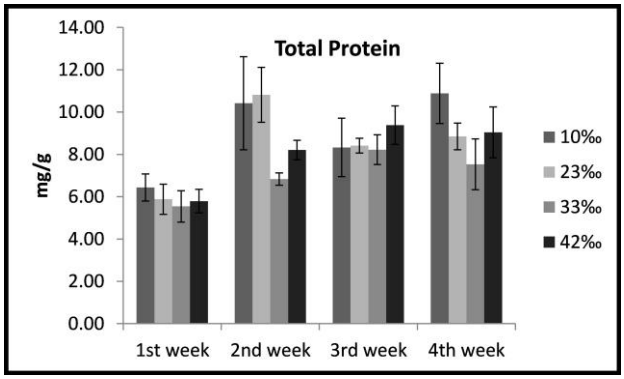

Figure 4. Total protein amounts of *P. morrowii* depending on time and salinity.

The change in the total phenol amount of *P. morrowii* was started to be measured from the 2nd week (Figure 5). Except for the 4th week, the

lowest total phenol values were reported in samples with 33‰, and the highest total phenol values were reported in samples with 42‰ salt concentration. The total phenol value reached the highest level in the samples with 10‰ salt concentration at the 4th week (2.18 ± 0.43 mg/g FW). The highest and the lowest total phenol values observed throughout the culture were observed in the samples with 42‰ salt concentration in the 2nd week and samples with 33‰ salt concentration in the 3rd week, respectively (2.28 ± 0.15 mg/g FW, 1.70 ± 0.15 mg/g FW). As a result, the total phenol values of the samples varied depending on salinity ($F=9.871$, $p<0.05$) and time ($F=4.148$, $p<0.05$). A significant difference was found between the total phenol values of the samples with 33‰ salt concentration and the total phenol values of the samples with 10‰ and 42‰ salt concentrations ($p<0.05$).

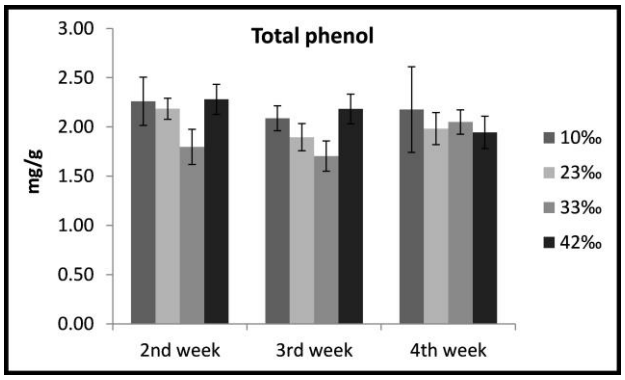

Figure 5. Total phenol amounts of *P. morrowii* depending on time and salinity.

3.4. Total oil-soluble and water-soluble antioxidant capacity

While the oil-soluble antioxidant contents of the samples cultured with 10‰ salt concentration in the 1st (0.84 ± 0.21 mg/g FW) and 2nd (0.92 ± 0.06 mg/g FW) weeks of the study showed close values to each other, these values increased in the 3rd (1.42 ± 0.21 mg/g FW) and 4th (1.27 ± 0.54 mg/g FW) weeks of the study. The low values observed in the samples with 23‰ salt concentration in the first week, approximately doubled in the 3rd week and reached 1.11 ± 0.58 mg/g FW. Except for the 4th week, the oil-soluble antioxidant contents of the samples exposed to 42‰ salt concentration increased gradually in the first 3 weeks (0.51 ± 0.13 mg/g FW, 0.64 ± 0.19 mg/g FW, 0.77 ± 0.26 mg/g FW, respectively) (Figure 6). Time affected the oil-soluble antioxidant content of the *P. morrowii* ($F=5.910$, $p<0.05$). In the 2nd, 3rd and 4th weeks of the study, the highest oil-soluble antioxidant contents were observed in the samples with 10‰ salt concentration (0.92 ± 0.06 mg/g FW; 1.42 ± 0.21 mg/g FW; 1.27 ± 0.54 mg/g FW, respectively). The oil-soluble antioxidant content of *P. morrowii* was affected by salinity changes. The oil-soluble antioxidant contents of the samples cultured at 10‰ salt concentrations differed from those of the samples exposed to 23‰ and 42‰ salt concentrations ($p<0.05$).

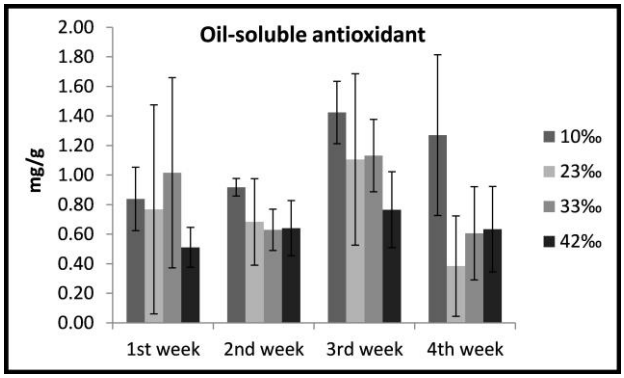

Figure 6. The amount of oil-soluble antioxidants of *P. morrowii* depending on time and salinity.

During the application period of 4 weeks, the highest water-soluble antioxidant contents were determined in the samples cultured at ‰10 salt concentration at each week of the study (Figure 7) and it reached the highest value in the 4th week (2.60 ± 0.17 mg/g FW). The lowest water-soluble antioxidant values were observed at 33‰ salt concentrations throughout the culture, and were determined to be 1.06 ± 0.12 mg/g FW in the 1st week. Although a similar situation was observed in all weeks, the amount of water-soluble antioxidants of the samples cultured at 10‰ salt concentration in the 4th week (2.60 ± 0.17 mg/g FW) reached approximately twice the amount of other applications. The effects of salinity ($F=148.017$, $p<0.05$), salinity and time together ($F=12.484$, $p<0.05$) were found to be significant on *P. morrowii*. On the other hand, the time factor alone was determined to be an ineffective variable

(F=2.588, p>0.05). Multiple comparison tests showed that the water-soluble antioxidant contents of the samples cultured at 10‰ and 33‰ salt concentrations were different from other treatments (p<0.05). The water-soluble antioxidant contents of the samples exposed to 23‰ and 42‰ salt concentrations did not differ statistically (p>0.05).

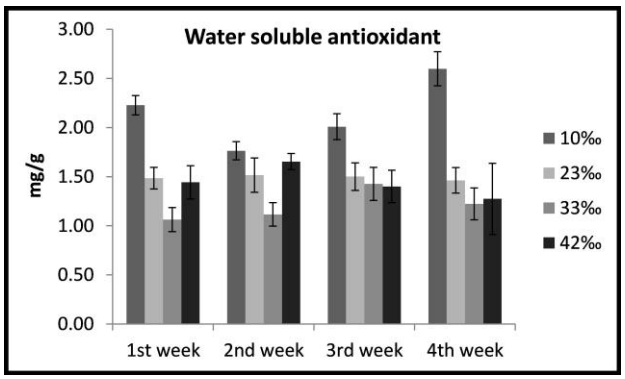

Figure 7. The amount of water-soluble antioxidants of *P. morrowii* depending on time and salinity.

3.5 Carbonic anhydrase activity

It was determined that the CA activity of *P. morrowii* samples was affected by salinity at the end of four weeks of culture (F=16.369, p<0.05). In the measurements made in the 4th week, the CA values of the samples cultured at 23‰ salt concentration were observed to be at the lowest level compared to other applications (1.35 ± 0.38 U/g FW, p<0.05). The CA values observed in the samples exposed to 10‰ salt concentration were found to be 3.4 times higher than the values of the samples exposed to 23‰ salt concentration. The highest CA value was detected in the samples cultured at 42‰ salt concentration (4.58 ± 0.96 U/g FW) (Figure 8). On the other hand, the difference between the CA values of the samples at 10‰, 33‰ and 42‰ salt concentrations was not significant (p>0.05).

3.6 Total solid organic matter

The total solid organic matter amount of *P. morrowii* was affected by salinity changes (F=8.966, p<0.05). Considering the total solid organic matter content of the cultured samples in the last week (Figure 9), it was observed that the values at high salt concentrations (42‰, 33‰) were higher than the other treatments (50.52 ± 6.56 g/g DW, 46.44 ± 2.79 g/g DW, respectively). The lowest total solid organic matter amount was found to be at 23‰ salt concentration (38.30 ± 3.59 g/g DW), and it was close to the values with 10‰ salt concentration (p>0.05). On the other hand, it differed significantly from the values of the samples with both 33‰ and 42‰ salt concentrations (p<0.05).

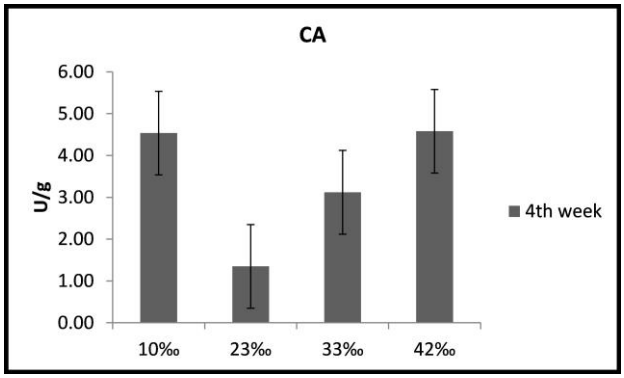

Figure 8. CA activity of *P. morrowii* depending on salinity.

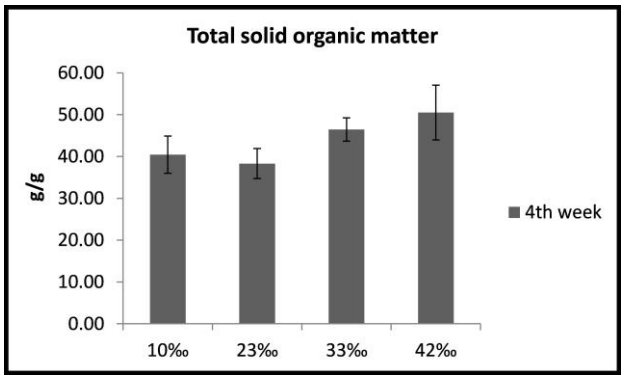

Figure 9. Total solid organic matter amount of *P. morrowii* depending on salinity.

In addition, some descriptive statistics of total protein, PE, PC, Chl-a, total oil- and water-soluble antioxidant, total phenol, CA, and total solid organic matter values of *P. morrowii* cultured in 4 different salt concentrations are given in Table 2.

Table 2. The measured mean values and standard deviation values of biochemical and physiological parameters depending on time in *P. morrowii* cultured in different salt concentrations.

		Salinity Concentration			
Parameters	**Week**	**10‰**	**23‰**	**33‰**	**42‰**
Total protein content (mg/g)	1	6.43±0.64	5.88±0.71	5.54±0.74	5.79±0.56
	2	10.42±2.20	10.82±1.30	6.83±0.29	8.21±0.46
	3	8.33±1.38	8.42±0.35	8.22±0.70	9.38±0.91
	4	10.88±1.42	8.85±0.63	7.53±1.20	9.04±1.20
Phycocyanin (mg/g)	1	0.08±0.02	0.05±0.01	0.05±0.01	0.06±0.01
	2	0.10±0.01	0.05±0.005	0.05±0.03	0.06±0.01
	3	0.10±0.02	0.06±0.01	0.04±0.01	0.08±0.02
	4	0.10±0.02	0.07±0.003	0.04±0.01	0.08±0.01
Phycoerythrin (mg/g)	1	2.69±0.43	2.74±0.28	1.84±0.23	2.34±0.41
	2	4.10±0.38	3.56±0.40	2.70±0.21	2.49±0.37
	3	3.73±0.56	3.28±0.27	2.47±0.36	2.65±0.50
	4	2.77±0.33	3.70±0.77	2.86±0.22	2.36±0.55
Chlorophyll-a (mg/g)	1	0.67±0.24	0.63±0.22	0.53±0.33	0.49±0.21
	2	0.80±0.17	0.64±0.28	0.49±0.50	0.47±0.21
	3	0.84±0.56	0.76±0.63	0.55±0.46	0.36±0.13
	4	0.94±0.13	0.80±0.33	0.71±0.22	0.49±0.10
Oil-soluble antioxidant (mg/g)	1	0.84±0.21	0.77±0.71	1.02±0.64	0.51±0.13
	2	0.92±0.06	0.68±0.29	0.63±0.14	0.64±0.19
	3	1.42±0.21	1.11±0.58	1.13±0.25	0.77±0.26
	4	1.27±0.54	0.38±0.34	0.61±0.32	0.63±0.29
Water-soluble antioxidant (mg/g)	1	2.23±0.10	1.48±0.11	1.06±0.12	1.44±0.17
	2	1.76±0.09	1.52±0.17	1.12±0.12	1.65±0.08
	3	2.01±0.13	1.50±0.14	1.43±0.17	1.40±0.17
	4	2.60±0.17	1.46±0.13	1.22±0.16	1.27±0.36
Total phenolic content (mg/g)	2	2.26±0.25	2.18±0.11	1.80±0.18	2.28±0.15
	3	2.09±0.13	1.90±0.14	1.70±0.15	2.18±0.15
	4	2.18±0.43	1.98±0.16	2.05±0.12	1.95±0.16
Carbonic anhydrase activity (U/g)	4	4.54±0.49	1.35±0.37	3.12±0.63	4.58±0.96
Total solid organic matter(g/g)	4	40.44±4.45	38.30±3.59	46.44±2.79	50.52±6.56

4. Discussion and Conclusion

Seaweeds maintain their homeostasis by osmotic adaptation to their environment through the intake, accumulation, destruction and disposal of various osmotic active substances against salinity changes (Kirst 1989). In the study, the high total solid organic matter amounts observed at high salt concentrations suggested that *P. morrowii* might have accumulated organic compatible solutes in order to increase its osmotic potential. Seaweeds and higher plants create low water potential in their cytoplasms by biosynthesis and accumulation of various organic osmolytes (e.g. in protein and/or carbohydrate structure) known as "Compatible solutes" in response to salinity (Brown and Simpson 1972, Karsten *et al.* 1996, Kirst 1989). In most cases, these substances are photosynthetic products as in red algae (Kremer 1978). In this study, the total amount of protein and the amounts of Chl-a, PE, and PC, which are protein-structured compounds, were generally lower at high salt concentrations suggested that the increase in total solid organic matter observed in *P. morrowii* may have been caused by the biosynthesis or accumulation of low molecular weight carbohydrate compounds rather than protein compounds. On the other hand, it was observed that the amount of total solid organic matter was not affected by the low salt

concentration. Similar to our findings, Reed et al. (1980) stated that fluoroidose carbohydrate increased as a compatible solute in *Porphyra purpurea* with increasing salinity. On the other hand, they found a decrease in low molecular weight carbohydrate levels with decreasing salinity as in the study of Munda and Kremer (1977). Karsten *et al.* (1996) and Eggert *et al.* (2007) showed that red seaweeds make sorbitol biosynthesis and accumulation in response to osmotic stress in high salinity conditions. Reed (1983) reported that *Polysiphonia lanosa* (L.) Tandy benefited from organic (DMSP) and inorganic solutes to maintain homeostasis against salinity conditions between 2% and 200%.

In this study, an increase was observed in the CA activity of the samples of *P. morrowiii* cultured at other salt concentrations compared to the samples cultured at 23‰ salt concentration, as in the total solid organic matter content. The thought that the increase in the total solid organic matter amount to ensure osmotic adaptation may be mostly due to carbohydrates is in harmony with the increased CA activity. CA may have allowed the seaweed to use inorganic carbon source by converting bicarbonate (HCO_3^-) to carbon dioxide (CO_2). In addition, the obtained results suggest that this seaweed uses HCO_3^- as an inorganic carbon source, converts more HCO_3^- to CO_2 with increased CA activity, so that it can absorb more CO_2 and synthesize more organic molecules such as carbohydrates. On the other hand, due to H_2O_2 formed under stress, changing in the functioning of the genes responsible for the operation of CA activity or removal of Reactive Oxygen Species (ROS) which negatively affects CA activity in the environment with increased antioxidant substance levels might be among the possible causes of high CA activity observed in samples with low and high salt concentrations. Similar to our findings, Booth and Beardall (1991) found in their study with *Dunaliella salina* that CA activity on the cell surface increased depending on increased salinity. They stated that increases in CA activity were closely related to increased affinity for CO_2 and $HCO3^-$ in photosynthesis. On the other hand, Liu *et al.* (2012) expressed that CA activity decreased as a result of H_2O_2 formed under low osmotic stress in *Dunaliella salina*.

In the study, Chl-a values of *P. morrowii* showed an increase depending on time in the medium with low salt concentration, but decreased as the salt concentration of the medium increased. The increase in Chl-a value of the samples at 23‰ salt concentration was higher than the values of samples cultured at 33‰ salt concentration suggested that the natural habitat of this species may be an environment with a salt concentration of 33‰; this species may have come from the Mediterranean to the Sea of Marmara in various ways and other salt concentrations cause stress. *P. morrowii* (Harvey 1856), which was first recorded in Japan, was later reported frequently in Japan and nearby waters (Kang 1966, Yamada and Tanaka 1944, Yoon 1986). This species, which entered the Mediterranean from the Northwest Pacific Ocean (Kim *et al.* 2004), has subsequently been reported in the Mediterranean and Çanakkale waters (Curiel *et al*. 2002, Erduğan *et al.* 2009, Marzocchi *et al.* 2001).

This species, which entered the Mediterranean from the Northwest Pacific Ocean (Kim *et al.* 2004), has subsequently been reported in the Mediterranean and Çanakkale waters (Curiel *et al*. 2002, Erduğan *et al.* 2009, Marzocchi *et al.* 2001). Until the thesis study on which this article data is based, no record of *P. morrowii* was found for the Sea of Marmara (Çetin 2014). *P. morrowii* was also detected in different studies in Marmara waters in the following years (Taşkın 2016, Taşkın *et al.* 2018, Taşkın *et al.* 2019, Taşkın 2022). It is stated that it was transported to this region by ship and ballast waters, and it is also an invasive/alien red algae species (Taşkın 2022). The fact that the first records of *P. morrowii* were mostly in regions with high salinity supports the view that the natural habitat of this species is environments with a salt concentration of 33‰. In addition, the salinity increases in the Sea of Marmara due to temperature rises suggest that this species has spread to these regions within the Mediterraneanization process of the Sea of Marmara. The data obtained from PE, PC, total protein, total phenol and water-soluble antioxidant

analyzes also seem to be compatible with these views.

In general, photosynthetic activity is suppressed in environments which has low and high salt concentrations (Seemann and Critchley 1985). The environment which has low salt concentration affect photosynthetic activity more negatively in marine species living in areas with high salt concentrations. Considering that the natural environment of the *P. morrowii* has 33‰ salt concentration in this study, *P. morrowii* may have increased the amounts of Chl-a, PE and PC (photosynthetic pigments) in order to counteract the effects of damage in photosynthetic activity as a response to stress at low salt concentration. The change in pigments was substantially similar. On the other hand, the decrease in Chl content detected under high salinity conditions (42‰) may be originated from changes in the lipid-protein ratio of the pigment-protein complex or increased chlorophylase activity. Similarly, Parida et al. (2004) found a decrease in the Chl and carotenoid contents of *Aegiceras corniculatum* depending on the increase in salinity. Also, Kakinuma et al. (2004) determined that the Chl and total pigment content of *Ulva pertusa* increased in low and high salinity treatments. They detected that this increase was especially more in the environment where the salt concentration was low. On the other hand, Israel *et al.* (1999) reported an increase in Chl-a, phycobiliprotein and total protein values in the sample when the *Gracilaria tenuistipitata* species was exposed to salt stress, especially in sample at high salinity environment. Consistent with our results, Kumar *et al.* (2010) reported in their study with *Gracilaria corticata* that in the environment which has low and high salt concentration Chl-a content decreased, PE and allophycocyanin content increased, and PC content increased only in environments with high salt concentration compared to the control. Contrary to Chl-a, a significant increase in PC values was determined also in the environment with high salt concentration (42‰). The fact that the PE and PC values of the samples cultured at low salt concentrations were higher showed that the species was more stressed in these environments than in environments with high salt concentrations. It was thought that *P. morrowii* tried to remove ROS, which were formed as a result of stress due to decreasing and increasing salinity, by increasing the amount of PE and PC pigments with antioxidant properties. Phycobiliproteins are antioxidant substances that prevent oxidative stress by neutralizing ROS through their nucleophilic abilities (Cano-Europa *et al.* 2010). On the other hand, we thought that there is an increase of the amount of phycobiliproteins during adaptation to unfavorable conditions because they play a role as protein stores in biosynthesis reactions, supply high energy needs, provide cell reorganization, maintain membrane fluidity under salt stress, and serve as nitrogen sources especially in low salt concentration conditions (Kumar *et al.* 2010). The total protein values of the samples were also similar to the phycobiliproteins and the lowest values were detected at 33‰ salt concentrations in all weeks. We can attribute the significant increase in protein at low salt concentrations to the increase in phycobiliproteins depending on time. As for the increase in high salt concentrations may resulted from the increase in phycobiliproteins as well as the increased cytoplasm:vacuole ratio in the cell and the accumulation of various proteins as organic solutes in the organism. In environments where salt concentration is high, while the cell begins to shrink by losing water rapidly, the vacuole becomes smaller. While inorganic ions accumulate in the vacuole, organic solutes mostly settle and accumulate in the cytoplasm, and they maintain the ion balance during osmotic stress and ensure the adaptation of the living thing to the environment. Similar to our findings, several researchers reported that *Ulva pertusa* increased the amount of proline (Kakinuma *et al.* 2006) and *Ulva prolifera* increased the amount of total soluble protein (Luo and Liu 2011) in their thalli under osmotic stress. On the other hand, contrary to our findings, Macler (1988) detected decrease in the Chl and phycobiliprotein amounts of the samples in both low and high salinity environments in *Gelidium coulteri*. The decrease in protein values at low salinity was attributed to the loss of phycobiliproteins and Chl pigment.

The fact that the total phenol content was generally the lowest in 33‰ salt concentration in this study suggested that *P. morrowii* was not stressed in this environment. The high total phenol values observed at 42‰ and 10‰ salt concentrations may be due to the fact that the organism increases the amount of antioxidant compounds to cope with oxidative stress as a response to ROS, which formed as a result of ionic effects in hyposaline and hypersaline environments. Phenolic compounds may have enabled *P. morrowii* to adapt to changing environmental conditions by removing ROS from the environment. Antioxidant activity in seaweeds is mainly due to phenolic compounds in most cases, and the amounts of these compounds vary depending on environmental conditions and species (Kumar *et al.* 2008). Polyphenolic compounds are antioxidant substances that exhibit effects to scavenge ROS, chelate metals and prevent lipid peroxidation (Rodrigo and Bosco 2006). On the other hand, the increased phenol values observed in high salt concentration in this study may indicate an adaptation mechanism for *P. morrowii* to maintain ionic balance during osmotic stress. Similar to our findings, Kumar *et al.* (2010) found that low and high salt concentrations cause oxidative stress in *Gracilaria corticata* and cause an increase in polyphenol content. The researchers stated that polyphenols may play an essential role in eliminating ROS, as well as an adaptive property in maintaining turgor pressure during salinity-induced osmotic stress. Parida *et al.* (2004) reported that increased polyphenol content is an adaptation mechanism to eliminate the negative ionic effects that occur in environments with low and high salt concentrations. Also, Matanjun *et al.* (2008) found relationship between phenolic content and antioxidant activity in eight seaweed species they examined.

Plants have enzymatic and non-enzymatic defense systems to cope with the negative effects of ROS formed under adverse conditions (Noctor and Foyer 1998). Water-soluble antioxidants (ascorbate and glutathione) and fat-soluble antioxidants (α-tocopherol and carotenoids) are essential non-enzymatic compounds that remove ROS from the environment (Munné-Bosch and Alegre 2002, Noctor and Foyer 1998). Ascorbate and glutathione play a fundamental role in detoxifying and removing H_2O_2 in the cell, thus protecting the living thing against oxidative stress (Noctor and Foyer 1998). As for α-tocopherol is an antioxidant found in the thylakoid membranes of chloroplasts and protects the fatty acids of the cell membrane against lipid peroxidation (Fryer 1992). In this study, the increase in water- and oil-soluble antioxidant values at 10‰ salt concentration showed that *P. morrowii* was more adversely affected by low salt concentration. While it is also supported by the water-soluble antioxidant values that the 23‰ salt concentration causes more stress on this species compared to the 33‰ salt concentration, the difference between the other salt concentrations in terms of fat-soluble antioxidants is insignificant. Consequently, it is thought that *P. morrowii* increases the amount of water-soluble antioxidant in response to H_2O_2 formed under stress and oil-soluble (α-tocopherol) antioxidant against lipid peroxidation. In addition, the increase in the α-tocopherol content of the samples in medium with low salt concentration may have protected the thylakoid membranes from oxidative stress and contributed to the increase in the chlorophyll (Chl) content. Supporting our findings, Lu *et al.* (2006) found that H_2O_2 accumulated in environments with lower and higher salt concentrations compared to the control in *Ulva fasciata* Delile exposed to salinity stress caused a stress in the organism, and while the total ascorbate content increased at low salinity concentrations, it decreased with increasing salinity concentration. They showed that the increase of ascorbate and glutathione content in low salinity conditions (15‰) is responsible for the removal of H_2O_2 accumulated in the environment thanks to antioxidant enzyme activities. Jahnke and White (2003) found in their study that, at low salinity concentrations, an increase in glutathione and α-tocopherol content of *Dunaliella tertiolecta* ensued and a decrease in total and reduced ascorbate content occurred. On the other hand, they reported that increasing salinity concentrations decreased the glutathione and α-

tocopherol levels while increasing the total ascorbate content. As a result, they suggested that the ascorbate content of the species is the compound that plays a key role in providing salt tolerance under NaCl stress. During salinity changes, the antioxidant content of salt-tolerant species increases, while antioxidant content of sensitive species decreases or does not change (Gossett *et al.* 1994, Hernández *et al.* 2000). In this context, due to the increase in antioxidant capacity of *P. morrowii*, it is thought that it tries to adapt to environments with low and high salt concentrations and is tolerant to salinity changes.

As a result, in this study, it was determined that *P. morrowii* was affected by salinity and that salinity caused some changes in the physiology of the species. In particular, the decrease of salinity in the environment was more effective on this species. In general, *P. morrowii* increased the amount of total protein, Chl-a (except 42‰), PC, PE, total phenol, and water-soluble antioxidants in media with low (10‰, 23‰) and high (42‰) salt concentrations. Similarly, it was detected that CA activity increased in both low (10‰) and high (33‰, 42‰) salt concentrations. However, while the amount of oil-soluble antioxidants increased in samples cultured at low salt concentration (10‰), the amount of total solid organic matter increased in samples cultured at high salt concentrations (33‰, 42‰). Therefore, this species has shown tolerance to salinity changes by adapting to changing environmental conditions with the various defense strategies mentioned. In this context, this study is important in terms of supporting that *P. morrowii* is a tolerant species that can easily spread in different ecosystems with physiological parameters. On the other hand, the fact that this species came from the Pacific and distributed to the Marmara Region supports the idea that it has a high salt tolerance. It is thought that the results obtained will contribute to the literature since there has not been any study on this species in the Marmara Region before. In the following years, it is expected that some species will disappear from the environment during salinity changes that may occur due to climate change, and *P. morrowii* will reach high populations in its environment thanks to its different adaptation characteristics. On the other hand, it is necessary to examine and investigate how salinity changes will affect this species in the long term and how other populations of species in the ecosystem will be affected by this change.

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