Phytoplankton in estuaries adapt to salinity stress by increasing the content and unsaturation of the thylakoid lipid MGDG

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Abstract

Life in estuaries, especially in surface waters, is a challenge, particularly due to changes in salinity. Environmental changes inevitably lead to acclimation or adaptation of phytoplankton in order to survive. Since membranes are the first to perceive changes in the environment, we focused on understanding how phytoplankton in estuaries adapt to salinity stress through lipid remodeling. Since photosynthesis is one of the most sensitive processes, we studied the response of phytoplankton thylakoid membrane lipids to salinity stress. The study was conducted in two estuaries with completely different environmental characteristics. Apart from hydrology, estuaries also differ in phytoplankton community compositions, nutrient status and temperature. Here we show that estuarine phytoplankton, regardless of environmental differences in the two estuaries studied, increase monogalactosyldiacylglycerol (MGDG) content and unsaturation in response to osmotic shock to protect photosynthetic machinery. This was particularly pronounced at the lowest salinities when freshwater phytoplankton encounter saline water and decreases with increasing salinity. Our results also suggest that increased concentrations of nitrogen nutrients have a positive effect on the increased unsaturation of MGDG. Finally, we speculate that the freshwater green algae are the major group responsible for the observed largest increased and content of unsaturated MGDG at the lowest salinity.

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Abstract

Life in estuaries, especially in surface waters, is a challenge, particularly due to changes in salinity. Environmental changes inevitably lead to acclimation or adaptation of phytoplankton in order to survive. Since membranes are the first to perceive changes in the environment, we focused on understanding how phytoplankton in estuaries adapt to salinity stress through lipid remodeling. Since photosynthesis is one of the most sensitive processes, we studied the response of phytoplankton thylakoid membrane lipids to salinity stress. The study was conducted in two estuaries with completely different environmental characteristics. Apart from hydrology, estuaries also differ in phytoplankton community compositions, nutrient status and temperature. Here we show that estuarine phytoplankton, regardless of environmental differences in the two estuaries studied, increase monogalactosyldiacylglycerol (MGDG) content and unsaturation in response to osmotic shock to protect photosynthetic machinery. This was particularly pronounced at the lowest salinities when freshwater phytoplankton encounter saline water and decreases with increasing salinity. Our results also suggest that increased concentrations of nitrogen nutrients have a positive effect on the increased unsaturation of MGDG. Finally, we speculate that the freshwater green algae are the major group responsible for the observed largest increased and content of unsaturated MGDG at the lowest salinity.

KEYWORDS

salinity stress, phytoplankton resilience, lipids, monogalactosyldiacylglycerol, fatty acids, estuaries

1 ?INTRODUCTION

Estuaries are highly dynamic systems, extremely important in providing a wealth of scientific information as they are exposed to a variety of stressors of natural (variable salinity, light attenuation, notable changes in nutrient availability and temperature) and anthropogenic origin (pollutants, various commercial activities in coastal areas, changes in water circulation due to construction, the effects of global warming, which are more easily felt in coastal areas than in the open oceans) (Miller et al., 2009; Sheela and Dhinagaran, 2023), They are also characterized by high productivity (Corell, 1978) and are often exploited as mariculture sites (Yang et al., 2017). In such environments, plankton develop mechanisms to acclimate or adapt to rapid environmental changes through physiological and/or community responses. Much of the productivity and microbiological diversity of estuaries is related to phytoplankton and its composition. Studies of phytoplankton communities in estuaries have shown that phytoplankton communities along the salinity gradient consist of marine phytoplankton at higher salinities, freshwater species at low salinity and species that develop at intermediate salinity (Lancelot and Muylaert, 2011). The osmotic shock caused by the increase in salinity influences the decline in abundance of freshwater phytoplankton downstream (Burić et al., 2007). The phytoplankton community is dependent on the flushing rate in the estuary (Lionard et al., 2008). Their studies in the Scheldt Estuary (Belgium) have shown that diatoms dominate the community at low flushing rates, while chlorophytes from the Scheldt are more important at high flushing rates. Diatoms have also been reported to develop in the lower parts of the Krka River Estuary, while the upper estuary due to the constant salinity changes short-lived nanoflagellates tend to develop (Burić et al., 2007).

Autotrophic plankton is the most important lipid producer in the seas (Gašparović et al., 2014). Lipid concentrations in seawater are relatively low, although they are involved in many important biological processes (Arts et al., 2001). Their quantity and quality depend on environmental factors and the stage in the life

cycle of the primary producers (Zhukova and Aizdaicher, 2001). One of the most important functions of lipids is the formation of membrane lipid bilayers for cells and organelles. The adaptability and flexibility of the membrane structure imposed by the nature of environment are only possible with a broad spectrum of lipid mixtures (Dowhan et al., 2008). Increased synthesis of glycolipids by phytoplankton has been observed under conditions of P scarcity, high temperatures and high light intensities (Gašparović et al. 2013; Novak et al., 2019).

The thylakoid membranes of chloroplasts consist mainly of glycolipids, monogalactosyldiacylglycerols (MGDG), digalactosyldiacylglycerols and sulfoquinovosyldiacylglycerols , with a small proportion of phosphatidyldiacylglycerols (Douce and Joyard, 1990). They are highly unsaturated (Selstam, 1998). These membranes are crucial for plant cell metabolism (Douce and Joyard, 1990), reflecting the importance of their adaptability to environmental changes. The effect of different stressors, such as changes in temperature and salinity, drought, and exposure to pollutants, on thylakoid membrane lipid remodelling has often been studied in higher plants (e.g. Ristic and Cass, 1991; Stefanov et al., 1995; Zheng et al., 2011; Omoto et al., 2016), but rarely in autotrophic plankton population. The literature indicates that different photosynthetic organisms use different strategies to cope with stress in terms of the quantity and quality of thylakoid membrane lipids.

Phytoplankton in estuaries are exposed to constant stress due to salinity changes. To maintain membrane homeostasis, the composition of membrane lipids is expected to change. The scientific question we tried to answer is: Does the change in salinity lead to membrane lipid remodeling in the estuarine phytoplankton to acclimate/adapt to such changes? Since photosynthesis is one of the most sensitive cellular processes, we hypothesized that regulating the state of the thylakoid membrane would contribute to the maintenance of photosynthesis under osmotic stress and thus to cells survival. To address this hypothesis, we analyzed the lipid profiles of particles from the subtropical, eutrophic Wenchang River Estuary and the temperate, mesotrophic Krka River Estuary. We used thin–layer chromatography–flame ionization detection (TLC/FID) to investigate the changes in the composition of lipid classes. To explore the stress responses, in which fatty acids play an important role, the redistribution of fatty acids within a single lipid class, was investigated, using high-performance liquid chromatography/electrospray ionization tandem mass spectrometry (HPLC/ESI/MS/MS). In addition, phytoplankton pigments were analyzed to gain insight into the phytoplankton community responses within the salinity gradients along the estuaries. This allowed us to propose a mechanism for cell stress acclimation through thylakoid membrane remodeling.

2 ?MATERIALS AND METHODS

2.1 ?Study sites and sample collection

Two estuaries, the Krka River Estuary and the Wenchang River Estuary, were selected because of their multiple differences, including temperature and riverine nutrient loading. We only sampled surface water to avoid other possible influencing factors on lipid synthesis, e.g. attenuated light at different depths.

The Krka River is a karst river that forms a 25 km long salt wedge estuary spreading from the Skradinski Buk waterfalls to the Šibenik Channel (Figure1a). Because of the physical barrier of the Skradinski Buk, riverine water, rich in phytoplankton developing at the Visovac lake flows into the estuary by the waterfall contributing to the estuarine phytoplankton community (Cetinić et al 2006, Šupraha et al 2016). The main sources of nutrients in this estuary are the Krka River and numerous submarine groundwater discharges connected to the karst aquifer. The Krka River is the most pristine and organic matter–poor river with a dissolved organic carbon (DOC) of only 0.5 mg L^{-1} (Louis et al., 2009; Hao et al, 2021), while the DOC concentration in the Krka River Estuary averages 1 mg L^{-1} (Lechtenfeld et al., 2013). The Wenchang and Wenijao rivers flow into the Wenchang River Estuary (Figure 1b). Input from the rivers, groundwater discharge and aquaculture wastewater are the main sources of nutrients entering the Wenchang River Estuary (Liu et al., 2011). The DOC content in the Wenchang River Estuary reaches values of up to 3.8 mg L^{-1} (Hao et al., 2021) and even up to 20 mg L^{-1} in ponds (Herbeck et al., 2013), which indicates eutrophic character.

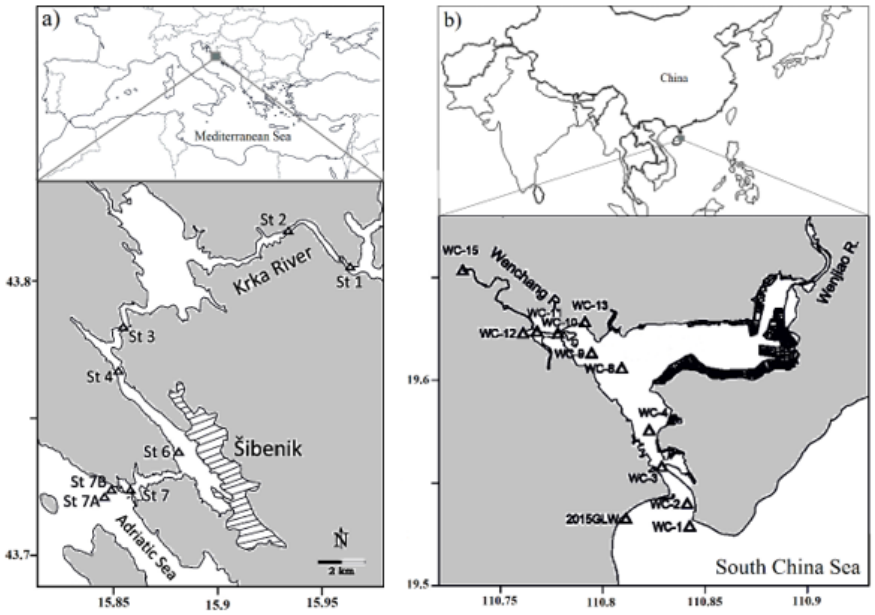

FIGURE 1 Sampling sites at (a) the Krka River Estuary and (b) the Wenchang River Estuary.

The water samples were collected in the Krka River Estuary from 4 to 9 September 2014 and in the Wenchang River Estuary from 8 to 10 May 2015. The samples were collected from the surface (0 m) using 5-litre Niskin bottles along the salinity gradient.

2.2 ?Basic environmental analysis

The salinity and temperature of the water in the Krka River Estuary were measured on site using an HQ40D portable probe (Hach Lange, Germany), while a portable multiparameter WTW multi 350i probe (Geotech Environmental Equipment, Denver, USA) was used for the Wenchang River Estuary.

The samples (50 mL) for the analysis of ammonium (NH_4^+) were stabilized by the addition of 2 mL phenol solution (1 mol L^{-1}; 95 % ethanol) (Ivančić and Degobbis, 1984) and stored in the dark at 4 °C. The samples (500 mL) for all other nutrients were stored at -20 °C. The concentrations of dissolved inorganic nitrogen (DIN=nitrate (NO_3^-), nitrite (NO_2^-), and ammonium (NH_4^+) and orthophosphate (PO_4^{3-}) were determined using spectrophotometric methods according to Strickland and Parsons (1972). Their method accuracies are ±3%, ±3%, ±5% and ±6%, respectively, while detection limits are 0.05 μmol L^{-1}, 0.01 μmol L^{-1}, 0.1 μmol L^{-1} and 0.05 μmol L^{-1}, respectively.

2.3 ?Pigment analysis

While Chlorophyll *a* (Chl *a*) is used as a suitable proxy for phytoplankton biomass, many other phytoplankton pigments show chemotaxonomic associations that can be used to characterize phytoplankton assemblages (Gibb et al., 2000). Pigment-based measurements can be a good tool to determine phytoplankton diversity (Schlüter et al., 2016 and references therein). Analyses of phytoplankton diversity in estuaries using microscopy can be very difficult due to the complex composition of marine and freshwater phytoplankton, cells degraded by osmotic shock in the halocline layer, and the usual taxonomic challenges of smaller cells and unresolved identification issues. Although the assignment of pigments to different phytoplankton groups has its drawbacks, e.g. if some of the phytoplankton groups have similar pigment profiles, the method can be used as a useful tool to describe the functional diversity of phytoplankton in combination with rapid screening of samples under the microscope to identify the dominant species (Sarmento and Descy, 2008, Schlüter et al., 2006). In the Krka River Estuary and in other saltwedge estuary studies, which have combined both

methods, pigment analyses have been proposed as a reliable method for determining phytoplankton diversity (Viličić et al., 2008, Šupraha et al., 2014).

We have detected the following marker pigments of the microphytoplankton: *fucoxanthin* (*Fuco* , diatoms),*peridinin* (*Perid* , dinophytes); mostly nanophytoplankton marker pigments: 19'–*hexanoyloxyfucoxanthin* (*Hex* , prymnesiophytes), *chlorophyll b* (*Chl b* , chlorophytes),*violaxanthin* (*Viola* , chlorophytes and prasinophytes),*alloxanthin* (*Allo* , chrysophytes and cryptophytes),*lutein* (*Lut* , chlorophytes and prasinophytes); and the marker pigment of picophytoplankton: *zeaxanthin* (*Zea* , cyanobacteria) (Jeffrey and Vesk, 1997).

For pigment determination, 1 L of seawater was filtered through 0.7 μm Whatman GF/F filters, which were pre-burned at 450°C/5h. Extraction was performed in 4 mL of cold 90% acetone by sonication, followed by centrifugation. The pigments were separated by reversed-phase HPLC (Barlow et al., 1997). The composition of the phytoplankton pigments was analyzed by HPLC following Barlow et al. (1997) method. The extracts were mixed 1:1 (v/v) with 1 M ammonium acetate and injected into the HPLC system with the 3-mm Thermo Hypersil-Keystone column MOS2, C-8, 120 A pore size, 150×4.6 mm (Thermo Hypersil-Keystone, Bellefonte, PA, USA). The pigments were separated at a flow rate of 1 mL min^{-1} using a linear gradient programme lasting 40 min, using solvents A and B. Solvent A consisted of 70:30 (v/v) methanol: 1 M ammonium acetate and solvent B was 100% methanol. Chlorophylls and carotenoids were detected by absorbance at 440 nm (SpectraSYSTEM, Model UV 2000, Thermo Fischer Scientific, USA). The qualitative and quantitative analyses of individual pigments were performed by external standard calibration with authentic pigment standards (VKI, Denmark).

2.4 ?Lipid class analysis

To determine the lipid classes, 3 L of seawater were collected in glass containers and passed through the 200 μm stainless steel screen to remove zooplankton and larger particles. Immediately afterwards, the seawater was filtered through 0.7 μm Whatman GF/F filters pre-burned at 450 °C for 5 h. The filters of Krka River Estuary samples were stored in liquid nitrogen for six months, while the samples from the Wenchang River Estuary were stored at -80 °C for 2-3 weeks before analysis. The particulate lipids were extracted with a modified one–phase solvent mixture of dichloromethane–methanol–water (Bligh and Dyer, 1959). N–hexadecanone was added to each sample as an internal standard to estimate recoveries in the subsequent steps of sample analysis. The extracts were evaporated to dryness under a nitrogen atmosphere, stored at -20 °C for one day and dissolved in 20 μL dichloromethane immediately prior to analysis.

The lipid classes were determined using TLC–FID (Iatroscan MK–VI, Iatron, Japan) (Gašparović et al., 2015). The lipid classes were separated on Chromarods SIII and quantified by an external calibration with a standard lipid mixture at a hydrogen flow of 160 mL/min and an air flow of 2000 mL/min. The standard deviation determined from duplicate runs accounted for 1–14% of the relative abundance of the lipid classes. Eighteen lipid classes were detected by this technique. The separation scheme comprised of successive elution steps in solvent systems of increasing polarity, followed by a subsequent partial combustion of Chromarods. Total lipid concentrations were determined by summing all lipid classes quantified by TLC-FID. Detailed procedures are described in Gašparović et al. (2015). However, in Gašparović et al. (2015) there was an error for the elution of the solvent mixture MGDG and DGDG (chloroform–acetone (72:28, v:v), which should be "chloroform–acetone (28:72, v:v)" (Gašparović et al. 2017). So, we tested the elution of the standards for MGDG, DGDG and SQDG with that incorrect protocol (Gašparović et al. 2015) and found that the MGDG standard mixture splits into two peaks (termed first and second MGDG, fMGDG and sMGDG, respectively), while DGDG and SQDG co-elute in one peak in the next elution step.

2.5 ?MGDG fatty acid composition

The separation of the MGDG lipids present in the sample mixture was carried out using the UltiMate 3000 Rapid Separation HPLC (Dionex, Germany) system. The Acquity UPLC BEH C18 (2.1 × 100 mm with 1.7 μm particles) (Waters, Milford, Massachusetts, USA) column was maintained at 50 ^{o}C while a gradient elution was employed. The solvent system comprised solution A: LC-MS-grade methanol:ultrapure water

(1:1, v:v; 10 mM ammonium-acetate/0.1 % formic acid) and solution B: LC–MS-grade isopropanol (10 mM ammonium-acetate, 0.1 % formic acid). The gradient started with 55% A/45% B, reached 90% B in 40 min, 99% B in 2 min and remained there for 10 min, then 45% B in 1 min, followed by equilibration for 22 min. The flow rate was 0.15 mL/min and sample mixture injected volume was 10 µL. Immediately before the analysis, dichloromethane was evaporated and the sample was redissolved in a solution of methanol:chloroform (1:2, v:v). The HPLC system was connected online to the amaZon ETD ion trap mass spectrometer (Bruker Daltonik, Bremen, Germany) for analyzing the fatty acid composition. The mass spectrometer was equipped with a standard electrospray ionization ion source (nebulizer pressure 8 psi; drying gas flow rate 5 L/min; drying gas temperature 250 ºC; the potential on the capillary –/+ 4500 V). Lipid profiling was performed in both positive and negative ion mode. Data were collected in the mass range of m/z = 100–1200. ESI MS/MS was performed using collision energy of 1 eV. The MGDG species were identified as $[M+NH_4]^+$ ions in the positive mode. The derived elemental compositions were matched with an internally compiled lipid library from LIPID MAPS (http://www.lipidmaps.org/).

2.6 ?Data analysis

Principal-component analysis (PCA) was performed to determine how the environmental variables (salinity (S), temperature (T), DIN and PO_4^{3}-) related to the accumulation unsaturated MGDG. It was performed using Origin 7 computer software. Linear fits (Origin 7 computer software, Origin Lab) were performed to analyze the correlations of interest.

3 ?RESULTS

3. 1 ?Environmental conditions

The environmental conditions differed significantly between the estuaries studied (Figure 2 and Table S1). Much higher T were measured in the Wenchang River Estuary (27.9-31.5 degC) compared to the Krka River Estuary (21.5-26.2 degC). The Wenchang River Estuary was enriched in DIN (4.0-154.9 µmol L^{-1}), while PO_4^{3-} concentrations were much lower (0.08-2.99 µmol L^{-1}), as previously observed (Liu et al., 2011). Both DIN (1.0-5.8 µmol L^{-1}) and PO_4^{3-} (0.21-0.70 µmol L^{-1}) were notably lower in the Krka River Estuary than in the Wenchang River Estuary.

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FIGURE 2 Environmental conditions along the salinity gradient. The distribution of (a) temperature (T), (b) dissolved inorganic nitrogen (DIN) and (c) orthophosphate (PO_4^{3-}) in surface water in the Krka River Estuary (triangles) and in the Wenchange River Estuary (squares).

Phytoplankton blooms developed in both estuaries. Much higher Chl*a* concentrations were detected in the Wenchang River Estuary than in the Krka River Estuary (Figures 3a-b and Table S2). The specific pigments roughly identified phytoplankton groups (Figures 3c-d and Table S2). The dominance of *fucoxanthin* indicates that diatoms were the main blooming group in both estuaries. The decrease in*violaxanthin* and *lutein* pigments with decreasing salinity suggests that riverine chlorophytes and prasinophytes were acclimated to less saline waters in the estuaries. In the Krka River Estuary, the significant contribution of 19'–*hexanoyloxyfucoxanthin* at intermediate salinities indicates an important involvement of prymnesiophytes, while lower salinities support the development of nanoflagellates. The pigment contribution in the Wenchang River Estuary suggests that different phytoplankton groups have evolved compared to the Krka River Estuary. In addition to diatoms, the largest contributions to the phytoplankton community in the Wenchang River Estuary were cyanobacteria (*zeaxanthin*), as well as chlorophytes, which are related to *chlorophyll b* .

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FIGURE 3 Phytoplankton pigments in the two estuaries. Surface waters along the salinity gradient in the Krka River Estuary (a, c and e) and in the Wenchang River Estuary (b, d and f). The distribution of Chl *a* (a and b). The inlet in (b) describes the Chl *a*distribution at higher salinities. The distribution of pigment markers (c and d) and their relative abundance (%) (e and f). The full names of the pigments can be found in Material and Methods.

3.2 ?Membrane lipid remodeling

The contribution of second MGDG to cell lipids, sMGDG (%), was low for the river endmembers in the Krka River Estuary (<1%) and Wenchang River Estuary (<3%). High sMGDG concentrations (0.2-112.8 μg L^{-1}) were detected in Wenchang River Estuary. Their concentrations in Krka River Estuary were lower, ranging from 0.2 to 3.9 μg L^{-1}. The sMGDG (%) increased significantly at the lowest S compared to river waters, and then its contribution was decreasing with increasing S. In contrast to other detected membrane lipids (not shown), sMGDG (Figure 4), showed a statistically significant negative correlation with the increase in salinity in both estuaries analyzed. All correlations were performed for estuarine samples, while freshwater endmembers were omitted.

There was a negative correlation trend between fMGDG and sMGDG, albeit not statistically significant (Figure 4b). These results suggest a change in MGDG fatty acid composition in response to salinity stress.

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FIGURE 4 The unsaturation of MGDG is highest at the lowest salinity. (a) The contribution of second monogalactosyldiacylglycerols (sMGDG) to cell lipids (sMGDG (%)) along the salinity gradient and (b) the relationship between first monogalactosyldiacylglycerols (fMGDG) and sMGDG in the Krka River Estuary (triangles) and the Wenchang River Estuary (squares). The lines represent linear fits without included salinity 0 of river waters. sMGDG at salinity 13.9 for the Krka River Estuary is an outlier and was excluded from the linear fit.

Although the primary source of MGDG was unconfirmed, we considered that it originates from phytoplankton. This assumption was supported by the fact that the main lipid producer in the oceans is autotrophic plankton (Gašparović et al., 2014) and MGDG is not a characteristic lipid for heterotrophic bacteria (e.g. Sebastián et al., 2016). Therefore, we analyzed in detail the MGDG species with HPLC/MS/MS that have FA with an even number of carbon atoms. The complete list of identified MGDGs for both estuaries can be found in the Table S3a and b. We found a linear correlation between the injected MGDG amount as calculated from the data obtained with TLC/FID and the cumulative MS/MS MGDG intensity (Figure S1).

MGDG fatty acid remodeling between fresh and estuarine waters is recorded (Table S3). Estuarine phytoplankton synthesize more unsaturated FA in MGDG than freshwater phytoplankton (Figure 5). Although there was a trend towards decreasing MGDG unsaturation with increasing salinity, the relationship between sMGDG (%) and average MGDG unsaturation was not statistically confirmed. This can be explained by the different ionization efficiency of the various MGDG from the different samples.

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FIGURE 5 Average MGDG unsaturation (number of double bonds (DB)) in the Krka River Estuary (triangles) and the Wenchang River Estuary (squares).

Our aim was to investigate the influential parameters responsible for the sMGDG accumulation, which MGDG species increase under salinity stress and which phytoplankton group is possibly responsible for their synthesis. To this end, we first performed PCA to determine the environmental variables (salinity (S), temperature (T), DIN and PO_4^{3-}) responsible for sMGDG accumulation (Figure 6a and b, Tables S4 and S5). For this analysis, we have taken data from the estuaries and not from the rivers. The SiO_4^{4-} concentrations were not considered in this analysis as diatoms did not appear to be important for sMGDG accumulation. First two principal components in the principal component analysis between five variables for the Krka River Estuary and the Wenchang River Estuary explained 90.95% and 92.37% of the total variability, respectively. The position of the salinity and PO_4^{3-} variables indicates that salinity changes and the availability of DIN contribute the most to the observed results. Temperature shows opposite trends in the PCA with salinity for both estuaries. The reason for this temperature distribution in the Krka River Estuary is the mixing of cold river water with warmer seawater in September, while the opposite occurred in the Wenchang River Estuary in May, when warmer river water mixed with colder seawater.

Second, we performed a PCA considering sMGDG (%), salinity, DIN, pigments and 28 variables of MGDG with fatty acid double bond combinations for the Krka River Estuary and Wenchang River Estuary, respectively. After a preliminary PCA, the MGDG species that correlated significantly with other variables were selected for further PCA (Figures. 6c and d, Table S6 and S7). First two principal components in the PCA for the Krka River Estuary explained 58.80% of the total variability among 34 variables, while for the Wenchang River Estuary they explained 53.81% of the total variability between 28 variables.

In the Krka River Estuary, in addition to sMGDG and DIN,*peridinin* and *violaxanthin* together with MGDG species with the fatty acid double bond combinations 6+6, 5+6, 1+4 and 0+5 have the largest negative PC1 loadings. These results indicate that dinoflagellates (pigment *peridinin*) and chlorophytes and prasinophytes (pigment *viola*) were probably the main contributors for the accumulation of sMGDG at low salinity.

PCA for the Wenchang River Estuary variables shows that the greatest positive PC1 loadings next to sMGDG (%) and DIN, have *lutein*(chlorophytes and prasinophytes), and 4+5, 3+4 and 1+3 MGDG species (Figure 6d). This indicates that chlorophytes and prasinophytes with the MGDG double bond combinations 4+5, 3+4 and 1+3 probably contributed to the accumulation of sMGDG at low salinity.

The main MGDG fatty acids that contributed to increased MGDG unsaturation (MGDG (%)) in the Krka River Estuary were fatty acids with the combination of double bonds 6+6, 5+6, 1+4 and 0+5. In the Wenchang River Estuary fatty acids with a combination of double bonds 4+5, 3+4 and 1+3.

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FIGURE 6 Principal component analysis (PCA). Variables (lines): salinity (S), temperature (T), nutrients (dissolved inorganic nitrogen, DIN and orthophosphate, PO4) and contributions of second monogalactosyldiacylglycerols to total lipids (sMGDG (%)) (a and b) and sMGDG (%), DIN, S, contributions of individual pigments (%) (see Materials and methods for abbreviations) and contributions of individual MGDG fatty acid combinations for the Krka River Estuary (%) (a and c) and the Wenchang River Estuary (b and d).

4 ?DISCUSSION

Phytoplankton assemblages in estuaries are determined by the season and river discharge dynamics (e.g. Chanand and Hamilton, 2001; Cetinić et al., 2006), which also influences succession between marine and freshwater phytoplankton taxa depending on the degree to which seawater intrusion into the estuary is impeded (Chanand and Hamilton, 2001; Zhu et al., 2015). The phytoplankton community in the studied

estuaries, the Krka River Estuary and the Wenchang River Estuary, differed both in abundance and in the contribution of the different groups between the estuaries (Figure 3), which is consistent with different environmental conditions (Figure 2). However, a similar response to salinity stress was observed in both estuaries when freshwater phytoplankton met saltwater in terms of MGDG remodeling (Figure 4a).

When the river water reaches the estuary, the freshwater phytoplankton either dies or acclimates to the increased ionic strength caused by mixing with the seawater. The change in ionic strength reflects in the composition of the membrane lipids, as membranes surrounding both the cell and the intracellular organelles, are the first to recognize and respond to the change/s in environmental conditions. The study on the influence of salinity on phospholipids in the same estuaries (Vrana Špoljarić et al., 2020) showed that the fatty acid composition of phospholipids phosphatidylcholine (PC), phosphatidylglycerol (PG) and phosphatidylinositol (PI) was similar in both estuaries. This was explained by the importance of these phospholipids for the membrane function(s) in which these phospholipids are involved. In contrast, the fatty acid composition of phospholipids phosphatidylethanolamine (PE), phosphatidic acid (PA) and phosphatidylserine (PS) differed along the salinity gradient and between the two estuaries. This is explained by the adaptability of the plankton to remodel these PL depending on the structure of the plankton community and the environmental conditions.

Photosynthesis is one of the most sensitive cellular processes. It has been shown that the photosynthetic capacity of the freshwater green alga*Chlamydomonas reinhardtii* is suppressed by increased ionic strength (the increase in NaCl concentration) (Husic and Tolbert, 1986). Our results suggest that phytoplankton communities in estuaries have the ability to acquire halotolerance by increasing the MGDG content and its unsaturation (Figures 4 and 5). This led to the conjecture that MGDG and particularly unsaturated MGDGs are crucially involved in the molecular organization and thylakoid membrane function under changing salinity conditions. Osmoregulation in phytoplankton is achieved and stress ameliorated, usually within an hour or two, by regulated ion uptake, by synthesis of osmotically active substances compatible with metabolic processes, by water expulsion via contractile vacuoles or, in species with rigid cell walls, by counterbalancing osmotic pressure with turgor pressure (Rai and Gaur, 2001). In order to respond to stress, sensing and signal transduction are important for the cell survival. Ca^{2+} is an essential component of signal transduction, which controls a large number of physiological processes (Edel et al., 2017). It is found that the cellular Ca^{2+} increase is essential for the survival of osmotic shock in the unicellular green alga *Chlamydomonas reinhardtii* (Bickerton et al., 2016) and the diatom *Phaeodactylum tricornutum* (Helliwell et al., 2021).

The increase in MGDG unsaturation was observed in the green microalgae*Dunaliella tertiolecta* exposed to low salinity (Vrana et al., 2022). Although it is generally known that unsaturated fatty acid content decreases with increasing temperature (e.g. Hernando et al., 2022), this was not evident in the much warmer Wenchang River Estuary, where MGDG has a higher unsaturated fatty acid content than in the case of the Krka River Estuary. This could be explained by the phytoplankton ecotypes of the Wenchang River Estuary, which are adapted to high temperatures and also higher DIN content there. We analyzed lipid classes in other seas with freshwater influence, such as the Baltic Sea and the northern Adriatic Sea, and also found elevated sMGDG (%), up to 8% (T. Novak, A. Penezić, pers. comm.).

The data suggest that the main influencing parameter for increased MGDG unsaturation at lowest salinities in estuaries is the increased ionic strength (salinity stress) compared to river waters. The PCA indicates that chlorophytes and prasinophytes, the pigments *violaxanthin*(the Krka River Estuary) and *lutein* (the Wenchang River Estuary) are the main groups, probably introduced into the estuaries with the river waters, responsible for increased contributions of sMGDG to cell lipids and for increased MGDG unsaturation in the Krka River Estuary and the Wenchang River Estuary, respectively.

We assume that chlorophytes are mainly responsible for this, which is explained in more detail in the following discussion. Green algae (chlorophytes) are known to have a high MGDG concentration in the thylakoids, e.g., compared to diatoms (Garab et al., 2016). According to our findings on the dominance of chlorophytes at low salinities, D'ors et al. (2016), who studied the short-term effects of low salinity on chlorophytes, a diatom and two dinoflagellates and found that chlorophytes were best able to adapt to low salinity in

terms of growth rate and photosynthetic activity. Bharathia et al. (2022) also found that green algae are an important component of the phytoplankton community in the upper low-salinity estuaries, as found in the 26 estuaries along the Indian coast. The chlorophyte *D. tertiolecta*(Vrana et al., 2022) was also shown to increase the unsaturation of MGDG by lowering salinity from 38 to 3. However, the contribution of other groups, including marine groups, cannot be excluded, as their contribution to increased MGDG unsaturation is probably smaller and therefore not confirmed by the PCA analysis. By increasing the unsaturation of the fatty acids in the membrane lipids,*Synechococcus* shows a greater tolerance to salt stress in the form of less damage to the photosynthetic apparatus compared to the more saturated fatty acids (Allakhverdiev et al., 2001). Changes in the proportion of MGDG are often observed in plants in response to changing environmental conditions (Harwood, 1998).

The decreasing unsaturation of MGDG fatty acids with increasing salinity could be explained by the disappearance of freshwater chlorophytes when salinity is too high in the lower estuary. Indeed, Zhu et al. (2015) found that chlorophytes in the Wenchang River make the largest contribution to Chl *a* , while their contribution in the estuary decreases with increasing salinity. We assume that freshwater chlorophytes cannot adapt to too high salinity because the time span for successful adaptation is too short. Long-term adaptation mechanism has been observed in the response of phytoplankton to increased temperature, in which phytoplankton resume their unsaturated fatty acid synthesis over a longer period of time after their initial loss (Jin et al., 2029). The higher MGDG unsaturation in the Wenchang River Estuary than in the Krka River Estuary could be explained by the positive role of the abundance of nitrogen nutrients on unsaturated MGDG fatty acid synthesis (Figures 6a and b).

Thylakoid membranes show remarkable structural flexibility, which plays an important role in various short-term adaptive mechanisms in response to rapidly changing environmental conditions (Garab, 2014). The most abundant lipid (˜50%) in the thylakoid membrane is MGDG (Douce and Joyard, 1990). MGDG may have several functions in the cellular response to salinity stress. In response to various abiotic and biotic stressors, marine phytoplankton and cyanobacteria synthesize oxylipins (oxidation products of unsaturated FA), the bioactive metabolites (Mosblech et al., 2009). For example, jasmonic acid (oxylipin) has been shown to play a role in salinity tolerance (Zhao et al., 2014). We hypothesize that polyunsaturated fatty acids from MGDG may be at least partially converted to oxylipins in salinity-stressed cells, which then play a role in phytoplankton adaptation to elevated salinity. In addition, MGDG strongly promote membrane stacking and increase the mechanical stability of the large light-harvesting complex (protein LHCII) located in the thylakoid (Seiwert et al., 2017; 2018). Therefore, we can speculate that lipid homeostasis of the thylakoid membrane of the phytoplankton in the estuary is promoted by an increased degree of MGDG unsaturation during salinity-stress.

The MGDG fatty acids that contributed to increased MGDG unsaturation in the Krka River Estuary (6+6, 5+6, 1+4 and 0+5) and in the Wenchang River Estuary (4+5, 3+4 and 1+3) were highly unsaturated. It is not easy to assign the mentioned combinations of double bonds in MGDG to chlorophytes, as there are almost no published articles providing data on the fatty acid composition of MGDG in chlorophytes. Our study on the green microalga *D. tertiolecta* (Vrana et al., 2022) has shown that the major fatty acids in MGDG are 18:3/16:4, the proportion of which increases with a decrease in salinity from 38 to 3, and 18:2/16:3, the proportion of which decreases with a decrease in salinity from 38 to 3.

The unsaturation of fatty acids in phytoplankton is crucial for maintaining survival and high growth reproduction rates of many aquatic organisms (Brett and Müler-Navarra, 1997). Since polyunsaturated fatty acids are synthesized exclusively by phytoplankton, the accumulation of more unsaturated MGDG in estuarine phytoplankton described here provides an advantage to organisms that feed on them and thus to higher trophic levels.

5 ?CONCLUSIONS

Although phytoplankton from temperate and subtropical estuaries live in completely different climate and environmental conditions, the mechanism of adaptation to salinity stress is similar in both cases as far as the

composition of thylakoid MGDG glycolipids is concerned. Here we demonstrate a mechanism of salinity stress tolerance utilized by phytoplankton in estuaries, namely the accumulation of the more unsaturated MGDG (sMGDG) to control thylakoid membrane function and finally to protect photosynthetic machinery. The unsaturation of MGDG is highest at the lowest salinity. This is likely a response of the river phytoplankton to the initial shock of exposure to salt, i.e. higher ionic strength at the upper estuary. Higher availability of DIN also plays a positive role in the synthesis of more unsaturated fatty acids in MGDG. Overall, the results of our studies have shown that the reorganization of MGDG fatty acids is an important survival strategy of phytoplankton in challenging environments such as estuaries where salinity changes are constant. The results of our research should be incorporated into predictions and modelling of the effects of global warming and the resulting changes to the future ocean existence. This is because the salinity in the seas and oceans is changing: reduced river inflows, e.g. in the Mediterranean Sea, lead to more saline estuaries and coastal seas, while the melting of glaciers leads to a lower salinity in the nearby ocean.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available in Institute Ruđer Bošković FULIR repository at https://data.fulir.irb.hr/islandora/object/irb:317 and in the Supporting information.

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REFERENCES

Allakhverdiev, S.I., Kinoshita, M., Inaba, M., Suzuki, I., Murata, N. (2001) Unsaturated fatty acids in membrane lipids protect the photosynthetic machinery against salt-induced damage in Synechococcus. *Plant Physiology* , 125, 1842–1853.

Arts, M.T., R.G. Ackman, & Holub, B.J. (2001) "Essential fatty acids" in aquatic ecosystems, a crucial link between diet and human health and evolution. *Canadian Journal of Fisheries and Aquatic Sciences* , 58, 122–137.

Barlow, R.G., Cummings, D.G. & Gibb, S.W. (1997) Improved resolution of mono– and divinyl chlorophylls *a* and *b* and zeaxanthin and lutein in phytoplankton extracts using reverse phase C–8 HPLC.*Marine Ecology Progress Series* ,161, 303–307.

Bharathia, M.D., Venkataramanaa, V. & Sarmaa, V.V.S.S. (2022) Phytoplankton community structure is governed by salinity gradient and nutrient composition in the tropical estuarine system. *Continental Shelf Research* , 234, 104643.

Bligh, E.G. & Dyer, W.J. (1959) A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology* , 37, 911–917.

Brett, M.T. & Müller-Navarra, D.C. (1997) The role of highly unsaturated fatty acids in aquatic foodweb processes. *Freshwater Biology* , 38, 483–499.

Burić, Z., Cetinić, I., Viličić, D., Caput Mihalić, K., Carić, M. & Olujić, G. (2007) Spatial and temporal distribution of phytoplankton in a highly stratified estuary Zrmanja, Adriatic Sea. *Marine Ecology* , 28 Suppl. 1. 169–177.

Cetinić, I., Viličić, D., Burić, Z. & Olujić, G. (2006) Phytoplankton Seasonality in a Highly Stratified Karstic Estuary Krka, Adriatic Sea.*Hydrobiologia* , 555, 31–40.

Chanand, T.U. & Hamilton, D.P. (2001) Effect of freshwater flow on the succession and biomass of phytoplankton in a seasonal estuary.*Marine and Freshwater Research* , 52, 869–84.

Correll, D.L. (1978) Estuarine Productivity. *BioScience* , 28**,** 646–650.

D'ors, A., Bartolomé, M.C. & Sánchez-Fortún, S. (2016) Repercussions of salinity changes and osmotic stress in marine phytoplankton species.*Estuarine, Coastal and Shelf Science* , 175, 169–175.

Douce, R. & Joyard, J. (1990) Biochemistry and function of the plastid envelope. *Annual Review of Cell and Developmental Biology* , 6, 173–216.

Dowhan, W., Bogdanov, M. & Mileykovskaya, E. (2008) Functional roles of lipids in membranes, In: Vance, D.E. & Vance, J.E. (Eds.)*Biochemistry of Lipids, Lipoproteins and Membranes* . Amsterdam: Elsevier, pp. 1–37.

Dyer, KR. (1991) Circulation and mixing in stratified estuaries.*Marine Chemistry* , 32, 111–120.

Edel, K.H., Marchadier, E., Brownlee, C., Kudla, J. & Hetherington, A.M. (2017) The evolution of calcium-based signalling in plants.*Current Biology* , 27, R667–R679.

Garab, G. (2014) Hierarchical organization and structural flexibility of thylakoid membranes. *Biochimica et Biophysica Acta* , 1837, 481–494.

Garab, G., Ughy, B. & Goss, R. (2016) Role of MGDG and non-bilayer lipid phases in the structure and dynamics of chloroplast thylakoid membranes. In: Nakamura, Y. & Li-Beisson, Y. (Eds.) *Lipids in plant and algae development* . Berlin: Springer Nature, 127-157.

Gašparović, B., Godrijan, J., Frka, S., Tomažić, I., Penezić, A., Marić, D. et al. (2013) Adaptation of marine plankton to environmental stress by glycolipid accumulation. *Marine Environmental Research* , 92, 120–132.

Gašparović, B., Frka, S., Koch, B.P., Zhu, Z.Y., Bracher, A., Lechtenfeld, O.J. et al. (2014) Factors influencing particulate lipid production in the East Atlantic Ocean. *Deep-Sea Research Part* I, 89, 56–67.

Gašparović, B., Kazazić, S.P., Cvitešić, A., Penezić, A. & Frka, S. (2015) Improved separation and analysis of glycolipids by Iatroscan thin–layer chromatography–flame ionization detection. *Journal of Chromtography* A, 1409, 259–267.

Gašparović, B., Kazazić, S.P., Cvitešić, A., Penezić, A. & Frka, S. (2017) Corrigendum to "Improved separation and analysis of glycolipids by Iatroscan thin-layer chromatography–flame ionization detection" [J.

Chromatogr. A, 1409 2015. 259–267]. *Journal of Chromtography* A, 1521, 168–169.

Gibb, S.W., Barlow, R.G., Cummings, D.G., Rees, N.W., Trees, C.C., Holligan, P. & Suggett, D. (2000) Surface phytoplankton pigment distributions in the Atlantic Ocean, an assessment of basin scale variability between 50°N and 50°S. *Progress in Oceanography* , 45, 339–368.

Hao, Y-Y, Zhu, Z-Y, Fang, F-T., Novak, T., Čanković, M., Hrustić, E., Ljubešić, Z., Li, M., Du, J-Z., Zhang, R-F. & Gašparović, B. (2021) Tracing Nutrients and Organic Matter Changes in Eutrophic Wenchang (China) and Oligotrophic Krka (Croatia) Estuaries: A Comparative Study.*Frontiers in Marine Science* , 8, 663601.

Harwood, JL. (1998) Involvement of chloroplast lipids in the reaction of plants submitted to stress. In: Siegenthaler, P.-A. & Murata, N. (Eds.)*Advances in photosynthesis. Lipids in photosynthesis* . Dordrecht: Kluwer, 287–302.

Helliwell, K.E., Kleiner, F.H., Hardstaff, H., Chrachri, A., Gaikwad, T., Salmon, D., Smirnoff, N., Wheeler, G.L. & Brownlee, C. (2021) Spatiotemporal patterns of intracellular Ca2+ signalling govern hypo-osmotic stress resilience in marine diatoms. *New Phytologyst* , 230, 155–170.

Herbeck, L.S., Unger, D., Wu, Y. & Jennerjahn, T. C. (2013) Effluent, nutrient and organic matter export from shrimp and fishponds causing eutrophication in coastal and back–reef waters of NE Hainan, tropical China. *Continental Shelf Research* , 57, 92–104.

Hernando, M.P., Schloss, I.R. De La Rosa, F. & De Troch, M. (2022) Fatty acids in microalgae and cyanobacteria in a changing world: Contrasting temperate and cold environments. *Biocell* , 46, 607-621.

Husic, H.D. & Tolbert, NE. (1986) Effect of osmotic stress on carbon metabolism in Chlamydomonas reinhardtii: accumulation of glycerol as an osmoregulatory solute. *Plant Physiology* , 82, 594–6.

Ivančić, I. & Degobbis, D. (1984) An optimal manual procedure for ammonia analysis in natural waters by the indophenol blue method. *Water Research* , 18, 1143–1147.

Jeffrey, S.W. & Vesk, M. (1997) Introduction to marine phytoplankton and their pigment signatures. In: Jeffrey, S.W., Mantoura, R.F.C. & Wright, S.W. (Eds.) *Phytoplankton pigments in oceanography, guidelines to modern methods, Monographs on Oceanographic Methodology* . Paris:UNESCO Publishing. p. 37–84.

Jin, P., Gonzàlez, G. & Agustí, S. (2020) Long term exposure to increasing temperature can offset predicted losses in marine food quality (fatty acids) caused by ocean warming. *Evolutionary Applications* , 13, 2497–2506.

Krumme, U., Herbeck, L.S. & Wang, T. (2012) Tide- and rainfall-induced variations of physical and chemical parameters in a mangrove-depleted estuary of East Hainan (South China Sea). *Marine Environmental Research* , 82, 28-39.

Lancelot, C. & Muylaert, K (2011) Trends in estuarine phytoplankton ecology. In: Wolanski, E. & McLusky, D. (Eds.) *Treatise on estuarine and coastal science: Vol 7. Functioning ecosystems at the land-ocean interface* . Amsterdam: Elsevier, pp 5-15.

Lechtenfeld, O.J., Koch, B.P., Gašparović, B., Frka, S., Witt, M. & Kattner, G. (2013) The influence of salinity on the molecular and optical properties of surface microlayers in a karstic estuary.*Marine Chemistry* , 150, 25–38.

Lionard, M., Muylaert, K., Hanoutti, A., Maris, T., Tackx, M. & Vyverman, W. (2008) Inter-annual variability in phytoplankton summer blooms in the freshwater tidal reaches of the Schelde estuary (Belgium).*Estuarine, Coastal and Shelf Science* , 79, 694–700.

Liu, S.M., Li, R.H., Zhang, G.L., Wang, D.R., Du, J.Z., Herbeck, L.S. et al. (2011) The impact of anthropogenic activities on nutrient dynamics in the tropical Wenchanghe Wenjiaohe Estuary and Lagoon system in East Hainan, China. *Marine Chemistry* , 125, 49–68.

Louis, Y., Garnier, C., Lenoble, V., Mounier, S., Cukrov, N., Omanović, D. & Pižeta, I. (2009) Kinetic and equilibrium studies of copper–dissolved organic matter complexation in water column of the stratified Krka Estuary Croatia. *Marine Chemistry* , 114, 110–119.

Miller, A.W., Reynolds, A.C. Sobrino, C. & Riedel, G.F. (2009) Shellfish face uncertain future in high CO_2 World: Influence of acidification on oyster larvae calcification and growth in estuaries. *PLoS ONE* , 4, e5661.

Mosblech, A., Feussner, I. & Heilmann, I. (2009) Oxylipins: structurally diverse metabolites from fatty acid oxidation. *Plant Physiology and Biochemistry* , 47, 511–517.

Novak, T., Godrijan, J., Marić Pfannkuchen, D., Djakovac, T., Medić, N., Ivančić, I., Mlakar, M. & Gašparović, B. (2019) Global warming and oligotrophication lead to increased lipid production in marine phytoplankton. *Science of The Total Environment* , 668, 171–183.

Omoto, E., Iwasaki, Y., Miyake, H. & Taniguchi, M. (2016) Salinity induces membrane structure and lipid changes in maize mesophyll and bundle sheath chloroplasts. *Physiologia Plantarum* , 157, 13–23.

Rai, L.C. & Gaur, J.P. (2001) *Algal Adaptation to Environmental Stresses, Physiological, Biochemical and Molecular Mechanisms* . Berlin: Springer, pp 6.

Ristic, Z. & Cass, D.D. (1991). Chloroplast structure after water shortage and high temperature in two lines of *Zea mays L* . that differ in drought resistance. *Botanical Gazette* , 152, 186–194.

Sarmento H. & Descy, J.P. (2008) Use of marker pigments and functional groups for assessing the status of phytoplankton assemblages in lakes.*Journal of Applied Phycology* , 20, 1001–1011.

Schlüter, L., Lauridsen, T.L., Krogh G. & Jørgensen, T. (2006) Identification and quantification of phytoplankton groups in lakes using new pigment ratios – a comparison between pigment analysis by HPLC and microscopy. *Freshwater Biology* , 51, 1474–1485.

Schlüter, L., Behl, S., Striebel M. & Stibor., H. (2016) Comparing microscopic counts and pigment analyses in 46 phytoplankton communities from lakes of different trophic state. *Freshwater Biology* , 61, 1627-1639.

Sebastián, M., Smith, A.F., González, J.M., Fredricks, H.F., Van Mooy, B.A.S., Koblížek, M. et al. (2016) Lipid remodeling is a widespread strategy in marine heterotrophic bacteria upon phosphorus deficiency. *The ISME Journal* , 10, 968–978.

Sheela, A.M. & Dhinagaran, G. (2023) Prevalence of microplastics, antibiotic resistant genes and microplastic associated biofilms in estuary - A review. *Environmental Engineering Research* , 28, 220325.

Seiwert, D., Witt, H., Janshoff, A. & Paulsen, H. (2017) The non-bilayer lipid MGDG stabilizes the major light-harvesting complex LHCII against unfolding. *Scientific Reports* , 7, 5158.

Seiwert, D., Witt, H., Ritz, S., Janshoff, A. & Paulsen, H. (2018) The nonbilayer lipid MGDG and the major Light-Harvesting Complex LHCII promote membrane stacking in supported lipid bilayers. *Biochemical Journal* , 57, 2278-2288.

Selstam, E. (1998) Development of thylakoid membranes with respect to lipids. In: Siegenthaler, P.A. & Murata, N. (Eds.) *Lipids in Photosynthesis: Structure, Function and Genetics* . Dordrecht: Springer, 139-154.

Stefanov, K. L., Pandev, S.D., Seizova, K.A., Tyankova, L.A. & Popov, S.S. (1995) Effect of lead on the metabolism in spinach leaves and thylakoid membranes. *Biologia Plantarum* , 37, 251–256.

Strickland, J. & Parsons, T.R. (1972) *A Practical Handbook of Sea Water Analysis* , 2nd ed. Ottawa: Fisheries Research Board of Canada, pp 45-90.

Šupraha, L., Bosak, S., Ljubešić, Z., Mihanović, H., Olujić, G., Mikac, I. & Viličić, D. (2014) Cryptophyte bloom in a Mediterranean estuary: High abundance of Plagioselmis cf. prolonga in the Krka River estuary (eastern Adriatic Sea). *Scientia Marina* , 78, 329-338.

Viličić, D., Terzić, S., Ahel, M., Burić, Z., Jasprica, N., Carić, M., Caput Mihalić K. & Olujić, G. (2008) Phytoplankton abundance and pigment biomarkers in the oligotrophic, eastern Adriatic estuary. *Environmental Monitoring and Assessment* , 142, 199-218.

Vrana, I., Bakija Alempijević, S., Novosel, N., Ivošević DeNardis, N., Žigon, D, Ogrinc, N. & Gašparović, B. (2022) Hyposalinity induces significant polar lipid remodeling in the marine microalga *Dunaliella tertiolecta* (Chlorophyceae). *Journal of Applied Phycology* , 34, 1457-1470.

Vrana Špoljarić, I., Novak, N., Gašparović, B., Kazazić, S.P., Čanković, M., Ljubešić, Z., Hrustić, E., Mlakar, M., Du, J., Zhang, R. & Zhu, Z. (2021) Impact of environmental conditions on phospholipid fatty acid composition: Implications from two contrasting estuaries. *Aquatic Ecology* , 5, 1-20.

Yang, C., Boggasch, S., Haase, W. & Paulsen, H. (2006) Thermal stability of trimeric light-harvesting chlorophyll a/b complex LHCIIb in liposomes of thylakoid lipids. *Biochimica et Biophysica Acta* , 1757, 1642-1648.

Yang, P., Lai, D.Y.F., Jin, B.S., Bastviken, D., Tan, L.S. & Tong, C. (2017a) Dynamics of dissolved nutrients in the aquaculture shrimp ponds of the Min River estuary, China: Concentrations, fluxes and environmental loads. *Science of the Total Environment* , 603-604, 256-267.

Zhao, Y., Dong, W., Zhang, N., Ai, X., Wang, M., Huang, Z. et al. (2014) A wheat allene oxide cyclase gene enhances salinity tolerance via jasmonate signaling. *Plant Physiology* , 164, 1068-1076.

Zheng, G., Tian, B., Zhang, F., Tao, F. & Li, W. (2011) Plant adaptation to frequent alterations between high and low temperatures: remodelling of membrane lipids and maintenance of unsaturation levels. *Plant, Cell & Environment* , 34, 1431–1442.

Zhu, Z.Y., Liu, S.M., Wu, Y., Li, Y., Zhang, J. & Hu, J. (2015) Phytoplankton dynamics and its further implication for particulate organic carbon in surface waters of a tropical/subtropical estuary. *Estuaries and Coasts* , 38, 905–916.

Zhukova, N.V. & Aizdaicher, N.A. (2001) Lipid and fatty acid composition during vegetative and resting stages of the marine diatom *Chaetoceros salsugineus* . *Botanica Marina* , 44, 287–293.