Marine Environmental Research 210 (2025) 107207

Contents lists available at ScienceDirect

Marine Environmental Research

journal homepage: www.elsevier.com/locate/marenvrev

Environmental impact of brine from desalination plants on marine benthic diatom diversity☆

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ARTICLE INFO

Keywords:
Brine
Cyprus
Desalination
Marine diatoms
DNA metabarcoding
Environmental impact

ABSTRACT

Benthic diatoms are sensitive indicators of environmental conditions at the seabed. In this study, benthic diatom communities at two brine outfall sites of reverse osmosis (RO) seawater desalination plants in Larnaca and Dhekelia, Cyprus, were investigated using a classical, microscopy-based approach and environmental DNA metabarcoding. In general, the diversity of diatoms measured by both methods (microscopy and eDNA metabarcoding), increased by distance from the brine discharge. Increased TOC and nutrient enrichment at brine outfalls contributed to decreased diatom diversity at the Larnaca outfalls, but the diatom diversity at Dhekelia was not driven by abiotic factors. The diatom communities at the outfalls were shown to be distinct and showed temporal variation across the sampling seasons with eDNA metabarcoding, but this was the case only for Dhekelia with microscopy. The results highlight the effect of local biogeography and different brine mixing methods on diatom diversity. The results revealed that conventional morphological methods and eDNA metabarcoding rarely leads to similar conclusions. However, the complementary results emphasise that more information can be derived when combining the methods for biodiversity impact assessments.

1. Introduction

Diatoms are a group of heterokont algae commonly found on or near the surface of aquatic sediments. They play a crucial role in coastal ecosystems by contributing to primary production, stabilising sediments, and supporting higher trophic levels (Andersen, 2004; Scholz and Einarsson, 2015). Their sensitivity to environmental impacts makes them valuable as bioindicators. Diatoms are used as a biological quality element (BQE) for the assessment of freshwater ecosystems according to the legal requirements of the Water Framework Directive (WFD) in the European Union.

Traditional morphological identification of diatoms is a complicated and laborious process. Recently, DNA metabarcoding has been developed as an alternative tool for identification of diatoms, particularly for assessment for ecological quality in freshwater systems (Vasselon et al., 2017). It has been concluded based on previous research that DNA metabarcoding and morphological identification should be seen as complementary rather than competing approaches (Bush et al., 2019; Dulias et al., 2017; Pereira et al., 2021). While freshwater diatoms are frequently used for quality assessment, this BQE remains less frequently applied in marine ecosystems. Previous research has demonstrated that diatoms can serve as indicators of nutrient enrichment in oligotrophic coastal systems, with nitrogen availability identified as a key driver of assemblage composition and diversity. This highlights the potential of

☆ The raw sequencing data generated for this study will be made available at the European Nucleotide Archive under project number PRJEB79159 upon publication.

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https://doi.org/10.1016/j.marenvres.2025.107207
Received 28 February 2025; Received in revised form 30 April 2025; Accepted 3 May 2025
Available online 6 May 2025

diatoms for detecting environmental changes in marine habitats, reinforcing the need for further research on their response to anthropogenic disturbances such as desalination brine discharge (Kafouris et al., 2019).

The application of metabarcoding of diatoms in marine ecosystems is scarcely explored. Compared to freshwater, marine environments pose additional challenges, such as higher diatom biodiversity and more complex ecological interactions, necessitating careful consideration in any metabarcoding studies (Tapolczai et al., 2019). Previous studies in marine ecosystems have found a strong correlation between sediment type and diatom community composition. Sandy sediments tend to support species that are adapted to more dynamic environments, while muddy sediments favour species that thrive in more stable, organic-rich environments (Virta et al., 2019). Nutrient availability seemingly plays a less important role in shaping species diversity compared to factors such as sediment type and light (Scholz and Einarsson, 2015).

The global market for desalination is rapidly growing. Any desalination technology leads to the discharge of a highly saline brine into the marine environment. The discharge of brine often leads to significant impact on the seabed. The ecological impacts of brine discharge will only increase in the years to come, especially in arid parts of the world. Additionally, desalination is increasingly relevant to provide clean water for the growing industry of electrolytic production of hydrogen and synthetic-organic fuels based on excess electricity from green energy sources, e.g. wind farms. Desalination plants that discharge brine directly at sea introduce variability in water quality, such as salinity fluctuations that can significantly impact the ecosystem. With specific regard to how the brine from RO desalination plants affects diatoms, little is known. Most biodiversity studies conducted at the outfalls of RO desalination plants in the Mediterranean focus on marine invertebrates (Al-Osaimi et al., 2020; Belatoui et al., 2017; de-la-Ossa-Carretero et al., 2016a, 2016b, Grammatiki et al., 2025), seagrasses (Blanco-Murillo et al., 2023; Gacia et al., 2007; Xevgenos et al., 2021) or bacteria (Belkin et al., 2015, 2017). Cyprus was selected as a case study of a vulnerable island that over the years has faced increasing water scarcity due to limited natural freshwater sources, water-intensive agriculture, hot summers, and a long-term decline in rainfall (Giannakopoulos et al., 2010; Tsiourtis, 2004). Its heavy reliance on desalination to meet freshwater supply, and its exposure to the Lessepsian migration of species tolerant to increased salinity and temperatures, make the marine environment of Cyprus particularly vulnerable to the environmental impacts of brine discharge. The shallow waters of Cyprus are home to diverse ecosystems, featuring extensive seagrass meadows of *Posidonia oceanica* and *Cystoseira* forests as climax communities (Kocak et al., 2002; Russo, 1997). In industrialised zones, however, opportunistic seaweeds have replaced these ecologically valuable *Cystoseiretum* climax communities (Kletou et al., 2018; UNEP/MAP-SPA/RAC, 2019). Cyprus hosts numerous Non-Indigenous Species (NIS), of which some are also invasive species and Lessepsian migrants from the Indo-Pacific, such as *Caulerpa taxifolia* var. *distichophylla, Synaptula reciprocans*, and the lionfish (*Pterois miles*) (Antoniadou and Vafidis, 2009; Aplikioti et al., 2016).

Cyprus has installed four desalination plants between 1997 and 2004 (Tsiourtis, 2004), with the two major freshwater producers located in Larnaca Bay, in the eastern part of Cyprus. Both plants have comparable daily production capacities of 60,000 m^3 but differ in the brine discharge methods: the plant of Larnaca releases brine via a single underwater pipe, while the plant of Dhekelia employs a submerged multi-diffuser pipe, which increases the brine-mixing zone on the seabed (Argyrou, 1999, 2000; Belatoui et al., 2017; Clark et al., 2018; Torquemada et al., 2009).

This study aims to fill the knowledge gap from the lack of understanding the impact of brine discharge from desalination plants on marine benthic diatoms. This was done by examining the diversity and composition of diatoms near outfalls from seawater Reverse Osmosis desalination plants by using diatom microscopic identification and eDNA as complementary approaches. It is hypothesised that the diversity of diatoms at, and near, brine outfalls will be lower compared to reference points, due to the impact of abiotic variables. It is additionally hypothesised that the impact will be localised at and near the outfalls and that temporal variation will shape the communities towards unique diversity pattern of taxa each season. The combination of the two approaches used in this study will reveal a comprehensive picture of the diatom diversity and community structure and their response to abiotic factors.

2. Materials and methods

2.1. Sample sites and collection

The sampling protocol for the site of Larnaca followed a grid that was used in previous monitoring studies in the area (Argyrou, 1999, 2000), with stations placed at 0, 50, 100, and 200 m from the discharge pipe (33°39.264′ E, 34°52.160′ N), with a reference station (33°39.113′ E, 34°51.393′ N), located at 1400 m upstream and south from the pipe, adjusted to the same isobath. Similarly, at Dhekelia, stations were placed at 0, 50, 100, and 150 m from the discharge pipe (33°45.599′ E, 34°58.773′ N) and a reference point situated 1245 m downstream to the east (33°46.414′ E, 34°58.726′ N). Sampling was conducted on 16–19 May, 29 August – 1 September, 14–17 November 2022, and 6–9 February 2023, to capture temporal variation. Details of the sampling stations used in this study, including coordinates, are the same as previously (Grammatiki et al., 2025).

Sediment samples were collected in triplicate from each sampling station and taken from the undisturbed top 3–4 cm layer of a Van Veen grab (0.05 m^2), homogenised in 50 mL Falcon tubes, and stored at 4 °C. Underwater images of the seabed at 50 m from the brine discharge points illustrate the observed differences in benthic conditions (Fig. 1), where a film of microalgae and an absence of macroscopic organisms were evident.

2.2. Measurement of environmental parameters

Conductivity and salinity measurements of bottom water (1 m from seabed) were conducted on board using the multiparameter instrument HANNA HI98194 (HANNA, UK), apart from Dhekelia in November 2022, due to probe malfunction and Larnaca and Dhekelia in February 2023, due to unavailability of the instrument. Salinity (as Total Dissolved Solids) and conductivity measurements for samples collected during February 2023 from both sites were alternatively performed at an external CYS-CYSAB accredited laboratory, following ISO/IEC 17025:2017 standards. Five hundred mL of seawater was collected from all sampling sites from both the surface and the overlaying water for analysis of dissolved silica following the same ISO.

Approximately 400 mL of top 3–4 cm sediment was collected in plastic jars for measurement of water-soluble Iron, Magnesium and Tin (except May 2022, where total Iron, Magnesium and Tin content was measured instead), and Total Organic Carbon (TOC), Total Kjeldahl Nitrogen (TKN). These measurements were performed at an external CYS-CYSAB accredited laboratory within three days of collection, following ISO/IEC 17025:2017 standards. Overall, eight subsamples from each Petri dish were taken with a shortened syringe (sample volume 1 cm^3). Two of these subsamples were used to determine water content and ash-free dry weight (AFDW) by oven drying at 60 °C, followed by combustion at 500 °C for determination of organic matter content (both for 24 h). Porosity was calculated from the weight loss of a known volume of wet sediment after drying to constant weight at 60 °C. With the third sediment sample, median grain size was determined with a Coulter Counter LS particle size analyser (LaserGranulometer, SediGraph, USA). Size ranges were classified according to an established protocol (Wentworth, 1992) and, in addition, to the phi (φ) scale (Krumbein and Sloss, 1963). Additional pigment analysis was conducted via high-performance liquid chromatography (HPLC). A

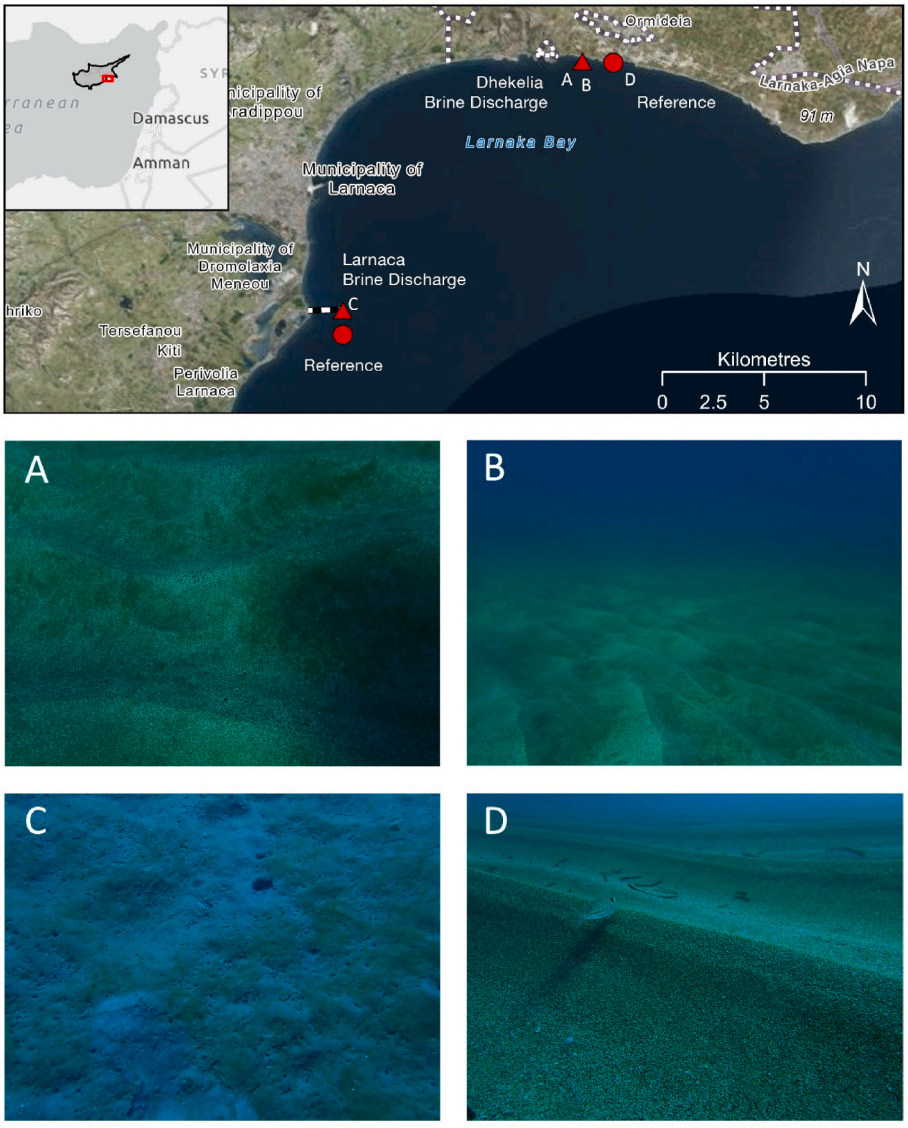

Fig. 1. Underwater images of sampling locations at 50 m from Dhekelia (A, B) and Larnaca (C) brine discharge points, and Dhekelia reference point (D). Images A, B, C show a film of microalgae covering the seabed in brine-affected areas, with an absence of macroscopic organisms. In contrast, the reference site at Dhekelia (D) shows no visible microalgal film and features a natural seabed structure of smooth texture and less organic enrichment. Images courtesy of Myrsini Lymperaki (February 2023).

spectrophotometric method adapted from (Parsons et al., 1984) using Lorenzen's equations (Lorenzen, 1967) was used to ascertain chlorophyll-*a* and phaeopigment content in the fourth sediment subsample. Measurements were conducted following the method described by Brito et al. (2009). Chlorophyll-*a* and phaeopigment concentrations were calculated in mg m^{-2}.

2.3. Microscopic identification of diatom communities

Sediment samples were collected in triplicate from 0 to 50 m from each outfall pipe and their respective reference points for microscopic identification of marine diatom communities. Samples were collected in a similar manner as the eDNA samples, from the top 3–4 cm of undisturbed sediment, placed in Petri dishes, covered in foil and kept at 4 °C until shipping to taxonomic facilities in Iceland. The identification was carried out using light microscopy at a specialised microphytoalgal taxonomy laboratory. Using a modified cleaning protocol (Schrader, 1973; Sabbe and Vyverman, 1991), sediment subsamples were oxidised with hydrogen peroxide (27 %) and acetic acid (99.9 %), cleaned valves were mounted in Naphrax™ (Dr. Thorns, Göttingen, Germany) and slides were analysed and examined by phase contrast light microscopy (Zeiss Axiophot, Oberkochen, Germany).

2.4. DNA extraction and metabarcoding

DNA was extracted from sediment samples using DNeasy PowerSoil Pro kit (Qiagen, USA). The *rbc*L gene, which encodes for the large subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) found in diatoms, was amplified using a primer cocktail (Tapolczai et al., 2019). The primers included Nanopore PCR barcoding overhangs. The concentration of DNA was measured using the Qubit dsDNA HS Assay kit (Invitrogen, USA). The PCR protocol comprised 2 μL of a 5 ng/μL DNA template, 10 μL of a primer mix (with each primer at a final concentration of 400 nM), 12.5 μL of PCR BIO Ultra Mix (PCR BIO Systems Ltd, UK), and 0.5 μL of Nuclease-Free water (Invitrogen, USA). The PCR conditions included an initial denaturation step at 95 °C for 2 min, followed by 35 cycles of 95 °C for 15 s, 54 °C for 15 s, and 72 °C for 1 min. A final extension was conducted at 72 °C for 5 min. Quality control

of randomly selected PCR products was conducted using a TapeStation 2200 with D1000 ScreenTapes (Agilent, USA). Amplicons were purified with CleanNGS (CleanNA, USA) at a sample-to-bead ratio of 0.8, and their concentrations were quantified using the Qubit dsDNA HS Assay (Invitrogen, USA) on a TECAN Infinite F200 PRO plate reader (TECAN, Switzerland).

2.5. Sequencing, bioinformatics and statistics

The final libraries of the *rbc*L barcodes that were generated by PCR were prepared for sequencing following the Nanopore protocol (PBAC96_9069_v109_revQ_14Aug2019). Samples were sequenced on a Mk1B MinION sequencer with a R9.4.1 flow cell for approximately 16 h (Oxford Nanopore Technologies). Raw sequencing data were obtained using live basecalling during the sequencing process, with Guppy v6.5.7 and the R9.4.1 High Accuracy (HAc) model. Demultiplexing and adapter removal were conducted using Porechop v0.2.3 (https://github.com/rrwick/Porechop), with stringent quality filtering settings: discard_middle, –require_two_barcodes and –barcode_threshold 85. The demultiplexed data underwent further quality control using NanoPlot v1.38 and quality-filtered to a specified size range (read length: 150–600 bp) using NanoFilt v2.6.0 (De Coster et al., 2018) with a minimum q-score of 10. Next, the reads were aligned with minimap2 v2.17 (Li, 2018) and polished with Racon v1.3.3 (Vaser et al., 2017). The reads were then clustered into Operational Taxonomic Units (OTUs) at 97 % sequence similarity using VSEARCH v2.13.4 (Rognes et al., 2016). Taxonomy was assigned using AMPtk v1.3.0 (Palmer et al., 2018). Additional manual curation was performed with BLAST (blastn suite), utilising standard databases, while excluding Models (XM/XP) and Uncultured/environmental sample sequences from the search. The search was optimised for highly similar sequences (megablast) (Zhang et al., 2000). Taxonomy was updated to reflect AlgaeBase (Guiry and Guiry, 2025) as of 15th November 2024.

Statistical analysis and data visualisation were performed in RStudio v2022.12.0 + 353 using R v4.3.0 (R Core Team, 2023) with ampvis2 (Andersen, 2004), ggplot2 (Wickham, 2016) and patchwork (Pedersen, 2024). Alpha diversity was measured as Shannon-Wiener H' index and Chao1 richness estimator. Prior to applying statistical tests, normality was tested using Shapiro-Wilk tests. Group comparisons and post-hoc pairwise tests were calculated using Kruskal-Wallis and Dunn's tests with Benjamini-Hochberg adjustment. Environmental parameters and diversity indices are reported as median and interquartile range, to facilitate visual comparison and to account for small sample sizes. Beta diversity calculated based on Jaccard distance was visualised with Principal Coordinate Analysis and Spearman correlations were performed between PCo axes scores and environmental variables. PERMANOVA was calculated with 999 permutations using the adonis2 model from the vegan package (Oksanen et al., 2022).

3. Results

3.1. Physicochemical parameters

At Larnaca sediment conductivity and salinity varied from 55.0 (Interquartile Range 9.4) mS cm^{-1} and 41.6 (IQR 1.9) g L^{-1} at 0 m to 55.0 (IQR 1.8) mS cm^{-1} and 40.2 (IQR 1.0) g L^{-1} at 200 m from the discharge point, with less variability in the measurements suggesting brine dilution within this radius (Table 1). Seasonal extremes were recorded, with salinity ranging from 36.7 g L^{-1} at the surface (reference point, autumn) to 47.0 g L^{-1} at the bottom (50 m, summer). The highest variability of measured conductivity and salinity was consistently recorded at 50 m. Iron concentrations followed a decreasing trend with distance ($P < 0.05$), while TOC was significantly higher at the discharge site compared to both 50 m ($P < 0.01$) and the reference point ($P < 0.001$), indicating brine discharge as a source of organic matter. Grain size and Φ (Phi) values showed sediment coarsening with increasing distance from the pipe. Chlorophyll-*a* and phaeopigment levels increased with distance ($P < 0.001$). All median values and their

Table 1
Median (Interquartile range) physicochemical parameters of sediment at 0 m from the brine discharge pipe to the reference points at Larnaca and Dhekelia Desalination Plants. Kruskal-Wallis tests for differences of parameters were performed among sampling stations and all statistically significant ($P < 0.05$) relationships were tested post-hoc using Dunn's pairwise tests. Statistical significance from Dunn's tests shown as; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Distance from discharge pipe (m)	0	50	100	150	200	Reference
Larnaca						
Conductivity (mS cm^{-1})	55.0 (9.4)	53.7 (11.3)	55.7 (2.3)	NA	55.0 (1.8)	53.9 (1.3)
Salinity (g L^{-1})	41.6 (1.9)	41.3 (5.0)	39.2 (5.5)	NA	40.2 (1.0)	40.4 (3.1)
Silica (mg L^{-1})	1.0 (0.0)	1.0 (0.0)	NA	NA	NA	1.0 (0.5)
Chlorophyll-*a* (μg m^{-2})	0.1 (0.0)	0.1 (0.0)	NA	NA	NA	0.1 (0.1)
Phaeopigment (μg m^{-2})	0.5 (0.3)	0.7 (0.4)	NA	NA	NA	0.9 (0.2)
Iron (mg kg^{-1})	3.2 (1.2)	5.4 (1.7) *	3.1 (1.1)	NA	1.3 (1.8) *	3.1 (1.6)
Magnesium (mg kg^{-1})	344.5 (159.0)	328.5 (142.4)	256.0 (239.3)	NA	332.0 (125.0)	213.0 (210.6)
Total Kjeldahl Nitrogen (%)	0.2 (0.2) ***	0.1 (0.0)	0.1 (0.0)	NA	0.1 (0.0)	0.1 (0.0) ***
Total Organic Carbon (%)	14.1 (16.9) ***	4.0 (1.5) **	3.4 (1.4)	NA	3.6 (0.9) **	3.3 (0.1) ***
Median Grain size (μm)	108.0 (1.2)	111.0 (1.0)	NA	NA	NA	113.0 (0.0)
Φ	3.1 (0.0)	3.1 (0.0)	NA	NA	NA	3.1 (0.0)
Porosity (%)	20.3 (0.2)	20.8 (0.4)	NA	NA	NA	21.3 (0.0)
Water content (% DW)	1.2 (0.0)	1.2 (0.0)	NA	NA	NA	1.2 (0.0)
Dhekelia						
Conductivity (mS cm^{-1})	74.5 (13.3)	56.3 (11.9)	56.5 (2.6)	51.9 (3.1)	NA	51.0 (9.8)
Salinity (g L^{-1})	42.0 (5.8)	41.4 (6.4)	40.8 (7.0)	40.9 (0.2)	NA	34.5 (5.7)
Silica (mg L^{-1})	1.0 (0.0)	1.0 (0.5)	NA	NA	NA	1.0 (0.0)
Chlorophyll-*a* (μg m^{-2})	0.0 (0.0)	0.0 (0.0)	NA	NA	NA	0.1 (0.0)
Phaeopigment (μg m^{-2})	0.1 (0.2)	0.4 (0.4)	NA	NA	NA	0.4 (0.4)
Iron (mg kg^{-1})	1.0 (0.3) **	0.8 (0.3)	0.9 (0.1)	1.4 (0.8)	NA	0.6 (0.5) **
Magnesium (mg kg^{-1})	260.5 (200.1)	195.0 (353.6)	289.5 (190.9)	281.5 (134.2)	NA	291.5 (284.8)
Total Kjeldahl Nitrogen (%)	0.1 (0.0)	0.1 (0.0)	0.1 (0.0)	0.1 (0.0)	NA	0.1 (0.0)
Total Organic Carbon (%)	2.8 (0.6) *	2.6 (0.4)	2.8 (0.3)	3.0 (0.9) *	NA	2.6 (0.6) *
Median Grain size (μm)	513.0 (3.0)	522.0 (1.0)	NA	NA	NA	533.0 (1.0)
Φ	0.9 (0.0)	0.9 (0.0)	NA	NA	NA	0.9 (0.0)
Porosity (%)	49.0 (0.3)	49.2 (0.2)	NA	NA	NA	49.3 (0.1)
Water content (% DW)	3.3 (0.5)	3.3 (0.4)	NA	NA	NA	3.2 (0.5)

interquartile ranges are presented in Table 1.

At Dhekelia, conductivity was highest at 0 m (74.5, IQR 13.3 mS cm^{-1}) and decreased to 51.0 (IQR 9.8) mS cm^{-1} at the reference point. Salinity showed a more complex pattern, peaking at 0 m (43.4, IQR 5.8 g L^{-1}), decreasing at 100 m, and increasing at 150 m before reaching its lowest average at the reference point (34.5, IQR 5.7 g L^{-1}). Salinity showed temporal variation, ranging from 31.6 g L^{-1} (winter, reference point) to 47.6 g L^{-1} (summer, 50 m). Iron concentrations decreased overall but peaked at 150 m, where they exhibited the highest variability. TOC levels were generally lower than at Larnaca but peaked at 150 m. Dhekelia sediments were coarser, with higher porosity and water retention compared to Larnaca.

Overall, Larnaca sediments were finer with lower porosity, while Dhekelia waters had higher salinity and conductivity. Larnaca showed a more linear response to brine discharge compared to Dhekelia, which displayed complex variations with distance.

3.2. Microscopy

Cell density exhibited a peak in spring (12.3 and 8.1 × 10^4 cells cm^{-2} at Larnaca and Dhekelia, respectively) and was the lowest in winter (6.5 and 3.8 × 10^4 cells cm^{-2} at Larnaca and Dhekelia, respectively). Alpha diversity (Shannon-Wiener and Chao1) was measured to be highest in spring and lowest in autumn for both sites, with the exception of Chao1 for Larnaca in spring, which had a low median but the highest variability. The effect of temporal variation on the alpha diversity was greater in the diatom communities of Dhekelia compared to Larnaca. Dunn's post-hoc tests for Dhekelia revealed differences between spring and autumn ($P < 0.001$) and between autumn and winter (Shannon-Wiener $P < 0.01$ and Chao1 $P < 0.05$), but no significant differences within seasons in Larnaca (Fig. 2).

Diversity in the sediment increased along the distance from the discharge pipe, with the highest average at the reference point (Fig. 2). The spatial effect on the alpha diversity was more prominent than the temporal effect. Shannon-Wiener and Chao1 were the most different between the sampling point at 0 m and the reference points for both sites ($P < 0.001$ for Larnaca and $P < 0.01$ for Dhekelia), and only slightly different between 0 and 50 m ($P < 0.05$ for both sites).

In Larnaca sediments, Principal Coordinate Analysis revealed that magnesium ($R^2 = 0.25$, $P < 0.01$) and iron ($R^2 = 0.22$, $P < 0.05$) concentrations were strong drivers of the variation observed in the diatom communities (Fig. 3A). Spearman correlations between PCo1 and the environmental variables, to further separate the biological variation on the first principal coordinate, showed Total Kjeldahl Nitrogen (TKN) and Total Organic Carbon (TOC) as significant drivers of the variation (rho = −0.607, $P < 0.001$ and rho = −0.603, $P < 0.001$, respectively). At Larnaca, 15.7 % of the beta diversity variance was due to temporal variation, although this was not statistically significant ($R^2 = 0.157$, $P = 0.08$) and that distance did not explain a significant portion of the observed variation ($R^2 = 0.008$, $P > 0.05$). A moderate effect of distance from the discharge pipe on the beta diversity was revealed from the PERMANOVA analysis of the Jaccard distance matrix ($R^2 = 0.16$, $P < 0.05$), as well as a small but significant effect of Total Kjeldahl Nitrogen ($R^2 = 0.05$, $P < 0.05$). Salinity measurements in overlaying water, which was used as proxy for porewater, explained a small portion of the variance ($R^2 = 0.05$), but this relationship was not significant ($P = 0.06$).

In the sediments of Dhekelia, Goodness of Fit testing (*envfit*) showed that none of the measured environmental variables significantly correlated to the beta diversity of the communities (Fig. 3B), however, further examination of the variation along the vertical axis (PCo2) showed the

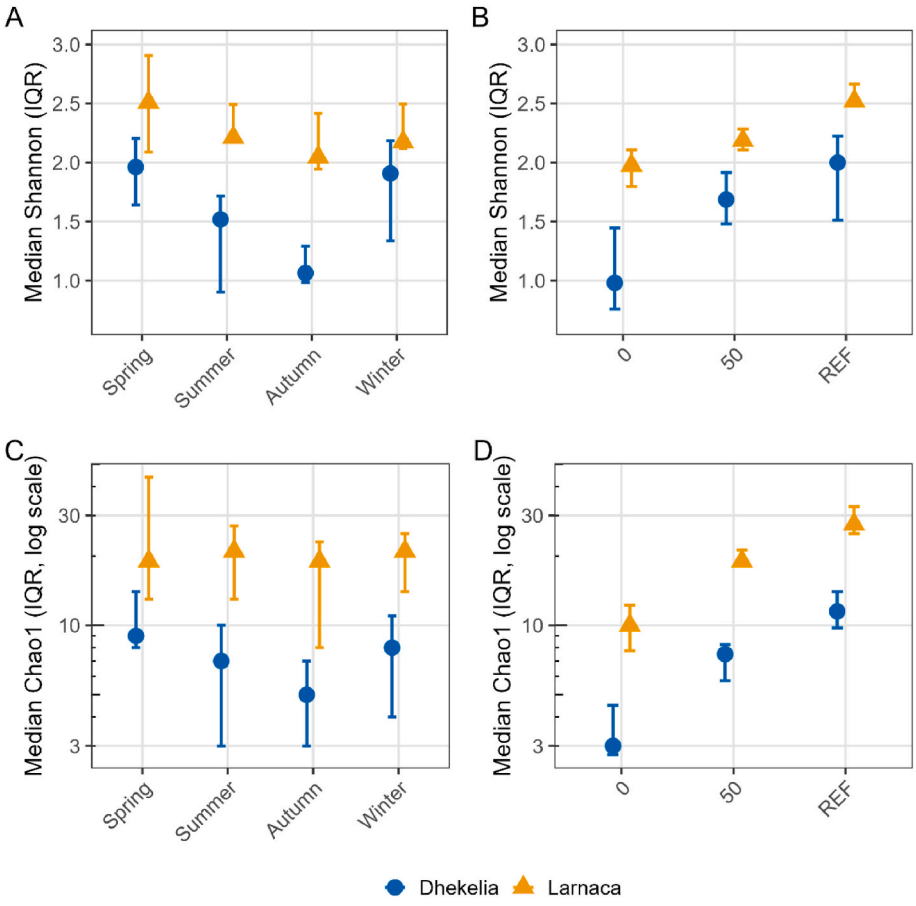

Fig. 2. Temporal and spatial variation of the alpha diversity of diatoms from microscopic identification, shown as Shannon-Wiener H' and Chao1 indices Median, with whiskers indicating the interquartile range.

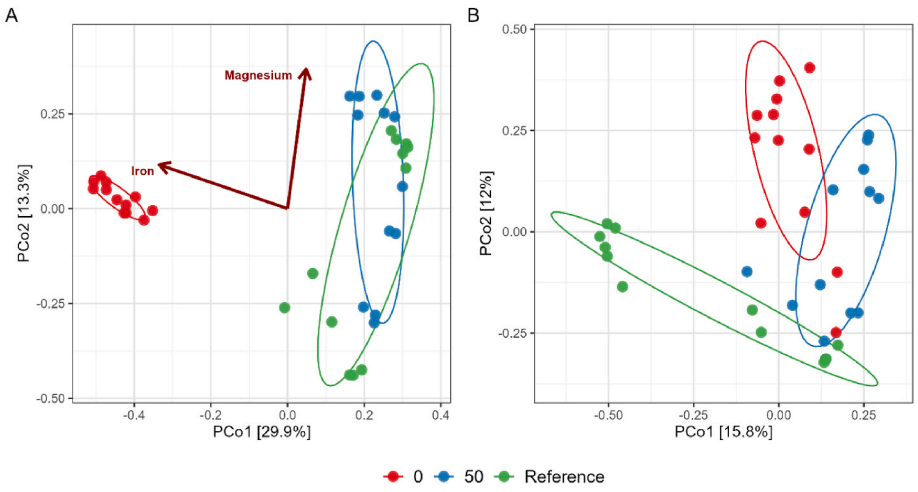

Fig. 3. Principal Coordinates Analysis (PCoA) based on the Jaccard distance measure of microscopy samples from A. Larnaca and B. Dhekelia. The ellipses are drawn around samples from the same sampling point at 70 % of the point dispersion.

iron content as a significant driver of the variation (Spearman correlation, rho 0.45, $P < 0.01$). Distance from the discharge pipe explained 20.4 % of the variation ($P < 0.05$) and temporal variation did not have an effect on the diversity variation ($R^2 = 0.15$, $P > 0.05$). From the PERMANOVA analysis, temporal variation and iron concentration had a significant effect on the variation ($R^2 = 0.12$ and 0.06, $P < 0.001$).

A total of 93 diatom species and subspecific taxa were found in the sampling sites. In particular, 61 epipelic, 13 epipsammic, 5 epiphytic, 7 pelagic and 7 diatom species with a mixed life-form, respectively, were identified from literature.

The order Naviculales displayed the greatest abundance in comparison to other major groups, with 27 species and a total annual abundance of 173.57 and 181.25 $\times 10^3$ cells cm^{-2} at Larnaca and Dhekelia, respectively. Achnanthales (8 species), Thalassiophysales (6 species) and Bacillariales (22 species) were further major contributing groups to the diatom diversity. The diatom orders Biddulphiales (1 species), Cymbellales (1 species), Licmophorales (1 species), Lithodesmiales (1 species), Paraliales (1 species), Rhopalodiales (1 species), Mastogloiales (2 species), Toxariales (2 species) and Triceratiales (1 species) were minor contributors to the distribution within the communities (Table 2).

The highest relative cell densities of the genera *Caloneis*, *Diploneis*, *Gyrosigma*, *Pinnularia* and *Pleurosigma* were recorded in Larnaca, at 50 m and at the reference point, but were absent from the discharge point (0 m). At Dhekelia, these genera were only found in traces at 0 and 50 m and at the reference point. At Larnaca, the highest relative cell densities were observed for *Achnanthes* spp. at the reference point (19.9 %), while *Cocconeis* spp. exhibited the highest relative proportions at 50 m (up to 7.6 %). Only *Achnanthes longipes* and *Cocconeis hoffmanni* were present year-round, with particularly high occurrences in autumn. A significant feature of the community structure at Dhekelia was the continuous increase of *Navicula* spp. from 0 m to the reference, with small species, such as *Navicula gregaria* and *Navicula phyllepta* reaching the highest relative proportions at the reference, particularly in winter (up to 89.8 %).

Overall, at both Larnaca and Dhekelia, 9 species of the Naviculales were observed over the whole sampling period and these exhibited differences in seasonal occurrence: *Navicula gregaria*, *Navicula phyllepta*, *N. complanata* and *Diploneis bombus* (Fig. 4E) had their highest recorded abundances in spring and followed an increasing abundance trend in both locations, while *Pinnularia claviculus*, *Gyrosigma acuminatum* and *Caloneis liber* were highly abundant during summer, and *Halamphora coffeaeformis* and *Gyrosigma attenuatum* recorded their highest abundances during autumn and winter, respectively. From the order of Bacillariales, *B. paxillifera* (Fig. 4A) was only present at the reference point of Larnaca, *T. punctata* (Fig. 4D) was found at 50 m from Larnaca. From the order of Paraliales, *P. sulcata* (Fig. 4G) was the most prominent at the discharge point of Dhekelia.

3.3. Sequencing data

A total of 431,556 high quality reads were generated across 185 samples. After stringent data filtering and curation, a total of 322,637 reads and 5154 OTUs were kept for alpha and beta diversity analysis. Of these OTUs, 71.4 % (3836 OTUs) could be assigned a family, 71.7 % (3693 OTUs) could be assigned a genus and 20.7 % (1069 OTUs) could be assigned to a species.

Alpha diversity (Shannon-Wiener and Chao1 index) of the diatom communities detected in the eDNA samples was the highest in spring and decreased by winter for Larnaca, and by autumn for Dhekelia (Fig. 5), with significant differences among all seasons ($P < 0.001$), except between autumn and winter ($P > 0.05$).

However, in contrast to the results from the microscopic identification (see Fig. 2), the effect of spatial variation was not as significant as temporal variation in the eDNA samples and it did not explain any of the alpha and beta diversity variation among the sampling points ($P > 0.05$).

The Principal Coordinate Analysis of eDNA sediment samples from Larnaca showed a strong effect of temporal variation on the beta diversity ($R^2 = 0.419$, $P < 0.001$). The *env*fit model fitted on the Jaccard distance in PCoA, showed no significant Goodness of Fit of environmental variables with the PCo1 and PCo2 axes (Fig. 6A), however, a negative correlation of the variation on PCo1 with salinity (rho −0.428, $P < 0.01$) and positive correlation of PCo1 with magnesium (rho 0.603, $P < 0.001$) were revealed with Spearman correlations. Distance from the discharge pipe had no effect on the beta diversity ($R^2 = 0.03$, $P > 0.001$).

At Dhekelia, Goodness of Fit testing showed a negligent effect of distance to the discharge point ($R^2 = 0.07$, $P = 0.07$), and no temporal effect ($R^2 = 0.03$, $P > 0.05$), salinity ($R^2 = 0.06$, $P > 0.05$) or any other environmental variable on the variation (Fig. 6B). However, salinity had a significant correlation with the variation on the PCo1 and PCo2 axis (rho = 0.28, $P < 0.05$ and rho = −0.3, $P < 0.05$ respectively), and TOC with the PCo1 (rho = 0.28, $P < 0.05$) and PCo2 (rho = −0.412, $P < 0.01$), and iron with PCo2 (rho = 0.259, $P = 0.05$). PERMANOVA analysis showed no significant effects of any of the environmental variables ($P > 0.05$) on the overall variation.

3.4. Community composition from eDNA metabarcoding

DNA metabarcoding from the 0 and 50 and sampling stations and the

Table 2
Temporal diatom abundances (orders) expressed as Mean values (±SD) 10^3 cells cm^{-2} identified through microscopy.

Diatoms	Spring	Summer	Autumn	Winter	Total
Larnaca					
Achnanthales	10.63 ± 1.92	9.42 ± 2.25	12.53 ± 1.92	8.25 ± 0.81	40.83
Bacillariales	27.75 ± 2.01	13.30 ± 0.85	8.74 ± 0.52	5.83 ± 1.16	55.62
Biddulphiales	0.01 ± 0.01	NA	NA	NA	0.01
Cymbellales	NA	0.02 ± 0.01	0.05 ± 0.01	NA	0.07
Eunotiales	0.03 ± 0.02	NA	0.03 ± 0.01	NA	0.06
Fragilariales	5.70 ± 0.25	0.76 ± 0.21	1.67 ± 0.59	1.23 ± 0.26	9.36
Licmophorales	0.33 ± 0.08	0.34 ± 0.05	NA	NA	0.67
Lithodesmiales	0.21 ± 0.05	NA	0.02 ± 0.01	NA	0.23
Mastogloiales	0.01 ± 0.01	NA	NA	NA	0.01
Melosirales	1.94 ± 0.15	1.70 ± 0.01	4.03 ± 0.22	6.77 ± 1.42	14.44
Naviculales	38.40 ± 6.45	71.73 ± 9.53	34.9 ± 3.61	28.54 ± 5.26	173.57
Paraliales	0.03 ± 0.02	NA	NA	NA	0.03
Rhopalodiales	1.90 ± 0.31	1.11 ± 0.15	4.14 ± 2.03	3.56 ± 0.18	10.71
Striatellales	6.44 ± 1.52	12.90 ± 4.32	0.08 ± 0.04	0.07 ± 0.15	19.49
Surirellales	4.81 ± 0.05	2.15 ± 0.27	0.44 ± 0.12	0.28 ± 0.13	7.68
Thalassiophysales	15.50 ± 4.01	12.60 ± 3.05	6.20 ± 2.23	2.70 ± 0.11	37.0
Thalassiosirales	5.44 ± 1.39	0.71 ± 0.15	7.03 ± 1.65	7.54 ± 1.30	20.72
Toxariales	3.49 ± 0.52	0.07 ± 0.04	0.06 ± 0.05	NA	3.62
Triceratiales	NA	1.15 ± 0.09	0.50 ± 0.25	NA	1.65
Total	122.62	127.96	80.42	64.77	395.77
Dhekelia					
Achnanthales	17.27 ± 5.03	15.48 ± 3.36	8.26 ± 4.22	5.31 ± 2.62	46.32
Bacillariales	5.16 ± 2.0	3.81 ± 0.84	0.25 ± 0.13	0.31 ± 0.01	9.53
Biddulphiales	0.02 ± 0.01	NA	NA	NA	0.02
Cymbellales	NA	0.01 ± 0.01	0.01 ± 0.01	NA	0.02
Eunotiales	NA	NA	NA	NA	NA
Fragilariales	0.29 ± 0.11	0.18 ± 0.15	NA	0.01 ± 0.01	0.48
Licmophorales	NA	0.03 ± 0.01	NA	NA	0.03
Lithodesmiales	NA	NA	NA	NA	NA
Mastogloiales	NA	NA	NA	NA	NA
Melosirales	NA	NA	NA	NA	NA
Naviculales	44.6 ± 6.15	51.2 ± 8.32	55.3 ± 4.17	30.15 ± 3.23	181.25
Paraliales	0.28 ± 0.13	NA	NA	NA	0.28
Rhopalodiales	2.86 ± 1.32	0.70 ± 0.09	0.02 ± 0.01	NA	3.58
Striatellales	0.30 ± 0.15	0.16 ± 0.08	NA	NA	0.46
Surirellales	0.27 ± 0.16	NA	NA	NA	0.27
Thalassiophysales	6.43 ± 2.04	4.77 ± 2.15	1.25 ± 0.57	1.78 ± 0.61	14.23
Thalassiosirales	3.46 ± 1.95	0.62 ± 0.33	0.64 ± 0.14	0.75 ± 0.25	5.47
Toxariales	NA	NA	NA	NA	NA
Triceratiales	NA	0.25 ± 0.10	NA	NA	0.25
Total	80.94	77.21	65.73	262.19	262.19

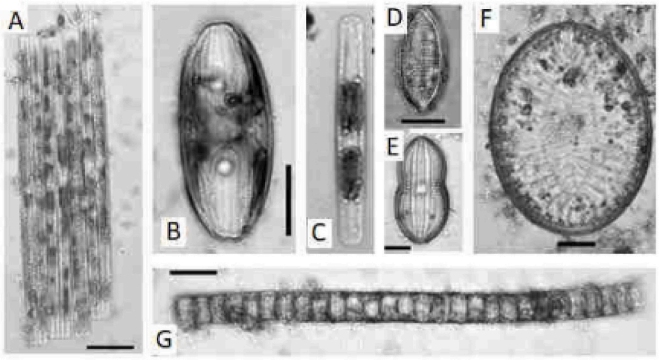

Fig. 4. Pictures of some of the diatoms observed during the first screening of the samples using the inverted microscope. A) *Bacillaria paxillifera* (colony), B) *Amphora proteus*, C) *Nitzschia distans*, D) *Tryblionella punctata*, E) *Diploneis bombus*, F) *Navicula fastuosa* and G) *Paralia sulcata* (colony).

reference points detected a total of 42 orders (89.4 % of total), and microscopy identified 16 orders (34.0 % of total).

Naviculales, Bacillariales, Achnanthales and Thalassiophysales were the dominant orders observed at both locations, based on relative abundances. Pelagomonadales were found at 0 and 50 m in Larnaca, Rhizosoleniales were only present at 50 m at Larnaca in spring. Stephanodiscales and Rhizosoleniales were only detected in the reference point of Dhekelia in winter. Contrary to the results from microscopy (Table 2), Mastogloiales and Melosirales were detected at both locations.

At genus level, *Aureococcus* was only found in Larnaca in spring, at 0 and 50 m. *Heterosigma*, *Chromopallida, Triparma* and *Pseudosolenia* were only detected at the Dhekelia reference point in winter (Fig. 7).

A total of 157 diatom species have been detected using metabarcoding. Species *Aureococcus anophagefferens* and *Pleurochloridella botrydiopsis* were only found in Larnaca in spring and in summer, respectively. *Heterosigma akashiwo*, *Chromopallida australis, Halamphora hyaline, Triparma mediterranea* and *Pseudosolenia calcar-avis* were only found in the reference point of Dhekelia and only in the winter. Within the samples from Larnaca, *Cymbellonitzschia banzuensis* was present only in the reference point of Larnaca in the summer.

At genus level, the majority of genera identified with microscopy (23.6 % of total) were also detected with metabarcoding (73.4 % of total, with undetected *Caloneis, Fragilaria, Frustulia, Grammatophora, Paralia, Rhopalodia, Surirella, Synedra, Toxarium, Tropidoneis*) (Fig. 7). The two methods identified eight common species, *Thalassiosira profunda, Nitzschia sigma, Nitzschia sigmoidea*, *Nitzschia longissima, Amphora helenensis, Bacillaria paxillifera, Entomoneis alata, Gyrosigma acuminatum* and *Cylindrotheca closterium*.

Nitzschia sigma, Entomoneis alata and *Bacillaria paxillifera* were only found at the reference point of Larnaca with the microscopy method, but metabarcoding revealed its presence in all sampling points.

4. Discussion

This study is the first to examine diatom communities at RO desalination plant outfalls using both traditional microscopy and DNA metabarcoding. Integration of these two methods enables the acquisition of a more complete picture of the diversity of diatoms. Our findings reveal

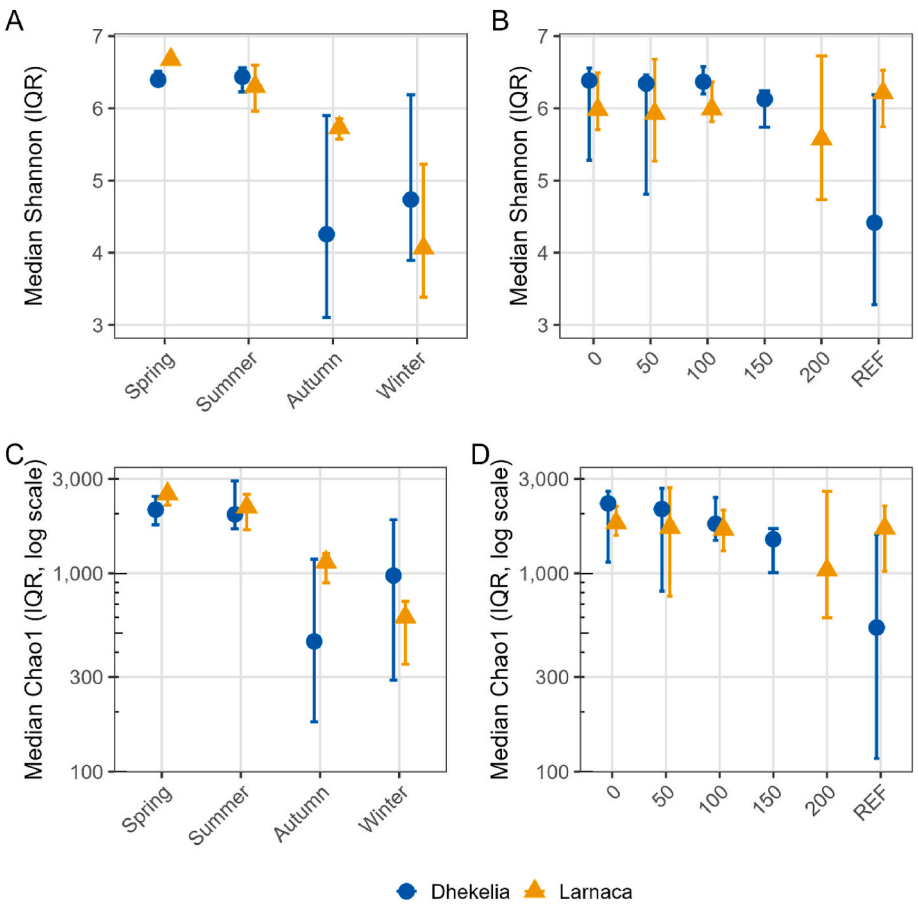

Fig. 5. Temporal and spatial variation of the alpha diversity of diatoms from eDNA metabarcoding, shown as Shannon-Wiener H' and Chao1 indices Median, with whiskers indicating the interquartile range.

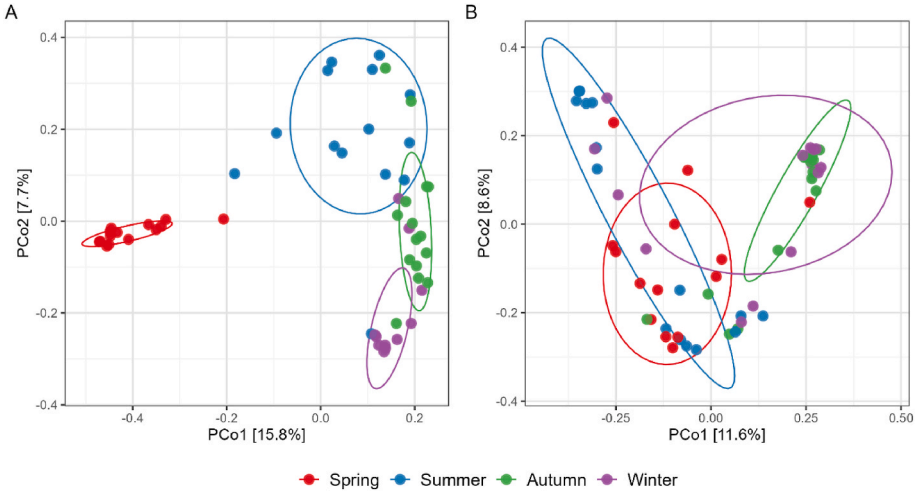

Fig. 6. Principal Coordinates Analysis (PCoA) based on the Jaccard distance measure for A. Larnaca and B. Dhekelia temporal patterns. The ellipses are drawn around samples from the same sampling point at 70 % of the point dispersion.

significant temporal as well as spatial variation of diatom communities, driven by increased organic deposits and nutrients and altered sediment characteristics near the brine outfalls. Microscopy captured fine-scale spatial variation, whereas metabarcoding revealed strong temporal patterns, emphasising the benefits of a synergistic multi-approach methodology.

Despite the functional importance of benthic diatoms in coastal marine ecosystems, little is known about the environmental factors driving their community structure and composition. The focus of previous studies was mainly on ecosystems characterised by tidal flats (Agatz et al., 1999), estuaries and brackish waters such as the Venice lagoon (Facca and Sfriso, 2007) or extreme environments (gas and thermal vents in the Aeolian Islands, Rogelja et al. (2016)). In such systems, salinity and light can play a significant role in driving benthic diatom assemblage structure besides currents. However, primary producers at mid-latitudes tend to be less limited by light and more by the

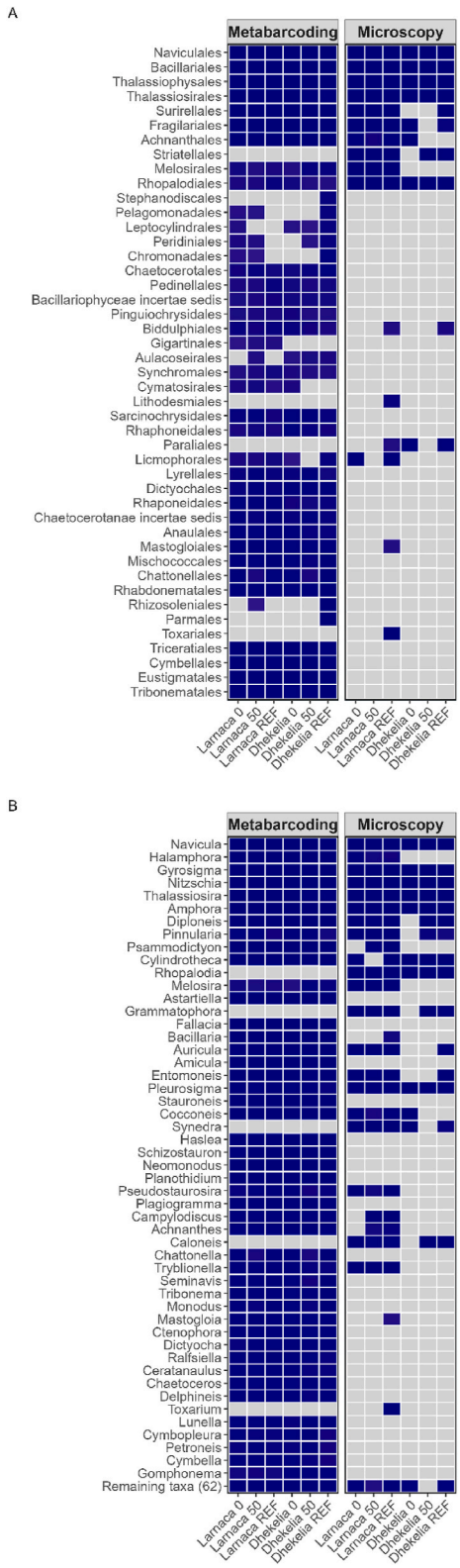

Fig. 7. Heatmap of presence/absence of the orders detected via metabarcoding and microscopy and (B) of the 50 genera with the highest relative abundance and at the two sites. Presence Absence.

availability of nutrients.

In this context, a recent study assessed the efficiency of benthic diatoms as bioindicators of nutrient enrichment in oligotrophic coastal systems, by investigating the effect of different physicochemical conditions and nutrient concentrations on the assemblage composition, diversity and individual species populations (Kafouris et al., 2019). The main drivers of assemblage composition, diversity and biomass of diatoms were observed to be nitrogen concentration and its temporal and spatial changes. While this study did not account for sediment composition, which depends on currents and deposition of finer organic rich material, the findings highlight the potential of diatoms used as bioindicators to account for nutrient pollution. This emphasises the relevance of our study in examining the impact of desalination brine on the diatom assemblages.

4.1. Physicochemical parameters at and around RO brine discharge points

The analysis of physicochemical parameters at Larnaca and Dhekelia revealed distinct environmental gradients at both sites, driven by differences in brine discharge methods and local hydrological conditions. At Larnaca, brine discharge led to pronounced gradients in total organic carbon (TOC), grain size, and salinity, while Dhekelia exhibited more homogeneous conditions, which could be explained by its diffuser pipe system. These differences were reflected in the diatom communities, with spatial variations being more prominent compared to temporal changes.

Diatom diversity increased with distance from the discharge points, suggesting less brine-related stress. At Larnaca, elevated TOC and nutrient enrichment near the outfalls influenced community composition, consistent with a previous study linking nutrient loading to reduced diversity and a shift toward opportunistic species (Brailsford et al., 2019). Dhekelia, with coarser sediments and lower organic enrichment, displayed weaker spatial gradients. This indicates that the responses to brine discharge are influenced by conditions specific to the site. In contrast, Larnaca shows more prominent gradients, while Dhekelia shows less prominent and more homogeneous gradients. The pattern observed at Dhekelia may also be explained by the greater dilution of brine discharge at Dhekelia, which is facilitated by the multi-diffuser pipe.

4.2. Environmental stressors affecting diatom communities at desalination brine outfalls identified via microscopy

The assessment of microphytobenthic diatom communities at Larnaca and Dhekelia sediments through microscopic identification reveals mostly spatial, but also temporal variations in diversity. Overall, it was observed that the diatom abundance was the lowest during winter before peaking in spring blooms, an effect more prominent at Dhekelia, whereas diatom abundance at Larnaca remained more stable across the sampling seasons. Spatial variation, however, was more prominent than temporal variation in the two sites. Measured alpha diversity increased with distance from the discharge point. Brine-associated nutrient enrichment by nitrogen and organic carbon at Larnaca resulted in relatively low chlorophyll-*a* which affected the local diatom communities, as shown in previous studies (Chintapenta et al., 2018; Virta et al., 2019). While most studies on diatom diversity and environmental variables relate to freshwater ecosystems (Bere et al., 2013; Leboucher et al., 2019), they all confirm that nutrient enrichment affects diversity negatively, due to the loss of specialists and replacement by opportunistic species. Alpha diversity was the highest at the reference points and beta diversity analysis confirmed for Larnaca the distinction of the communities at the outfalls, with magnesium and iron content, TKN, TOC as strong environmental stressors. While alpha diversity at Dhekelia was the highest at the reference point, there were no environmental stressors that could immediately explain the variation in beta diversity. This could be due to the differences in local conditions due to sediment characteristics or currents. In any case, given that the two desalination plants operate on the same production level (Xevgenos et al., 2021), it is evident that the effects of brine discharge as observed

via microscopic identification are site-specific. A previous study at Larnaca on the impact of brine on the health of seagrass meadows showed deterioration of their physiology near the outfalls (Xevgenos et al., 2021) and revealed localised impact up to 150 m from the discharge, which is also confirmed by the data presented here.

4.3. Environmental stressors affecting diatom communities at RO desalination brine outfalls detected by eDNA metabarcoding

Metabarcoding revealed temporal variation across seasons as the primary driver of community composition at both sites, rather than spatial variation or environmental factors. Observed alpha diversity of the communities identified by eDNA analysis at Larnaca was the highest in spring, declining in the cold season, but at Dhekelia, diversity peaked in summer and was the lowest in autumn. This discrepancy is likely attributed to the sensitivity of the technique to the environmental DNA signal from static benthic organisms as well as dynamic pelagic organisms that move freely, allowing a broader taxonomic coverage. This is supported by our results where eDNA identifications showed a low resolution at the species level (20.5 %), however, it detected a greater number of taxonomic groups, and a greater number of epiphytic and pelagic species compared to epipelic and epipsammic ones. Contrary to the microscopy results, beta diversity observed from the metabarcoding data did not reveal spatial variation as a significant driver, highlighting the importance of combining spatial with temporal variation in analysing the beta diversity of complex ecosystems, such as coastal environments, where reduced primary productivity or changes in sediment conditions (Douglas et al., 2019; Bellino et al., 2019).

The taxonomy results from metabarcoding were complementary to the results from microscopic identification, with an overlap of >70 % of genera being observed with both methodologies.

4.4. Description of diatom communities at RO desalination brine outfalls as derived from both methodologies

Naviculales is the most prominent order observed across both Larnaca and Dhekelia and comprises several potential bioindicators of ecological health. *Navicula* spp., previously described to be tolerant to environmental stressors (Kelly and Whitton, 1995) was abundant at all sites. At Larnaca, the increasing abundance of *Achnanthes* and *Cocconeis* (Falasco et al., 2019; Rimet and Bouchez, 2012) along the distance gradient suggest less impact of brine further from the pipe. In Dhekelia, *Cylindrotheca* spp. dominate the discharge point and *Navicula* was dominantly present at the reference point, indicating differences in environmental conditions compared to Larnaca, which is supported by the measured physicochemical parameters. The genera *Navicula*, *Halamphora* and *Nitzschia* were present at both locations, indicating their resilience and ability to adapt to nutrient enrichment, as well as temporal changes. *Navicula gregaria* and *Nitzschia sigma* have been previously shown to be tolerant of pollution from sewage discharge and to be highly mobile (Agatz et al., 1999). *Paralia sulcata,* a species that consists of thick silicified walls, also forms colonies and was observed at the discharge point of Dhekelia, as well as the reference point (Fig. 4). This species' morphology as well as behaviour facilitate its tolerance of nutrient enrichment (Mosquera et al., 2023).

The unique taxa found at each location (E.g. *Psammodictyon* at Larnaca and *Cylindrotheca* at Dhekelia, respectively) suggest site-specific communities and reinforce the idea that the two sites have different hydrogeological conditions.

A study of the impact of various anthropogenic activities on the riverine diatoms of Cyprus gave useful context on the diatom communities and can reflect some of the impact of brine on the marine diatom communities (Pissaridou et al., 2021). The study highlighted the effect of nutrient loads on perennial rivers, contrary to the effect on intermittent rivers, that better reflect the dry climate of inland Cyprus. Tolerant genera of *Navicula* and *Nitzschia* were most abundant in the impacted streams, while indicators of good ecological status, such as *Achnanthidium minutissima*, were found mostly in intermittent streams. Even though the ecological and geographical context is different, these findings are still relevant to the results of the present study, as they show how diatom assemblages may adapt to the changing environmental stressors.

4.5. Methodological complementarity

The results from this study highlight that conventional morphological methods and eDNA metabarcoding rarely provide identical results. Typically, differences are explained by (1) the lack of appropriate reference sequences; (2) the mismatch of molecular specificity of applied DNA barcodes and conventional method specificity; (3) traces of DNA from adjacent sites (upstream) that may be detectable by eDNA are not observed by conventional methods; and (4) diluted traces of target-DNA in the sample are too low to amplify in the metabarcoding PCR, often referred as "The needle in the haystack" problem (Harper et al., 2018). These factors may lead to some taxa observed by eDNA and not by the conventional technique and vice versa. Measuring diatom diversity using microscopic identification and enumeration allowed for only the morphologically intact cells to be accounted for. The cleaning protocol followed included only intact silica diatom valves in the identification process, as opposed to eDNA metabarcoding that accounted for any DNA present, including that of partially broken diatoms and cells carried over from the brine plume, as seen in similar riverine studies comparing these two methodologies (Zimmermann et al., 2015). The osmotic stress and the force of the plume directly at brine discharge points may have contributed to broken diatom walls and hence, which then translated as reduced diversity at outfalls, compared to 50 m from the pipe and the reference point, a trend that was not evident in the eDNA diversity.

Additionally, molecular techniques can differentiate species that are indistinguishable under a microscope. Hence, identical data are not expected from the two different approaches to biodiversity monitoring. This is an expected and common finding seen in numerous other aquatic DNA studies (Bush et al., 2019; Kuntke et al., 2020; Pereira et al., 2021), as well as terrestrial (Kestel et al., 2024). DNA metabarcoding typically provides extensive diversity information beyond the conventional method, from the pool of unidentified sequences. The vast amount of additional and unidentified data obtained from DNA metabarcoding may include organisms, which are also useful as indicators for impact assessment of the studied ecosystem but are currently not included in conventional environmental assessment strategies.

5. Conclusions

The aim of this study was to investigate the diversity and composition of diatoms by conventional microscopy and by eDNA metabarcoding near brine discharges from seawater desalination plants.

The study revealed that the diversity of diatoms, measured by microscopy increased significantly along the distance from the brine discharge. This was not the case for the eDNA metabarcoding method, which did not show a significant difference in diversity along the distance gradient. Increased nutrient enrichment and reduced grain size at brine outfalls contributed to reduced microscopic diversity, especially at Larnaca. This supports our hypothesis that the diversity of diatoms was reduced at brine outfalls, therefore this hypothesis is only accepted for the microscopy dataset. The microscopy results also revealed site-specific impact, which was evident in both sites as distinct communities at the outfalls, we therefore accept our second hypothesis for the microscopy results, but this was not seen as clearly from the metabarcoding results. Lastly, our third hypothesis is supported by both methods, as temporal variation was a significant driver of diversity, with the exception of microscopy diversity at Larnaca.

Metabarcoding and microscopy provided complementary insights

into the diatom communities. Microscopy identified higher taxonomic resolution for sediment-associated species, while metabarcoding captured a broader range of taxa, including pelagic and cryptic species. The results highlight that conventional morphological methods and eDNA metabarcoding rarely provide identical results. However, the complementary results emphasise that more information can be derived when combining the methods for biodiversity impact assessments.

The global market for desalination is rapidly growing and the ecological impacts from brine discharge will only increase in the years to come. Environmental Impact Assessment (EIA) of brine discharge will be an essential part of approval for most new desalination facilities. In our study we have confirmed diatom diversity as a useful biological quality element (BQE) for brine discharge in the marine environment. For future improved impact assessment from brine discharge, we suggest a multi-method approach for biodiversity assessment and inclusion of seasonality in the monitoring scheme.

CRediT authorship contribution statement

K. Grammatiki: Formal analysis. **N. de Jonge:** Formal analysis. **J.L. Nielsen:** Formal analysis. **B. Scholz:** Formal analysis. **E. Avramidi:** Investigation. **M. Lymperaki:** Investigation. **M. Hesselsøe:** Conceptualization, Supervision. **Dimitris Xevgenos:** Funding acquisition. **F.C. Küpper:** Writing – original draft, Supervision, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We are grateful to the European Commission for supporting the activities carried out in the framework of the Horizon 2020 project WATER-MINING (project under grant agreement No. 869474). We would equally like to thank the TOTAL Foundation (Project "Diversity of brown algae in the Eastern Mediterranean"). This work also received support from the Marine Alliance for Science and Technology for Scotland pooling initiative. MASTS is funded by the Scottish Funding Council (grant reference HR09011) and contributing institutions. Work by JLN, NDJ, and MHES was supported by Industrial post doc grant #9066-00046B from Innovation Fond Denmark donated to Aalborg University and NIRAS A/S. We are also grateful to representatives of competent authorities in Cyprus providing logistics and laboratory support, especially the Department of Fisheries & Marine Research, Ministry of Agriculture, Natural Resources & Environment and the Civil Aviation Authority. We would also like to thank Pieter van West (Univ. Aberdeen) for useful discussions within the framework of his role as co-supervisor of KG's PhD, as well as Spyros Sfenthourakis at the University of Cyprus, Nicosia.

Data availability

Data will be made available on request.

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