



## Article

# Unveiling Microalgal Diversity in Slovenian Transitional Waters (Adriatic Sea): A First Step Toward Ecological Status Assessment

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## Abstract

This study presents the first comprehensive assessment of microalgal diversity in two Slovenian transitional waters (TWs): the shallow brackish lagoon of the Škocjanski Zatok Nature Reserve (SZNR) and the Rižana River estuary within the Port of Koper (PK) area. Between 2018 and 2021, water samples collected with a phytoplankton net were analyzed using light and scanning electron microscopy. In total, 240 species from 117 genera were identified in TW, dominated by diatoms and dinoflagellates, surpassing the diversity at a marine coastal station (91 species, 59 genera). Species richness was higher in PK (226) than in SZNR (154), mainly due to dinoflagellates and coccolithophores. Marine taxa predominated along the salinity gradient, with moderate contributions from brackish taxa and few freshwater forms, reflecting both natural and anthropogenic influences. Planktonic taxa dominated at all sites, while benthic forms were abundant in the lagoon, particularly in spring. Thirty-two taxa were recorded for the first time in Slovenian TW, mostly benthic or tychopelagic diatoms. The detection of *Coolia monotis* and five cyanobacterial genera with potentially harmful traits highlights the role of TW as an ecological interface. The taxonomic sufficiency analysis showed that the order level is sufficient to distinguish transitional from marine assemblages, beyond which ecological information is lost. Overall, this study highlights the importance of detailed taxonomic resolution for detecting microalgal diversity, including harmful and non-indigenous species to ensure robust ecological assessments under the WFD and MSFD directives.

**Keywords:** transitional waters; microalgae; diversity; phytoplankton; harmful algal blooms; non-indigenous species; Adriatic Sea



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## 1. Introduction

Transitional waters (TWs), including estuaries and coastal lagoons, are highly productive ecosystems located at the interface between land and sea. These environments receive nutrient inputs from freshwater, tides, atmospheric deposition and sediments, resulting in fluctuating salinity and other environmental variables [1]. According to the European Communities' Water Framework Directive (WFD 2000/60/EC), TW are naturally stressed water bodies due to the interaction of marine and freshwater influences [2]. The unique hydrodynamic and geomorphologic features of the TW, such as shallow isolated waters, create diverse environmental conditions that promote high biodiversity, enhance ecological value, and support provisional and cultural ecosystem services. In these environments, microalgae together with seagrasses and macrophytes play a crucial role as primary producers. In shallow lagoons, microalgal communities include both benthic and planktonic

forms that overlap functionally. Benthic microalgae, which typically form biofilms at the sediment-water interface, can be resuspended into the water column, further contributing to phytoplankton diversity and primary production [3].

Despite their ecological importance, TW are classified by the IUCN as critically endangered due to anthropogenic impacts such as habitat degradation, aquaculture, eutrophication, pollution, biological invasions and climate change. The decline of these ecosystems has led to the need for restoration and protection measures [4]. The effective implementation of these measures requires accurate ecological assessments as required by the WFD and other international regulations such as the European Habitats Directive (92/43/EEC) and the European Nature Restoration Law (EU) 2024/1991. Since the adoption of the WFD in 2000, significant scientific and management efforts have been undertaken in European countries [5], with positive effects observed in some Mediterranean TW (e.g., [6,7]).

The Mediterranean Sea, with one of the largest areas of coastal lagoons in Europe, is considered a biodiversity hotspot and is home to about 11% of all marine species, of which about 20% are endemic [8,9]. However, the Mediterranean also has the highest number and fastest growing rate of non-indigenous species (NIS) introductions in European seas, with an annual average of 17.7 new species between 2012 and 2017 [10]. While estimates vary in the literature (e.g., around 820 NIS reported by [11]), there is a consensus on the main pathways of introduction: maritime transport, corridors, contaminants and unaided introductions, with climate change further influencing species establishment and dispersal [12]. Not all NIS are inherently harmful; only about 12% of the ~800 marine NIS in the Mediterranean is classified as invasive [13]. According to the IUCN definition, invasive alien species (IAS) are species that have a negative impact on biodiversity, ecosystem functions, socio-economic values and human health. In the context of microalgae, negative impacts arise mainly from species that cause harmful algal blooms (HAB) [14], regardless of whether they are native or non-native.

To address these growing threats, the EU has introduced targeted legislation, including the Marine Strategy Framework Directive (MSFD 2008/56/EC), which aims to achieve and maintain good environmental status (GES) in European marine waters. Under this Directive, NIS are assessed within Descriptor 2 (D2) and HAB under Descriptor 5 (D5—Eutrophication).

While extensive research has focused on microalgal communities in the Mediterranean TW, these environments are still largely unexplored on the Slovenian coast (northeast Adriatic). The available studies, many of which are from coastal lagoons on the western Adriatic coast, show considerable variability in species composition, abundance, seasonal and inter-annual dynamics and ecological preferences [15–19] and the genetic diversity of microalgae [20,21]. These variations are influenced by environmental factors such as salinity and nutrient levels. Some studies have specifically investigated the presence and dynamics of potential HAB species in estuaries [22,23].

The lack of research in Slovenian TW represents a critical gap in the understanding of microalgae diversity and ecology in the region. This is particularly worrying considering that both the MSFD and the WFD require comprehensive monitoring to assess ecological and environmental status.

The main objective of this study was to assess microalgal diversity and habitat preferences along a salinity gradient using a high-frequency sampling scheme and a detailed microscopic approach. Particular attention was paid to the detection of NIS, as required by MSFD Descriptor D2, and to HAB species that can proliferate in these confined environments. The study focused on two Slovenian TW: a coastal brackish lagoon and a heavily modified small estuary exposed to intensive shipping activities.

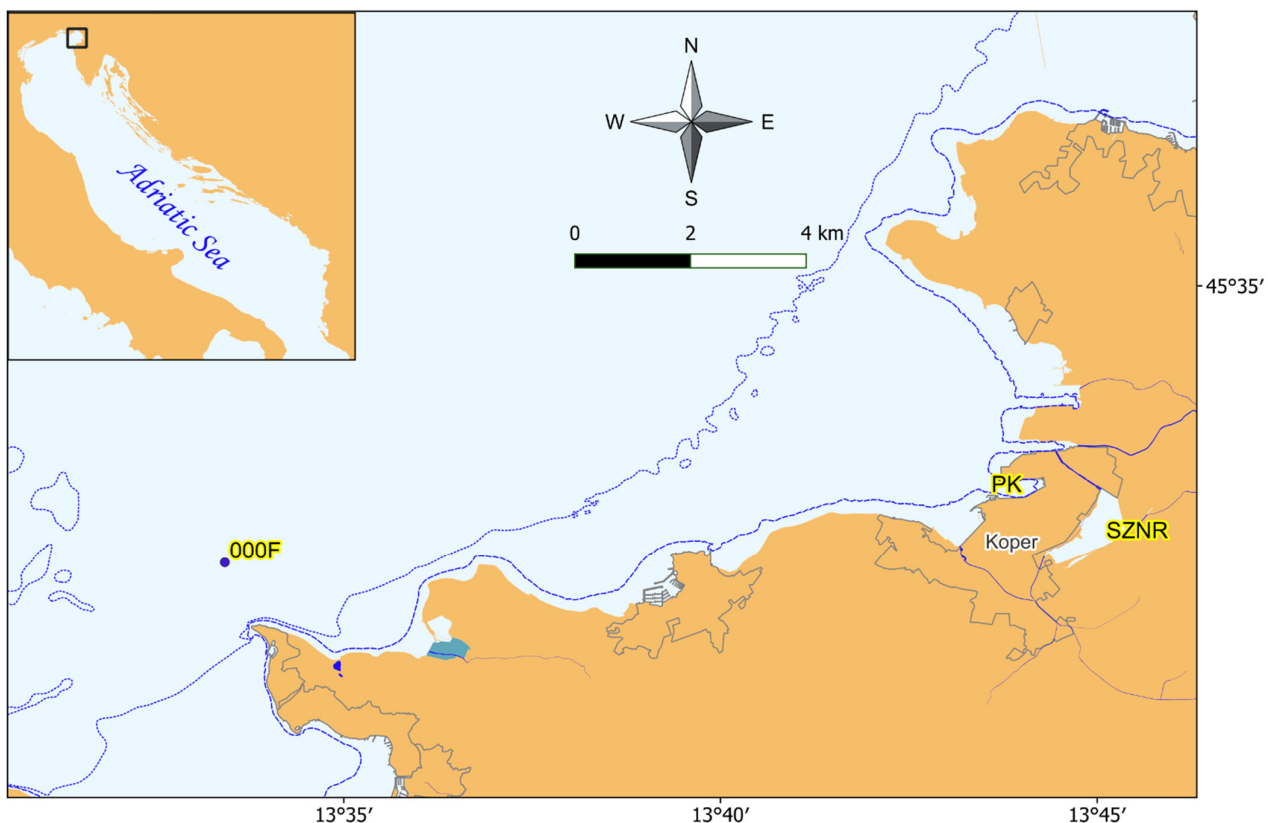
Another objective was to compare the microalgal diversity in these TW with that of a coastal marine station that is regularly monitored for its ecological status in accordance with the WFD. We hypothesized that microalgal diversity differs between these environments, highlighting the need for an expert-led monitoring program. Such a program, aligned with the WFD, has only recently been initiated in the Slovenian TW and has yet to be fully implemented, drawing on results from this study as well.

## 2. Materials and Methods

### 2.1. Description of the Study Area and Sampling Locations

The Slovenian sea is located in the southeastern part of the Gulf of Trieste (GoT), the shallowest (maximum depth ~25 m) and northernmost region of the Adriatic Sea. The GoT is known for having the largest tidal variations in the Mediterranean Sea, with semidiurnal amplitudes reaching up to 30 cm, and the lowest winter temperatures, often dropping below 10 °C [24]. In recent decades, the effects of climate change have become evident through increasing seawater temperatures and alternating drought and flood cycles. These changes have led to nutrient imbalances in coastal waters and a general decline in phytoplankton biomass throughout the northern Adriatic [25]. The Slovenian coastal sea is also subject to significant anthropogenic pressures due to its highly urbanized environment, characterized by intensive tourism, aquaculture industry, and the presence of a major cargo port.

Only a few small transitional areas are found along the Slovenian coast, including the Stjuža lagoon, the Dragonja and Rižana estuaries, and the brackish lagoon of Škocjanski Zatok Nature Reserve. The latter two areas were selected for this study (Figure 1). A detailed location of the sampling sites in these two areas can be found in Supplementary Figure S1.



**Figure 1.** Map of the Slovenian coastal area and adjacent sea, the Gulf of Trieste, showing the locations of transitional waters (PK and SZNR) and the marine monitoring station (000F).

The Škocjanski Zatok Nature Reserve (SZNR) is the largest semi-saline wetland in Slovenia and offers a distinctive blend of brackish and freshwater habitats surrounded by a highly urbanized environment [26]. Before its restoration in 2007, the area was significantly influenced by anthropogenic activities [27]. Approximately three-quarters of the reserve consists of a brackish lagoon, which receives freshwater inflow from the east via a river channel and seawater inflow from the north through artificial sluices connected to the Port of Koper.

The Rižana River is the largest river on the Slovenian coast, with a catchment area of approximately 212 km<sup>2</sup>, predominantly composed of karst rock, and an average discharge of around 4 m<sup>3</sup>/s [28]. Its estuary discharges into the Bay of Koper and forms one of the three basins of the cargo Port of Koper (PK). Here, treated wastewater from a sewage treatment plant mixes with freshwater and seawater, resulting in brackish conditions extending to the middle of the bay.

Station 000F, located at a depth of 21 m and approximately 1 NM off the coast, was selected for comparing transitional waters with the coastal sea (Figure 1). This station represents a reference point for near-natural, minimally disturbed conditions within the GoT and has been designated as a marine reference station for national ecological status monitoring under the WFD.

## 2.2. Sampling and In Situ Measurements

For the qualitative analysis of microalgae in the two transitional waters, SZNR and PK, water samples were collected using a phytoplankton net with a 20 µm mesh size (KC Denmark).

In SZNR, samples were obtained from the deepest part of the lagoon (ca. 1 m depth), specifically from the main channel connecting the lagoon to the coastal sea (Supplementary Figure S1), using a canoe. Sampling was conducted every other month from April 2019 to January 2020 and five times between August 2020 and February 2021 (Supplementary Table S1). Water samples were collected using two horizontal plankton net hauls just below the water surface, with each haul lasting approximately one minute in one direction. Care was taken to avoid overlapping transects for the two hauls.

In PK, three sampling sites were selected: PBS1 and PBS2, located in the first and second basins (maximum depth 14 m), and PBS5 in the anchorage area (depth 18 m) (Supplementary Figure S1). From April 2018 to March 2019, monthly water samples were collected at all three sites. Between October 2020 and May 2021, monthly sampling was limited to PBS2 (Supplementary Table S1). At PBS1 and PBS2, vertical net hauls were conducted, while at PBS5, horizontal hauls were performed at a depth of approximately 2 m along a 120 m long transect.

The diversity and seasonal distribution of microalgae, together with environmental data in the TW, were compared with the data from monthly samplings at station 000F (Figure 1), which is part of the coastal sea monitoring program funded by the Slovenian Environmental Agency—ARSO. Of all sites in the TW, the sampling patterns at 000F and PBS2 were the most synchronized, with comparable sampling frequencies and the longest study period (April 2018 to May 2021, including temporary gaps; see Supplementary Table S1).

Basic environmental parameters, such as temperature and salinity, were measured at PK and station 000F using a fine-scale multiparameter CTD probe (Conductivity Temperature Depth; Sea & Sun Technology GmbH, Trappenkamp, Germany). Because temperature and salinity were not recorded in SZNR, monthly data for these parameters were derived from the publicly available ARSO database (<http://www.arso.gov.si/vode/podatki/>, accessed on 27 May 2025).

### 2.3. Microscopic Analysis

The net samples were collected in the field in opaque containers and fixed with neutralized formalin (final concentration 2%) immediately after arrival in the laboratory and stored in the dark until analysis.

For light microscopy (LM), the sample was thoroughly mixed, and a subsample of 2.5 mL was allowed to settle in a sedimentation chamber for at least two hours. The examination was performed using an Axio Observer Z1 inverted epifluorescence microscope equipped with an integrated AxioCam Mrc5 digital camera (Carl Zeiss Microscopy GmbH, Jena, Germany). The entire chamber bottom was first examined at 200 $\times$  magnification, while smaller taxa were subsequently observed at 400 $\times$  magnification and occasionally at 1000 $\times$  magnification with immersion oil. Armored dinoflagellates were stained with Calcofluor white M2R to facilitate visualization of their plates, which is crucial for taxonomic identification [29].

For specimens that were difficult to identify under LM, a scanning electron microscope (SEM; Tescan Mira, TESCANA HOLDING, a.s., Brno, Czech Republic) was used. The standard protocol involved filtration of 2 mL of the formalin-fixed sample onto a 10- $\mu$ m polycarbonate filter, followed by immersion in ethanol increasing gradations (20, 30, 50, 70, 80, 90, 95 and 100%). After absolute ethanol, the dehydrated samples were treated with hexamethyldisilazane (HMDS), and filters were mounted on stubs. The dried organisms on the filters mounted on a SEM stub were coated with a 5 nm thick Au-Pd layer (Q150R S/E/ES Plus sputter (Quorum, San Jose, CA, USA)) and examined. An alternative method based on Trobajo and Mann [30] has been shown to be effective for diatoms. The diatom samples were cleaned directly on coverslips. After fixation with 30% ethanol, thorough washing to remove salts, and sedimentation overnight, a small volume of the sample was placed on a coverslip. After drying, the coverslips were treated with 65–70% HNO<sub>3</sub> and processed similarly to the other samples prior to examination with SEM. All SEM examinations were conducted under high-vacuum conditions, typically at an energy of 2 keV (occasionally 5 keV), a beam current of 100 pA, and using a secondary electron (SE) or In-Beam SE detector.

Taxonomic identification of the observed organisms was carried out to the lowest possible level, typically to genus or species, using a range of phytoplankton identification keys and literature to ensure a comprehensive understanding of the microalgal community. References consulted included Dodge [31], Tomas [32], Faust and Guedes [33], Vilićić [34], McDermott and Raine [35], and Kraberg et al. [36], as well as various scientific papers and online resources.

The species names, ecological group affiliation (planktonic, benthic, tychopelagic or mixed) and salinity-based habitat classification (marine, brackish, freshwater or mixed) of the identified organisms were checked for accuracy using AlgaeBase [37], WoRMS (2025) [38] and other relevant publications. The classification of identified taxa according to ecological group and environmental affinity was performed up to the genus level, at most. HAB species were determined based on the IOC-UNESCO Taxonomic Reference List of Toxic Microalgae ([39] onwards) and the global overview of HAB [40]. NIS and cryptogenic species status were assigned based on the procedure outlined by Mozetič et al. (2019) [41], which incorporates extensive scientific literature on NIS in the Mediterranean and online information systems such as EASIN (<https://easin.jrc.ec.europa.eu/>, accessed on 10 December 2025).

### 2.4. Data Analysis

The diversity of microalgae in TW (PK, SZNR) and in the coastal sea (000F) was expressed as species richness (S), which included all observed organisms identified to

the species level, as well as those recognized only at the genus level and reported as sp. Species richness in PK was assessed using pooled samples from stations PBS1, PBS2 and PBS5. These estimates were made using the entire datasets and aggregated seasonally, with seasons defined as follows: spring (April–June), summer (July–September), autumn (October–December), and winter (January–March).

To determine the appropriate taxonomic depth needed to distinguish ecologically different environments, a taxonomic sufficiency analysis [42,43] was performed using data from PK (station PBS2) and 000F, for which sufficiently long datasets were available. Specimens identified only above the genus level were excluded from the analysis. For this analysis, we used five taxonomical levels (genus, family, order, class, and phylum). Similarities between samples from two study areas were assessed using Sørensen similarity and nMDS plots were created for all relevant taxonomical levels. To check whether location means were statistically different, we used the PERMANOVA routine (9999 permutations,  $\alpha < 0.05$ ). To test whether dispersion of samples within groups differed, we used the PERMDISP routine (9999 permutations,  $\alpha < 0.05$ ). Finally, we performed the RELATE routine to compare the sample patterns among different taxonomic levels and used the genus level as the baseline. Afterwards, the taxa that contributed the most to the differences between the two locations were checked at the most appropriate taxonomic level (SIMPER). Statistical analyses were performed using the PRIMER-7 with PERMANOVA+ software package and Microsoft Excel.

### 3. Results

#### 3.1. Environmental Characteristics of Study Sites

The basic environmental parameters—water temperature and salinity in the surface layer—measured at sampling sites PK and 000F and retrieved for SZNR from the ARSO website are presented in Table 1. For SZNR, a longer time span (February 2018–December 2020) than the actual sampling period (April 2019–February 2021; see Supplementary Table S1) was considered in order to better represent the average environmental conditions in the deepest part of the lagoon. Of the three sites, SZNR showed the widest range in both temperature and salinity, with water temperatures ranging from near freezing to above 30 °C. On average, the water temperature in SZNR and PK was about 3 °C lower than in 000F. The average salinity values of 21.6 (SZNR) and 24.3 (PK), together with their wide ranges of variation, reflect the brackish character of the two sites. In contrast, station 000F had a much higher average salinity (36.96) and a narrower range.

**Table 1.** Temperature (T, °C) and salinity characteristics at study sites based on monthly measurements.

Study Site	Period	T (°C)			Salinity		
		Min	Max	Mean $\pm$ SD	Min	Max	Mean $\pm$ SD
SZNR *	2018–2020	0.2	32.2	14.7 $\pm$ 7.7	1.9	35.9	21.6 $\pm$ 9.6
PK	August 2020–May 2021	9.4	25.4	14.2 $\pm$ 4.8	12.0	36.5	24.3 $\pm$ 10.2
000F	April 2018–May 2021	9.79	26.92	17.65 $\pm$ 5.82	29.68	38.42	36.96 $\pm$ 1.56

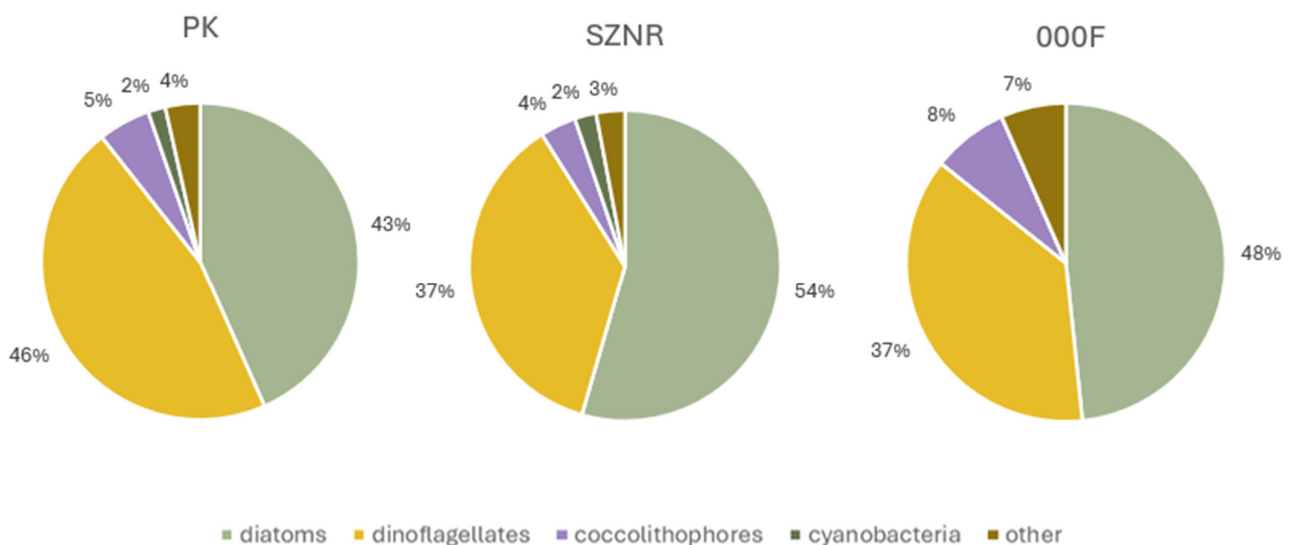
\* The data for SZNR were obtained from <https://www.arso.gov.si/vode/podatki/>, accessed on 27 May 2025. Based on data availability, the ranges were calculated for the period February 2018–December 2020.

#### 3.2. Diversity of Microalgae in Transitional Waters and Coastal Sea

The diversity of microalgae is expressed as species richness (S), which includes all taxa identified to species level as well as those recorded only as sp. In Slovenian transitional waters (TW), a total of 240 species from 117 genera, representing six algal phyla

and 12 classes were recorded in the period 2018–2021 (Supplementary Table S2). In addition, one heterotrophic flagellate from the phylum Cercozoa (*Hermesinum adriaticum*) was recorded but was not included in further analyses. The two most species-rich groups—diatoms and dinoflagellates—were almost equally represented, with 108 and 107 taxa, respectively. At the genus level, however, diatoms were more diverse (62 genera), followed by dinoflagellates (33 genera), coccolithophores (10 genera), and cyanobacteria (5 genera) (Supplementary Table S3).

When analyzed separately, species richness was higher in PK (226 taxa) than in SZNR (154 taxa), due to a greater diversity of dinoflagellates (104 vs. 61 taxa), diatoms (98 vs. 81), and coccolithophores (12 vs. 5). Other phyla, including Cyanobacteria, Chlorophyta, Euglenophyta and classes such as Dictyochophyceae, Chrysophyceae and Centrohelea, contributed no more than four taxa in either environment. These results show that diatoms were the dominant group in SZNR (52.6%), whereas dinoflagellates dominated in PK (46.0%) (Figure 2).



**Figure 2.** Species richness in Slovenian transitional waters (PK, SZNR) and in the coastal sea (000F), shown as relative proportions (%) of the most important algal groups. Less diverse phyla (Chlorophyta, Euglenophyta) and classes (Dictyochophyceae, Chrysophyceae, Centrohelea) are combined under “other”.

In both TW, the most diverse diatom genus was *Chaetoceros*, while among the dinoflagellates, *Dinophysis*, *Gonyaulax*, *Prorocentrum*, *Tripos*, and especially *Protoperidinium* were most diverse, with *Protoperidinium* having 24 species recorded in PK compared to 17 in SZNR. Overall, the species diversity of these genera was consistently higher in PK. In addition, 26 genera were found exclusively in PK, while only 8 were found exclusively in SZNR (Supplementary Table S3).

In contrast, species richness (91 taxa) and genus diversity (59 genera) were considerably lower at the marine coastal station 000F than in the two TW. Similar to SZNR, diatoms were the predominant group (48.4%), followed by dinoflagellates (37.4%). The relative contribution of coccolithophores (7.7%) and other taxa (6.6%) was lower than that of diatoms and dinoflagellates but the highest among the three water bodies. Cyanobacteria were not recorded at station 000F. The most obvious difference between the TW and the marine station was the higher species diversity of *Pseudo-nitzschia* (four species at station 000F compared to one in PK), together with the exclusivity of the genera *Haslea*, *Karenia* and *Parapedinella*, all found only at station 000F (Supplementary Tables S2 and S3).

Species richness also varied seasonally (Figure 3). As expected from the total number of taxa identified in TW (Supplementary Table S2), diversity was consistently higher in PK across all seasons—about 1.4 to 5 times higher than in SZNR. In PK, autumn and winter were the most species-rich seasons, whereas in SZNR the highest richness occurred in winter and spring; summer was the least species-rich season in both TW.

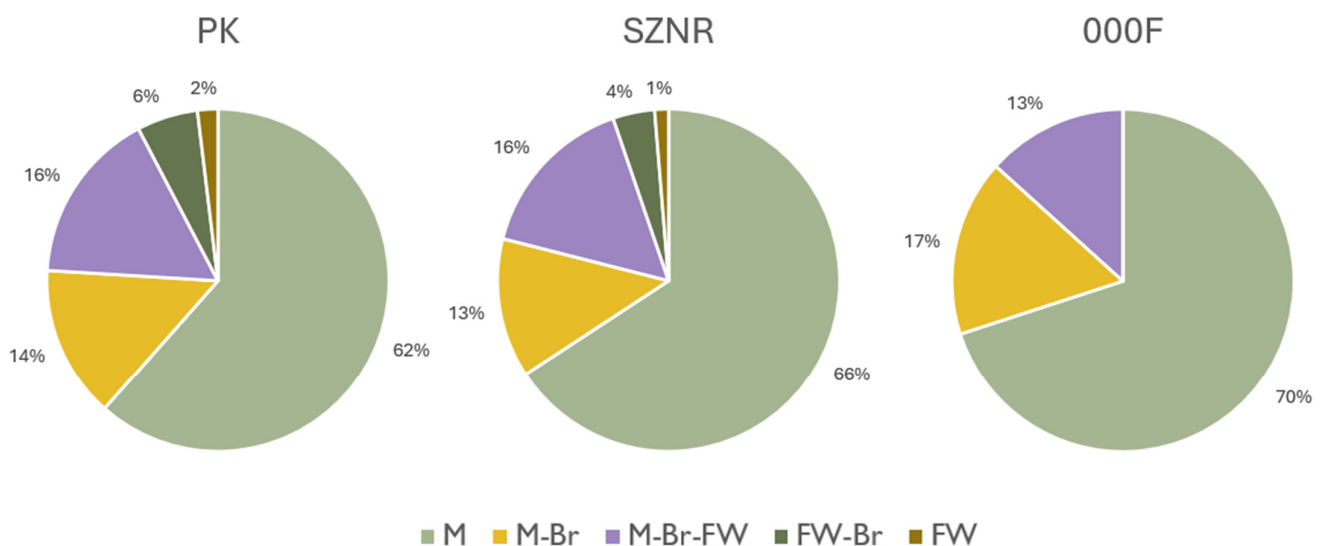


**Figure 3.** Seasonal distribution of the diversity of the main algal groups, expressed as species richness (S), in Slovenian transitional waters (PK, SZNR) and in the coastal sea (000F).

The two most diverse groups alternated in dominance in PK, with dinoflagellates predominating in spring and summer, and diatoms in autumn and winter. In SZNR, diatoms prevailed in terms of species richness across all seasons except summer, when their diversity, although low, matched that of dinoflagellates. Dinoflagellate diversity showed only minor seasonal variation in PK (ranging from 63 taxa in winter to 67 in spring), whereas pronounced seasonal changes were observed in SZNR (from only 13 taxa in summer to 40 in spring). In contrast, diatoms exhibited greater seasonal fluctuations at both TW, reaching maximum diversity in autumn (76 taxa in PK) and in winter (58 taxa in SZNR). At both locations, coccolithophores, cyanobacteria and other taxa contributed only marginally to the microalgal diversity, although a slight increase in coccolithophore richness was observed in autumn and winter.

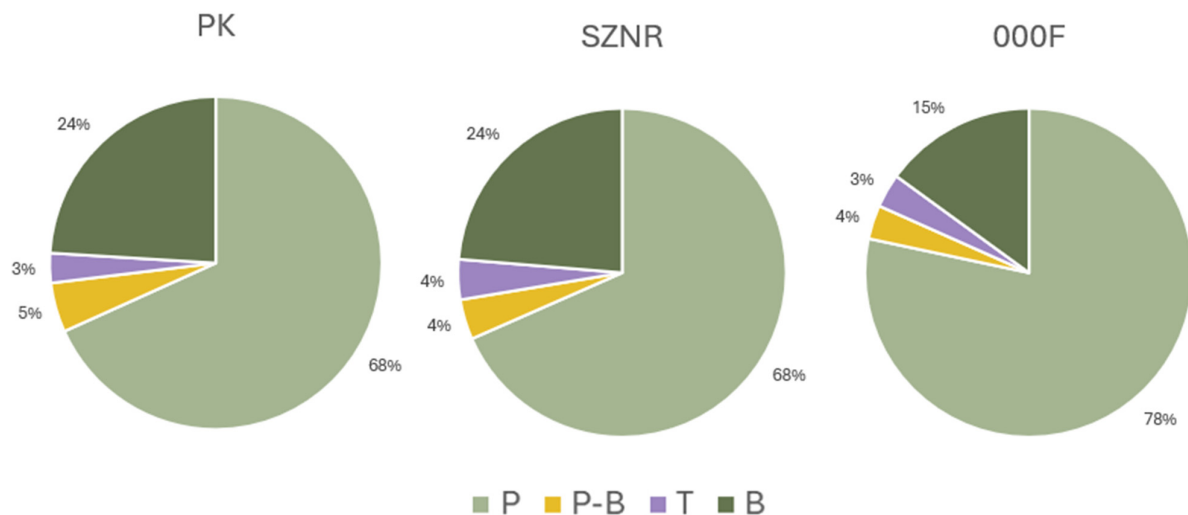
At the marine station 000F, diatoms were the most diverse group in all seasons except spring, when dinoflagellates prevailed in species richness. However, the richness of both groups was significantly lower than in TW. However, this comparison should be interpreted with caution, as methodological bias, such as different analysts, may have influenced the results. For a clearer assessment of the community structure between the TW and the 000F, the relative proportions (%) of species richness among algal groups and their seasonal changes are more informative (Supplementary Figure S2).

The diversity of microalgal communities was also analyzed with regard to habitat preferences along the salinity gradient and affiliation with ecological groups in the two TW and compared with the coastal station. The results showed a clear and largely consistent pattern along the salinity gradient in both TW, with marine taxa (M) dominating (up to 66%), while strictly freshwater taxa (FW) were less common (at most 2% in PK) (Figure 4). Mixed groups of taxa, freshwater–brackish (FW-Br), marine–brackish (M-Br), and marine–brackish–freshwater (M-Br-FW), capable of occupying habitats across a wide salinity range, accounted for 4–16%. Although the relative proportions in PK and SZNR were very similar, the share of brackish and freshwater taxa (in the groups FW-Br and FW) was slightly higher in PK at the expense of marine taxa. As expected, marine taxa were even more dominant at the coastal station (000F) (70%), while mixed groups (M-Br, M-Br-FW) had a comparable proportion to that in TW (13–17%). Pure freshwater taxa (FW) were not detected at this site.



**Figure 4.** Relative proportions of taxa in habitats defined by the salinity gradient in Slovenian transitional waters (PK, SZNR) and the coastal sea (000F). Legend: marine (M) and freshwater (FW) taxa, and taxa with broad salinity tolerance occurring along a wide gradient: marine–brackish (M-Br), marine–brackish–freshwater (M-Br-FW), freshwater–brackish (FW-Br).

Most taxa in both TW were planktonic ( $\approx 70\%$ ) (Figure 5). Benthic taxa followed (24%), and in SZNR their proportion was the highest in summer, reaching about 50%. Tychopelagic taxa (e.g., *Paralia sulcata*, *Striatella unipunctata*, *Diplopsalis* sp.) and taxa with both planktonic and benthic affinities accounted for up to 5% in both TW. The latter category (P-B) mainly comprised organisms that were not identified to species level and reported as sp. Plankton was also the dominant ecological group at the coastal station (000F), with an even higher proportion (78%) than in TW.



**Figure 5.** Relative proportions of taxa assigned to different ecological groups in Slovenian transitional waters (PK, SZNR) and in the coastal sea (000F). Legend: planktonic (P), benthic (B), tychopelagic (T), and taxa occurring in both plankton and benthos (P-B).

### 3.3. First Recorded, NIS and HAB Species in Transitional Waters

Thirty-two taxa were recorded for the first time in Slovenian transitional waters: 24 in PK and 17 in SZNR (Table 2). In this context, “first record” refers to taxa identified either unambiguously or tentatively (cf.) at the species or genus level, while acknowledging that some may have been previously present but remained unrecognized or misidentified. Most of these were diatoms (15), followed by dinoflagellates (8), cyanobacteria (5), coccolithophores (2), and chlorophytes (2). Several species observed under LM were further resolved with SEM.

Among the diatoms, most of the new taxa were benthic to occasionally tychopelagic (11) (e.g., *Gomphonema* cf. *acuminatum*, Figure 6a; *Navicula* cf. *subrostellata*, Figure 7a). Six were marine (e.g., *Odontella aurita*, Figure 6b), three were freshwater, and the remainder occurred across a wide salinity range from freshwater to marine habitats. Only three of the newly observed taxa were found in both transitional environments (e.g., *Actinocyclus* sp., Figure 7b).

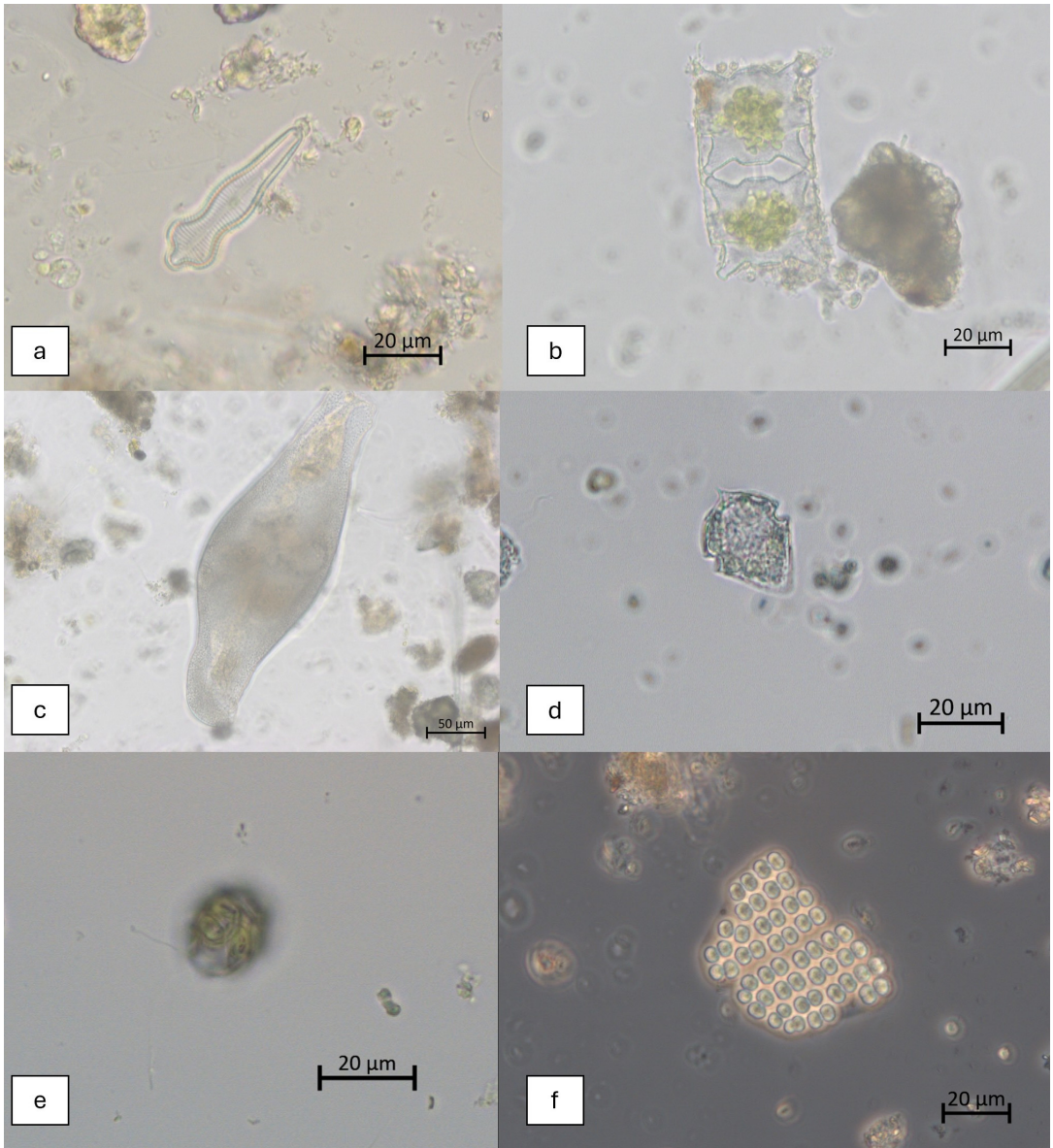
First recorded dinoflagellates were more numerous in PK (7) than in SZNR (3), with planktonic and marine taxa predominating (e.g., *Scaphodinium mirabile*, Figure 6c). The most notable records were *Azadinium caudatum* var. *margalefii* (Figure 6d), *Coolia monotis* (Figure 7c), and *Prorocentrum* cf. *formosum*. *C. monotis*, a benthic or epiphytic marine species that occasionally occurs in plankton, was only found in SZNR and was included in the HAB list as potentially toxic due to the production of cooliatxin. *P.* cf. *formosum* was classified as cryptogenic due to uncertainties regarding its origin, and due to the unresolved taxonomy (reported as cf.). Concurrently, *Alexandrium insuetum* (Figure 7d) is not listed as a “first recorded” as it had previously been annotated as *A.* cf. *insuetum* in coastal seas, also due to difficulties in distinguishing it from *Protoceratium reticulatum* under LM. However, it was reliably identified to species level for the first time using SEM.

**Table 2.** List of species recorded for the first time in Slovenian transitional waters (PK and SZNR), together with non-indigenous (NIS) or cryptogenic species and species causing harmful algal blooms (HAB). A question mark (?) indicates species of uncertain origin and is therefore categorized as cryptogenic. Genera labeled with (+) are considered potentially toxic/harmful, as they also contain non-toxic species.

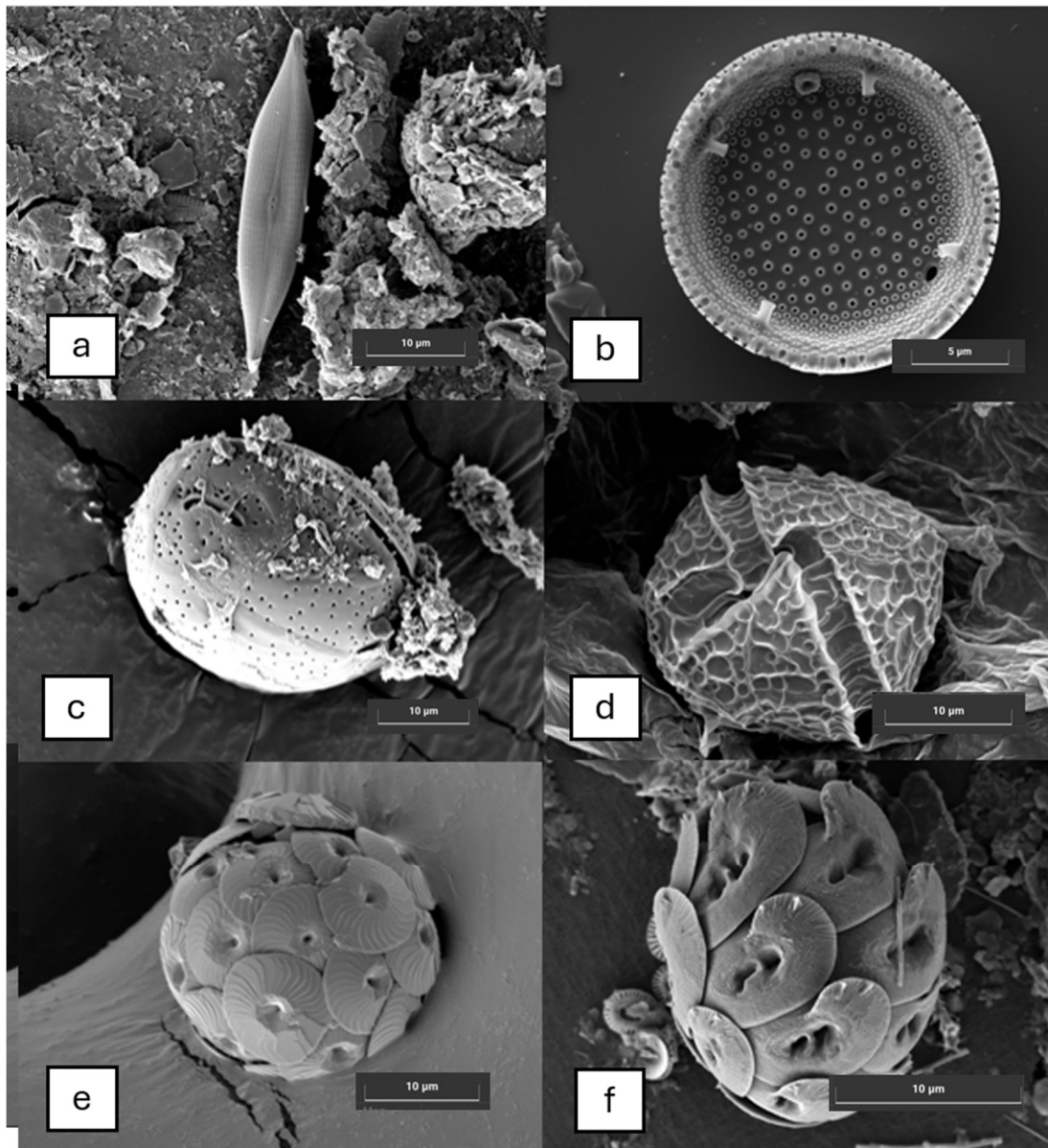
Taxon	First Record		NIS/Cryptogenic		HAB	
	PK	SZNR	PK	SZNR	PK	SZNR
<b>DIATOMS</b>						
<i>Actinocyclus</i> sp.	+	+				
<i>Asteromphalus</i> cf. <i>parvulus</i>		+				
<i>Chaetoceros</i> cf. <i>subtilis</i>	+	+				
<i>Cocconeis</i> cf. <i>sawensis</i>		+				
<i>Corethron</i> sp.		+				
cf. <i>Craticula cuspidata</i>		+				
<i>Cymbella</i> sp.	+	+				
cf. <i>Diatoma vulgare</i>	+					
<i>Eupyxidicula turris</i>		+				
<i>Gomphonema</i> cf. <i>acuminatum</i>	+					
<i>Gyrosigma</i> cf. <i>fasciola</i>	+	+				
<i>Navicula</i> cf. <i>subrostellata</i>	+					
<i>Nitzschia</i> cf. <i>sigmoidea</i>		+				
<i>Odontella aurita</i>	+					
<i>Pseudo-nitzschia multistriata</i>			+		+	
<i>Pseudo-nitzschia</i> spp.					(+)	(+)
<i>Tryblionella</i> sp.	+					
<b>DINOFLAGELLATES</b>						
<i>Akashiwo sanguinea</i>					+	+
<i>Alexandrium insuetum</i>					+	+
<i>Alexandrium minutum</i>					+	
<i>Alexandrium</i> cf. <i>tamarense</i>					+	
<i>Alexandrium pseudogonyaulax</i>					+	+
<i>Alexandrium</i> spp.					(+)	(+)
<i>Azadinium caudatum</i> var. <i>margalefii</i>	+	+				
<i>Blixaea quinquecornis</i>	+					
<i>Coolia monotis</i>		+				+
<i>Dinophysis acuminata</i>					+	
<i>Dinophysis caudata</i>					+	+
<i>Dinophysis fortii</i>					+	+
<i>Dinophysis ovum</i>					+	
<i>Dinophysis sacculus</i>					+	+
<i>Dinophysis</i> spp.					(+)	
<i>Dinophysis tripos</i>					+	
<i>Gonyaulax polygramma</i>					+	+
<i>Gonyaulax spinifera</i>					+	+
<i>Gymnodinium</i> cf. <i>fuscum</i>	+					
<i>Heterocapsa</i> spp.					(+)	(+)
<i>Lingulaulax polyedra</i>					+	+
<i>Pentaparsodinium</i> cf. <i>dalei</i>	+					
<i>Phalacroma mitra</i>						+
<i>Phalacroma rotundatum</i>					+	+
<i>Prorocentrum</i> cf. <i>formosum</i>	+		(?)			
<i>Prorocentrum lima</i>					+	+
<i>Protoceratium reticulatum</i>					+	+
<i>Scaphodinium mirabile</i>	+	+				
<i>Tripos teres</i>	+					
<b>COCCOLITHOPHORES</b>						

Table 2. Cont.

	First Record	NIS/Cryptogenic	HAB
<i>Calcidiscus leptoporus</i>	+		
<i>Helicosphaera carteri</i>	+		
CHLOROPHYTA			
<i>Pediastrum</i> sp.	+		
<i>Scenedesmus</i> sp.	+	+	
CYANOBACTERIA			
cf. <i>Anabaena</i> sp.	+		(+)
<i>Lyngbya</i> sp.	+	+	(+)
<i>Nostoc</i> sp.		+	(+)
<i>Merismopedia</i> sp.	+	+	(+)
<i>Oscillatoria</i> sp.	+		(+)



**Figure 6.** Representative light microscopy images of some of the first recorded taxa: diatoms (a) *Gomphonema* cf. *acuminatum* and (b) *Odontella aurita*; dinoflagellates (c) *Scaphodinium mirabile* and (d) *Azadinium caudatum* var. *margalefii*; (e) coccolithophore *Helicosphaera carteri*; and (f) cyanobacteria *Merismopedia* sp.



**Figure 7.** Scanning electron microscopy (SEM) images of some of the first recorded taxa: diatoms (a) *Navicula* cf. *subrostellata* and (b) *Actinocyclus* sp., dinoflagellates (c) *Coolia monotis* and (d) *Alexandrium insuetum*, and coccolithophores (e) *Calcidiscus leptoporus* and (f) *Helicosphaera carteri*.

The SEM examination also enabled the first identification of two coccolithophores, *Calcidiscus leptoporus* (Figure 7e) and *Helicosphaera carteri* (Figures 6e and 7f), both of which were restricted to PK.

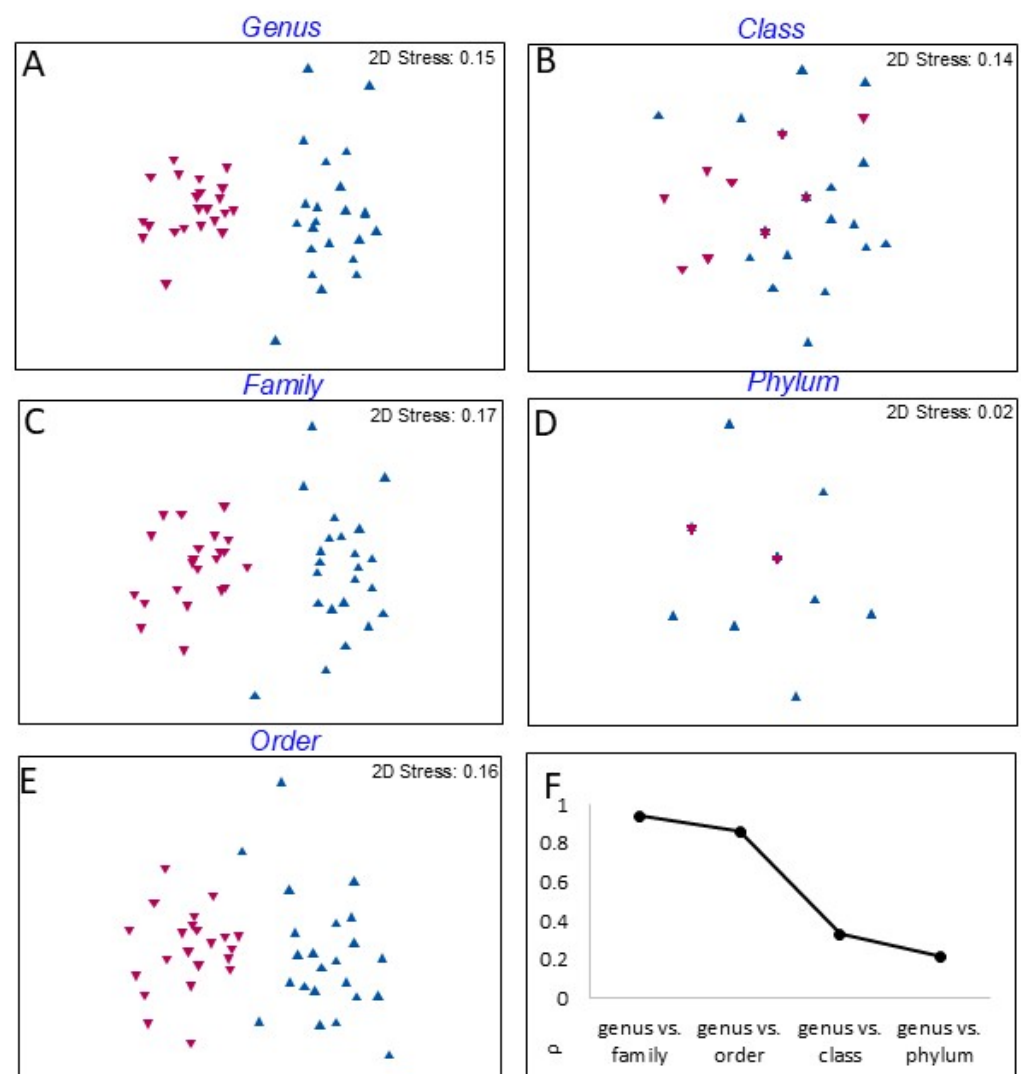
Further first records were two colonial freshwater chlorophytes (*Pediastrum* sp., *Scenedesmus* sp.) and five genera of cyanobacteria. The cyanobacteria exhibited great morphological and ecological diversity, ranging from filamentous to colonial forms (e.g., *Merismopedia* sp., Figure 6f), benthic or planktonic life forms, and habitats from freshwater to marine and even terrestrial. Among the newly recorded cyanobacteria, filamentous, benthic, mat-forming taxa typically found in freshwater to brackish habitats predominated. All cyanobacteria were also included in the HAB list as potentially toxic, as certain species are known to produce different cyanotoxins.

In addition to these new entries in the HAB list, i.e., *C. monotis* and five cyanobacteria, the remaining 23 HAB taxa had already been documented in previous studies of the Slovenian coastal sea, many of them as regular members of the phytoplankton. The largest

HAB category comprised toxic species producing amnesic toxins (genus *Pseudo-nitzschia*), diarrhetic toxins (genera *Dinophysis*, *Phalacroma*, *Prorocentrum*), paralytic toxins (genus *Alexandrium*), and yessotoxins (*Lingulodinium polyedra*, *Protoceratium reticulatum*, *Gonyaulax spinifera*). *Akashiwo sanguinea*, *Alexandrium pseudogonyaulax*, and *Heterocapsa* spp. were categorized as ichthyotoxic, while *Gonyaulax polygramma* was classified as high-biomass-forming species. Due to the limited resolution at the species level and toxic potential, entire genera such as *Pseudo-nitzschia*, *Alexandrium*, *Dinophysis* and *Heterocapsa* were categorized as potentially toxic. To date, only *Pseudo-nitzschia multistriata* can be classified as a non-indigenous species (NIS), while also being toxic.

### 3.4. Comparison of Diversity Between Transitional and Coastal Marine Waters

As an additional aim, we investigated taxonomic sufficiency, determining the depth of taxa identification required to yield suitable results for future monitoring purposes. The comparison of similarity patterns between different taxonomic levels is shown in Figure 8.



**Figure 8.** Taxonomic sufficiency analysis. (A–E) nMDS plots showing the differentiation of microalgal samples from transitional waters (PK, blue) and coastal marine waters (000F, red) at different taxonomic levels. (F) Similarity of patterns among genus and higher taxonomic levels (RELATE).

Already at the genus level, two groups of samples (PK and 000F) were very well separated, and the samples from PK were more dispersed compared to the ones from 000F (Figure 8A). The separation between the two groups remained clear up to the class level,

where they began to merge. This is further supported by the correlation between sample patterns across taxonomic levels, which shows the largest drop in correlation between genus and order, and between genus and class (Figure 8F). Differences between PK and 000F were statistically significant up to the class level (Table 3, PERMANOVA). On the other hand, the dispersion between samples from the two locations was mostly comparable, except when comparing samples at the order level (Table 3, PERMDISP).

**Table 3.** Results of PERMANOVA and PERMDISP tests comparing microalgal assemblages from transitional (PK) and coastal marine waters (000F) when using different taxonomic levels.

Level	PERMANOVA		PERMDISP	
	pseudo-F	p (perm)	F	p (perm)
genus	26.51	0.0001	14.56	0.0009
family	26.53	0.0001	7.18	0.0123
order	27.75	0.0001	3.33	0.1005
class	4.91	0.0078	5.0557	0.0322
phylum	1.99	0.1577	11.375	0.0057

Based on these results, the order level represents a suitable compromise between capturing ecological patterns accurately (Figure 8F) and minimizing the effort required for morphological identification under the microscope and was therefore selected for the SIMPER analysis. Table 4 shows orders, which contributed the most (cumulatively up to 70%) to the dissimilarities between two locations. Some orders, though not all shown in Table 4 due to their low contribution (<2.80%), were present only in PK (Surirellales, Melosirales, Striatellales, Rhaphoneidales, Ebriiida, Eupodiscales, Dinophyceae ordo incertae sedis, Oscillatoriales, Zygodiscales, Eutreptiales, Prymnesiales, Lyrellales, Cymbellales, Synechococcales, Nostocales, Coccolithales, Fragilariiales, Sphaeropleales) and only one was exclusive to 000F (Pedinellales).

**Table 4.** Orders which contributed the most (up to 70% cumulatively) to the dissimilarities between two locations—Port of Koper (PK) and coastal marine waters (000F). FO—frequency of occurrence.

Order	FO PK	FO 000F	Contribution (%)
Isochrysidales	0.26	1.00	5.40
Stephanodiscales	0.17	0.86	5.21
Dinophysales	1.00	0.27	5.16
Lithodesmiales	0.83	0.14	5.01
Thalassiosphaerales	0.04	0.73	4.88
Licmophorales	0.78	0.14	4.76
Surirellales	0.70	0.00	4.62
Achnanthes	0.70	0.14	4.30
Paraliales	0.78	0.32	4.18
Melosirales	0.61	0.00	4.05
Mischococcales	0.35	0.59	3.72
Dictyochales	0.74	0.45	3.62
Thoracosphaerales	0.48	0.55	3.52
Probosciales	0.87	0.55	3.35
Striatellales	0.52	0.00	3.35
Asterolamprales	0.39	0.14	2.83
Gymnodiniales	0.61	1.00	2.80

#### 4. Discussion

This study provides the first inventory of microalgal diversity in Slovenian transitional waters (TW), revealing that both the Škocjanski Zatok Nature Reserve (SZNR) and the Port

of Koper (PK) host rich and taxonomically complex assemblages that differ markedly from those of the adjacent coastal sea. The observed pattern—higher species richness in confined and anthropogenically influenced TW compared to open marine waters—reflects a general feature of Mediterranean lagoons and estuaries, where fluctuating salinity and nutrient inputs create heterogeneous niches promoting coexistence of marine, brackish and even freshwater taxa [44,45].

#### 4.1. Microalgal Diversity and Ecological Gradients

A total of 240 species from 117 genera were recorded in Slovenian transitional waters. Since our study focused on the microplanktonic size fraction collected with a 20 µm phytoplankton net, we presume that the total number of taxa would be higher if the nanoplanktonic and picoplanktonic fractions were also sampled. Nevertheless, some nanosized taxa (e.g., coccolithophores) were captured in the net samples. In addition, a number of taxa were identified only to the genus level, indicating that further analyses are needed to refine species-level resolution.

The dominance of diatoms and dinoflagellates observed in this study (Figure 2) aligns with previous findings from the adjacent coastal waters of the GoT [46,47]. Diatom species richness peaked in autumn and winter in both transitional waters (Figure 3), reflecting the seasonal pattern typical of the GoT, where cooler, mixed conditions and nutrient replenishment favor diatom diversity [46,47]. In SZNR, diatom richness was also high in spring.

In contrast, dinoflagellates were more diverse in warmer seasons and dominated in PK during spring-summer, largely due to the high diversity within the genera *Protoperidinium*, *Prorocentrum*, *Dinophysis*, and *Tripes*. This may reflect the influence of the Rižana River plume mixed with sewage effluent discharging into the estuary, creating stratified and nutrient-enriched conditions favorable for this group [48,49]. SZNR, a shallow semi-isolated lagoon, was characterized by a larger proportion of benthic and tychopelagic diatoms, typical of low-energy environments where sediment resuspension contributes to the phytoplankton pool. This benthic–pelagic coupling is a common feature of Mediterranean lagoons and contributes to total diversity [44].

Coccolithophores, although a minor component overall, were more diverse in PK—including first records of *Calcidiscus leptoporus* and *Helicosphaera carteri*—than in the SZNR lagoon with large variations in salinity (1.9–35.9), reflecting the group-wide preference for clearer, more marine and colder conditions with highest richness in autumn and winter.

The group “other” (chlorophytes, euglenophytes, chrysophyceans, dictyochophyceans), together with cyanobacteria, contributed less than 10% to total diversity. The seasonal pattern changed little, whether expressed as the absolute number of taxa (Figure 3) or as relative proportions (Supplementary Figure S2). Despite seasonal shifts in diatoms and dinoflagellates, these minor groups remained consistently low. Many of these taxa, particularly cyanobacteria, chlorophytes, and euglenophytes, were absent from the marine station 000F but present in both PK and SZNR, indicating greater environmental variability and occasional eutrophic influence typical of transitional environments. However, this alone does not fully account for the much lower species richness at station 000F (91) compared with TW (240), as differences in taxonomic resolution among analysts with varying experience may also have contributed.

The prevalence of marine–brackish taxa in both transitional waters suggests strong connectivity with the coastal sea, while the presence of brackish–freshwater species indicates intermittent freshwater influence, especially in SZNR after rainfall or freshwater inflow events. Similar transitional gradients have been reported for other European estuaries and lagoons [49,50].

A similar group-level dominance pattern was observed during the earlier BALMAS project survey, which was based on only five sampling campaigns in the Port of Koper and did not include the brackish lagoon [41,51]. The BALMAS study identified 139 taxa in PK, dominated by diatoms (57%) and dinoflagellates (37%), whereas the present study recorded 226 taxa, an increase of over 60%. This difference underscores the importance of higher temporal resolution and extended seasonal coverage (24 samplings in this study) for capturing microalgal diversity and confirms the ecological significance of the Slovenian port–lagoon system as a key monitoring area and reservoir of biodiversity [41,51].

A total of 32 taxa were recorded for the first time in Slovenian transitional waters, including 15 diatoms, 8 dinoflagellates, 2 coccolithophores, 2 chlorophytes, and 5 cyanobacteria. These newly recorded taxa do not necessarily represent new introductions to the area but rather previously overlooked species, now detected due to enhanced sampling coverage, higher taxonomic resolution, and the use of electron microscopy, which allowed for the recognition of small or morphologically similar forms. In addition, some of the newly identified taxa are freshwater or brackish species, which likely occur transiently or persist in low-salinity microhabitats typical of transitional systems.

Most of the first recorded taxa were benthic or tychopelagic diatoms, such as *Asteromphalus* cf. *parvulus*, *Cocconeis* cf. *sawensis*, *Gyrosigma* cf. *fasciola*, and *Navicula* cf. *subrostellata*, reflecting the influence of sediment resuspension in shallow, low-energy environments such as the SZNR lagoon. Several marine planktonic dinoflagellates were also identified for the first time, including *Azadinium caudatum* var. *margalefii*, *Blixaea quinquecornis*, *Pentapharsodinium* cf. *dalei*, and *Scaphodinium mirabile*. Notably, *Coolia monotis* was recorded for the first time in Slovenia, confirming its establishment in the northern Adriatic. Two coccolithophore species, *Calcidiscus leptoporus* and *Helicosphaera carteri*, were observed exclusively in PK, where marine influence and higher salinity favor calcareous taxa.

Among other groups, *Pediastrum* sp. and *Scenedesmus* sp. represent the first chlorophyte records in Slovenian transitional waters, as do five cyanobacterial taxa (cf. *Anabaena*, *Lyngbya*, *Nostoc*, *Merismopedia*, and *Oscillatoria*). Since cyanobacterial genera are primarily freshwater and brackish, their first records in Slovenian transitional waters and coastal ecosystems, in general, are not unexpected. Their absence from a truly marine environment is consistent with this pattern, where unicellular *Synechococcus*-type cyanobacteria nevertheless represent important components of the bacterial assemblages [52] and the phytoplankton community [53].

Among all first records, only one species was classified as cryptogenic: *Prorocentrum* cf. *formosum*, which closely resembled the taxon *Prorocentrum formosum*, first described from the Caribbean [54]. However, due to uncertainties regarding its exact taxonomic position, we consider its occurrence in Slovenian coastal ecosystems cryptogenic rather than introduced. The diatom *P. multistriata* is still regarded as a likely non-indigenous species. It was detected outside its native range (Japanese seas) in 1985 in the northeastern Atlantic [55] and in 1992 in the Tyrrhenian Sea [56], with subsequent records across the Mediterranean (in Zingone et al., 2021 [57]), including the GoT [58] and the Port of Koper [41]. Its nearly simultaneous occurrence in ballast water tanks from ships arriving in the Port of Koper and in water samples from the same port suggests a possible introduction pathway via maritime transport [59].

It is important to note that some taxa remain identified only to the genus or tentative species level (e.g., taxa marked as “cf.” or “sp.”), as further morphological or molecular confirmation is needed to validate their status. Overall, these results emphasize that the new records reflect the combination of improved analytical methods, greater sampling effort, and the natural heterogeneity of transitional waters, rather than recent colonization events.

#### 4.2. HAB Taxa in Transitional Waters

In Slovenian transitional waters, 29 taxa were classified as HAB species, which may adversely affect ecosystems, human health, or the economy (Table 2). Although not all have been unequivocally linked to harmful events, caution is warranted when species-level identification is incomplete (e.g., designated as cf., sp., or spp.), and a conservative classification approach is justified. In such cases, the term “potentially harmful” is more appropriate. Most HAB taxa recorded in this study are already known from the Slovenian coastal sea and have previously been observed in the Port of Koper [41,60]. Exceptions are the dinoflagellate *Coolia monotis*, detected in SZNR, and all cyanobacteria—presumably (cf.) *Anabaena* sp., *Lyngbya* sp., *Merismopedia* sp., *Nostoc* sp., and *Oscillatoria* sp.—found in either PK or SZNR, all identified for the first time in Slovenian coastal ecosystems.

*Coolia monotis* (Figure 7c) is a benthic and epiphytic marine dinoflagellate frequently reported from Mediterranean coastal waters and lagoons (e.g., [61,62]), including the Adriatic Sea [63]. *C. monotis* often co-occurs with species of the genus *Ostreopsis* [61,63,64]. Unlike the three Mediterranean *Ostreopsis* species known to produce palytoxin-like compounds associated with respiratory symptoms in humans [57,65], the toxicity of *C. monotis* remains uncertain. Although some strains have been reported to produce a yessotoxin derivative (cooliatoxin) in culture, several European isolates have shown no toxicity [63,64]. The species is not included in the IOC–UNESCO list of harmful algal species (Lundholm et al., 2009 onwards) [39] but is mentioned by Lassus et al. (2016) [40] as potentially toxin-producing. Given this uncertainty, *C. monotis* was included in our list of potential HAB species. Caution is also warranted due to the reported co-occurrence of *Coolia* and *Ostreopsis*, which suggests that the latter may already be present in Slovenian coastal waters but has remained undetected, likely because of morphological similarity requiring SEM for accurate identification, or the absence of mass proliferations such as the event observed in autumn 2009 on the nearby Italian coast of the GoT [66].

Of the five cyanobacterial taxa identified for the first time in our coastal brackish environments, classification as HAB species remains tentative—i.e., potentially harmful—as they were identified only to the genus level. However, some toxic species within these genera are listed in the IOC–UNESCO reference list (Lundholm et al. 2009 onwards) [39] as toxin producers or as being associated with harmful events [40]. Toxicity has also been inferred from the detection of cyanotoxin-related genes, for example, in a *Merismopedia* bloom in a Cypriot lake [67]. Cyanobacteria from these genera are known to produce various bioactive compounds, including microcystins and nodularins with hepatotoxic effects, which are the most widespread cyanotoxins in European brackish-water environments [68]. Since representatives of these genera were found in our transitional waters, their presence indicates a potential, though currently low, risk of cyanotoxin accumulation, which could be assessed more reliably once cyanobacterial species are identified with greater taxonomic resolution. Nevertheless, toxic cyanobacterial blooms are well documented in Slovenian freshwater systems [69], where microcystin production is frequently associated with *Microcystis* and *Planktothrix* [70], genera that were not identified in this study.

The remaining HAB taxa are either potentially toxic or non-toxic, such as *Gonyaulax polygramma*, which can form high-biomass blooms that cause deleterious effects on fish and invertebrates [40]. To date, such events have not been recorded in Slovenian coastal waters, nor have fish kills been reported in association with ichthyotoxic species such as *Alexandrium pseudogonyaulax*, *Heterocapsa* spp., or *Akashiwo sanguinea*, known to produce surfactant-like compounds. These compounds can also cause plumage fouling and hypothermia in marine birds, leading to mass strandings reported elsewhere [71,72]. The proliferation of *A. sanguinea* under favorable conditions could therefore represent a real-

istic threat, particularly to the coastal brackish lagoon (SZNR), an important nesting and wintering area for birds within the Natura 2000 network.

In contrast, food poisoning linked to algal toxins occurs relatively frequently along the Slovenian coast, although it is limited to diarrhetic shellfish poisoning (DSP). Episodes of elevated diarrhetic shellfish toxin concentrations have occasionally resulted in temporary closures of mariculture activities when regulatory limits were exceeded, thereby preventing human intoxication [60]. Paralytic shellfish toxins produced by *Alexandrium* spp. have never been detected in Slovenian bivalves, while domoic acid, the causative agent of amnesic shellfish poisoning, has only been identified in cultures of certain *Pseudo-nitzschia* species, including *P. multistriata* [73]. In this category of toxic species, transitional waters are more likely to serve as entry points for non-indigenous harmful organisms than as areas where mariculture is at immediate risk.

#### 4.3. Comparison with Coastal Marine Waters as a Framework for Monitoring in Transitional Waters

The coastal reference station (000F) exhibited lower richness and a community dominated by typical offshore diatoms (e.g., *Pseudo-nitzschia*, *Thalassiosira*) and fewer dinoflagellates, confirming that open-sea assemblages are more stable but less diverse [46]. In contrast, both transitional systems (PK and SZNR) hosted more heterogeneous and dynamic communities reflecting their variable hydrological conditions.

To better understand how distinct these environments are and how precisely phytoplankton needs to be identified for effective ecological assessment, a taxonomic sufficiency analysis was performed on the two datasets (PK and 000F). The analysis revealed that order-level was sufficient to discriminate transitional from marine assemblages, with statistically significant differences remaining up to the class level. This was further confirmed by testing the similarity between taxonomic levels (RELATE test), which showed the largest decline in correlation between the genus and order, and between genus and class, indicating that ecological information is rapidly lost when identification becomes too coarse. Indeed, the orders that contributed most to discrimination between the transitional and marine assemblages (see Table 4) represented either rare species, which require more sampling effort to be found (net samples vs. Niskin samples) or species with a benthic habitat preference that were found exclusively in PK, such as diatoms from the genera *Surirella*, *Entomoneis*, *Melosira*, *Podosira* and *Striatella*.

Although order-level identification can reliably reflect ecologically distinct site characteristics in relation to salinity gradients and general habitat preferences with reduced taxonomic effort, it lacks the specificity needed to assess the diversity and abundance patterns of particular organism groups on which ecological and environmental status assessments are based. Our results indicate that at least genus-level identification—also shown to be discriminatory in the taxonomic sufficiency analysis—is the minimum requirement, while species-level identification is currently used in indices specifically developed for transitional waters (Facca et al., 2014) [74]. Indeed, the approach of identifying microalgae as accurately as possible is adopted for monitoring Slovenian transitional waters under the WFD and MSFD, as recently implemented in SZNR.

For HAB and NIS, species-level identification remains essential. Despite the increasing use of environmental DNA metabarcoding in diversity and ecological studies (e.g., Bazin et al. 2014 [21], Hering et al. 2018 [75], Minicante et al. 2020 [20], Neri et al. 2025 [76]), microscopy remains widely used and requires well-trained taxonomic expertise.

The diversity patterns observed here strengthen the argument that transitional waters are key components of the northern Adriatic ecosystem and should be incorporated into national long-term observation programs. As demonstrated by the Italian LTER network [45,77], consistent time-series data are essential to distinguish natural variability from

anthropogenic change. Integrating Slovenian TW into these frameworks would enhance regional assessments of ecological and environmental status under both the WFD and MSFD.

## 5. Conclusions

This study presents the first comprehensive inventory of microalgal diversity in Slovenian transitional waters, revealing exceptionally high species richness (240 species) and complex community structures compared to the adjacent coastal sea. Both the Rižana River estuary in the Port of Koper and the brackish lagoon in the Škocjanski Zatok Natural Reserve supported diverse assemblages dominated by diatoms and dinoflagellates. Despite the broad salinity range of transitional waters, which would favor euryhaline, brackish species, marine taxa remained the most abundant, indicating strong biological and human-mediated physical connectivity with the open sea.

A total of 32 taxa were recorded for the first time in Slovenian coastal ecosystems. Several were freshwater or brackish species, while others, such as *Coolia monotis* and *Prorocentrum cf. formosum*, are ecologically relevant as potentially harmful or cryptogenic species, respectively. To date, *Pseudo-nitzschia multistriata* is likely the only non-indigenous species with an established, stable population in coastal marine waters. The first detection of five cyanobacterial genera with potentially harmful traits, requiring precise taxonomic and toxicological verification, exclusively in transitional waters, highlights the role of these systems as ecological interfaces linking freshwater and marine biodiversity.

The taxonomic sufficiency analysis showed that order-level identification is adequate to distinguish transitional from marine microalgal assemblages, confirming the need to monitor ecologically different water bodies in accordance with WFD and MSFD frameworks, which in turn require finer taxonomic resolution depending on the objective of the analysis. The small number of non-indigenous and cryptogenic species suggests a low risk of Slovenian transitional waters serving as recipient environments for alien microalgal taxa. Nevertheless, vigilance remains essential given the harmful potential of newly recorded, ecologically tolerant taxa and their possible spread into adjacent coastal waters, as well as that of already established HAB species.

Overall, this study highlights transitional waters as reservoirs of biodiversity and previously unrecognized hotspots for potential harmful algal blooms. Their inclusion in long-term national and regional monitoring programs is crucial for understanding ecosystem connectivity, tracking biological introductions, and supporting effective management and conservation of coastal biodiversity.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/d18010021/s1>, Figure S1: Sampling sites in the Port of Koper sampling area (PK) and the coastal brackish lagoon (SZNR); Figure S2: Relative proportions (%) of the number of species of microalgal groups by season in Slovenian transitional waters (PK, SZNR) and coastal sea (000F); Table S1: Dates of sampling at sampling sites in Slovenian transitional waters - the heavily modified estuary (PK) and the coastal brackish lagoon (SZNR) - and the coastal sea (000F) during 2018–2021; Table S2: List of taxa (species, genera) found in Slovenian transitional waters (PK, SZNR) and in the coastal sea (000F) along with their habitat preferences and ecological group affinities; Table S3: List of genera found in Slovenian transitional waters (PK, SZNR) and coastal sea (000F).

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