UNIVERZA NA PRIMORSKEM
FAKULTETA ZA MATEMATIKO, NARAVOSLOVJE IN INFORMACIJSKE TEHNOLOGIJE

MAGISTRSKO DELO
(MASTER'S THESIS)

BIODIVERZITETA MIKROALG V SLOVENSKEM OBALNEM MORJU IN BRAKIČNIH VODAH

(BIODIVERSITY OF MICROALGAE IN SLOVENIAN COASTAL SEA AND BRACKISH WATERS)

PETRA SLAVINEC

2022

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Biodiverziteta mikroalg v Slovenskem obalnem morju in brakičnih vodah

(Biodiversity of microalgae in Slovenian coastal sea and brackish waters)

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Študijski program: Varstvo narave, 2. stopnja
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Koper, september 2022

Ključna dokumentacijska informacija

Ime in PRIIMEK: Petra SLAVINEC

Naslov magistrskega dela: Biodiverziteta mikroalg v Slovenskem obalnem morju in brakičnih vodah:

Kraj: Koper
Leto: 2022
Število listov: 82 Število slik: 27 Število tabel: 10
Število prilog: 1 Št. strani prilog: 8
Število referenc: 84
Mentor: izr. prof. dr. Patricija Mozetič
Somentor: dr. Janja Francé

UDK: 574.1:591.9(26)(043.2)
Ključne besede: biodiverziteta, mikroalge, obalno morje, brakični habitati, tranzitni habitati, tujerodne vrste

Izvleček: V sklopu magistrske naloge smo ugotavljali, kakšna je biodiverziteta mikroalg v brakičnih in antropogeno preoblikovanih habitatih – v Luki Koper, Stjuži in Škocjanskem zatoku, ter jo primerjali z referenčno postajo LTER 000F. Mikroalge smo vzorčili enkrat mesečno med aprilom 2018 in majem 2021 s fitoplanktonsko mrežico. Vzorce smo fiksirali in pregledali z invertnim epifluorescentnim mikroskopom Axio Observer Z1 (ZEISS), ter vrstičnim elektronskim mikroskopom (SEM) TESCAN MIRA. Diverziteto mikroalg z različnih območij smo analizirali s pomočjo programa PRIMER-7, iskali pa smo podobnosti med leti, sezonami in lokacijami. Skupno smo identificirali 284 taksonov, od tega jih največ pripada razredoma Dinophyceae (126) in Bacillariophyceae (125). Precej manj jih pripada skupinam Coccolithophyceae (15), Dictyochophyceae (4), Ebriida (1), Euglenophyceae (2), Chlorophyta (3), Ochrophyta (2), Cyanophyceae (6). 24 vrst, ki smo jih določili lahko povzroča škodljiva cvetenja alg (HAB), ena vrsta (*Pseudo-nitzschia multistriata*) je bila identificirana kot tujerodna (NIS), dva taksona (*Azadinium caudatum* var. *margalefii* in *Merismopedia* sp.) pa kot kriptogena. 35 od 284 taksonov je bilo določenih prvič, od tega jih je nekaj značilnih predvsem za sladkovodna in brakična okolja. Ugotovili smo, da si je biodiverziteta mikroalg v različnih brakičnih, oz. antropogeno spremenjenih okoljih podobna in se razlikuje od biodiverzitete na referenčni postaji v obalnem morju. Vzorci iz LTER so si bili po vrstni sestavi mikroalg tudi bolj podobni, kot vzorci iz Luke Koper, Stjuže in Škocjanskega zatoka. Rezultati bodo pomembni za pripravo nove presoje stanja morskega okolja po deskriptorju D2 - tujerodne vrste, kot to določa Okvirna direktivi o morski strategiji 2008/56/ES (ODMS).

Key document information

Name and SURNAME: Petra SLAVINEC

Title of the thesis: Biodiversity od microalgae in Slovenian coastal sea and brackish waters

Place:Koper
Year: 2022
Number of pages: 82 Number of figures: 27 Number of tables:10
Number of appendix: 1 Number of appendix pages: 8
Number of references: 84
Mentor: Assoc. Prof. Patricija Mozetč, PhD
Co-Mentor: Janja Francé, PhD

UDC: 574.1:591.9(26)(043.2)
Keywords: biodiversity, microalgae, coastal sea, brackish habitats, transit habitats, non-indigenous species

Abstract: We determined the species diversity of microalgae in brackish and heavily modified environments - in Port of Koper, Stjuža lagoon and Škocjanski Zatok Nature Reserve - and compared it with the reference station in the Slovenian coastal sea. Microalgae were sampled once a month between April 2018 and May 2021 using a phytoplankton net. Samples were fixed and examined under an inverted epifluorescence and a scanning electron microscope. We analysed the diversity of microalgae from different areas using the program PRIMER-7 and looked for similarities between seasons and locations. We identified a total of 284 taxa, most of which belong to the classes Dinophyceae (126) and Bacillariophyceae (125). Significantly fewer belong to the groups Coccolithophyceae (15), Dictyochophyceae (4), Ebriida (1), Euglenophyceae (2), Chlorophyta (3), Ochrophyta (2), Cyanophyceae (6). We found 24 species that can cause harmful algal blooms, one species (*Pseudo-nitzschia multistriata*) was classified as non-indigenous, and two taxa (*Azadinium caudatum* var. *margalefii* and *Merismopedia* sp.) as cryptogenic. 35 of the 284 taxa were identified for the first time, some of which are primarily characteristic of freshwater and brackish environments. We found that the species diversity of microalgae in different brackish water environments was similar on average, but differences were observed in the percentage of true marine, brackish, and freshwater species. When comparing a limited number of samples, differences were observed between these environments and a coastal sea. In addition, phytoplankton in the Port of Koper showed a more pronounced seasonal pattern compared to the coastal sea. The results will be relevant for the preparation of a new assessment of the status of the marine environment according to descriptor D2 - non-indigenous species, as set out in the Marine Strategy Framework Directive 2008/56/EC (MSFD).

AKNOWLEGMENTS

This study was carried out at the Marine Biology Station Piran (National Institute of Biology) as part of the project "Monitoring the diversity and abundance of non-indigenous species in the Slovenian sea" commissioned by the Ministry of Agriculture, Forestry and Food and as part of the ARRS research program "Coastal Sea Research".

I would like to thank my mentor Assoc. Prof. Patricija Mozetič, PhD and comentor Janja Francé, PhD for mentoring me and all the knowledge and expirience they have given me throughout the years of my studies.

I would like to thank the Nacional Institute for Biology – Marine Biology Station for being able to use the laboratories and other equipment, needed for this study. I would also like to thank my coworkers who helped me in any way, especially Ana Fortič and Milijan Šiško for all the help with statistics and fieldworks. Scanning electron microscope TESCAN MIRA, which was used for this study was co-financed by project RI-SI- LifeWatch, Ministry of Education, Science and Sport, Slovenia and the European Union, European Regional Development Fund.

I would like to thank all the professors and assistants at UP FAMNIT.

I would also like to thank my parents, my family, my boyfriend and friends for all of their patience and support.

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BALMAS - Ballast Water Management System for Adriatic Sea Protection
HAB – Harmful Algal Bloom
HAOP – Harmful Aquatic Organisms and Pathogens
IUCN - International Union for Conservation of Nature
LTER – Long Term Ecological Research
MSFD - Marine Strategy Framework Directive
NIS – non-indigenous species
SS1 – sampling site 1
SS2 – sampling site 2
WFD - Water Framework Directive

1 INTRODUCTION

1.1 Brackish habitats, NIS and HAB species

Brackish areas or brackish waters are waters with a salinity between 0.5 and 30, which means these are waters that have a higher salinity than inland or fresh water and a lower salinity than sea water. Brackish areas are areas where salinity varies greatly due to the mixing of seawater and freshwater, and they are often heavily modified by humans. Brackish water bodies can have natural or anthropogenic origin.

Natural brackish water bodies include, besides brackish seas (e.g. Baltic Sea, Black Sea) and lakes also transitional coastal areas where such water mix. The term "transitional waters" first came to prominence in 2000 with the publication of the European Communities Water Framework Directive, which defines transitional waters as "bodies of surface water in the vicinity of river mouths which are partially saline in character as a result of their proximity to coastal waters but which are substantially influenced by freshwater flows" (European Communities 2000; Lusky and Elliot 2007).

Transitional waters, such as brackish estuaries and lagoons are highly productive ecosystems that play an important ecological role due to their location at the interface between sea and land, receiving nutrients from freshwater, tides, the atmosphere, and bottom sediments. Nevertheless, according to the IUCN classification, they are among the most threatened ecosystems worldwide, especially due to various anthropogenic impacts (Šajna et al. 2005). Because of the high variability of environmental parameters and extreme conditions resulting in empty ecological niches, these types of anthropogenically altered environments can provide a favourable environment for non-native species (NIS). Ports also provide a receiving environment for NIS because of the likelihood that they will be introduced with ballast water and sediments (Kraus et al. 2019).

A non-native species (NIS) is a species, subspecies, or taxon of a lower category that has been intentionally or unintentionally introduced into a new area by human intervention from outside its natural range. This includes any developmental stage of an organism that is capable of survival and reproduction (Pyšek et al. 2009). Species whose origins are unclear and cannot be designated as non-native or native are referred to as cryptogenic species (Carlton 1996). The subcategory of non-native species (NIS) that have become established outside their natural range (historically or currently) and have negative impacts on biodiversity, ecosystem functions, socioeconomic values, and human health are referred to as invasive alien species (IAS). The cryptogenic species can also demonstrate invasive characteristics and should therefore be included in IAS assessments (Olenin et al. 2010). These species usually reproduce rapidly and can expand their range very quickly (Carlton et al. 1996; Pyšek et al. 2009). The above categories of potentially harmful organisms can be grouped together with indigenous harmful organisms (HAB species - harmful algal blooms) under the designation HAOP (Harmful Aquatic Organisms and Pathogens) (David et al. 2013), which is defined in the IMO International Convention for the Control and Management of Ships' Ballast Water and Sediments (IMO 2004).

As part of this thesis, we sampled three brackish and anthropologically modified habitats in transitional waters along the Slovenian coast. These bodies are the ship's basins in the Port of Koper, the semi-saline wetland in the Škocjanski zatok Nature Reserve and the Stjuža sea lagoon. According to Lusky and Elliot (2007) partition of physiographic forms to be included under the term transitional waters, the Port of Koper is a heavily modified transitional water body, which also includes the river mouth of Rižana. The lagoon in Škocjanski zatok, where the samples were taken and Stjuža are both lentic microtidal lagoons, with limited exchange with the coastal sea through a restricted mouth and tidal range bigger than 50 cm.

1.2 Microalgae in coastal ecosystems

Microalgae are a highly diverse group of microscopic organisms found in both marine and freshwater environments. They can be classified as eukaryotic microorganisms or prokaryotic cyanobacteria, with more than 25,000 species already isolated and identified (Vale et al. 2020). They form the basis of the food webs of many ecosystems. Besides taxonomy, the criteria for classifying microalgae into groups also include their morphological, physiological and ecological characteristics (Hemaiswarya et al. 2013; Salmaso et al. 2015). The major taxonomic groups of marine microalgae, both in number of species and abundance, are diatoms (phylum Bacillariophyta), dinoflagellates (superclass Dinoflagellata), coccolithophorids and other haptophytes (phylum Haptophyta, class Coccolithophyceae), cryptophytes (phylum Cryptophyta), unicellular green algae (phylum Chlorophyta), and cyanobacteria (Algaebase 2022).

They can also be divided into two ecological groups depending on their habitat: benthic microalgae or microphytobenthos and planktonic microalgae - phytoplankton. Benthic microalgae (example in Fig. 1) can be found on the surface of the rocky sediment bottom (epilithic), on sand grains (epipsammic), on mud (epipelic), inside sediment (endopelic), inside rock (endolithic), on leaves of seagrass or stems of microalgae (epiphytic), on animals (epizooic) or on ice (psychrophilic), while planktonic ones float freely in the water column

(Round et al. 1971). Benthic microalgae often form mats at the sediment-water interface, but vertical migration, physical disturbance, and bioturbation can distribute viable microalgae not only to upper layer of the water column, but also to depths far below the photic zone in sediments (Cahoon 1999).

Phytoplankton are small photosynthetic protists that float in the water column, although they are often capable of swimming by themselves. Phytoplankton range in cell diameter is on average from 1 μm to 70 μm, with a few representatives up to 1 mm and are pragmatically divided into three size classes: picoplankton (2 μm or less), nanoplankton (2-20 μm) and microplankton (20-200 μm). The term phytoplankton is also misleading in some respects because some groups, such as the dinoflagellates, include species that are not photosynthetic (Miller 2004; Hoppenrath et al. 2009). The various life forms observed in phytoplankton are functionally interpreted as adaptations to survive in an unstable and turbulent environment (Margalef 1978).

Interactions between wind, solar radiation, tides, and Earth's rotation result in different forms of water movement that affect the distribution of physical and chemical quantities of seawater and consequently the characteristics of planktonic communities (Graham et al. 2009). Interactions between differential access to light and nutrients and losses due to sinking and grazing of zooplankton form characteristic seasonal dynamics of pelagic phytoplankton assemblages (Miller 2004).

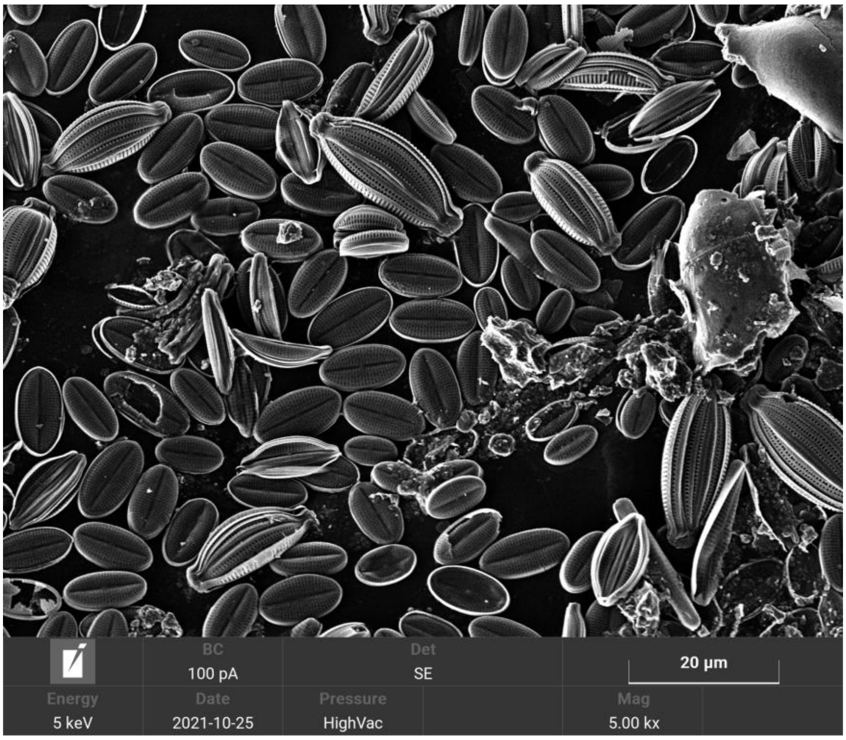

Figure 1: Benthic diatoms *Amphora* sp. and *Cocconeis* sp. photographed with scanning electron microscope.

1.3 Biodiversity in microalgae in the Slovenian sea

The Slovenian sea is part of the Gulf of Trieste, which has been known as a Mediterranean region with comparatively high pelagic biomass at several trophic levels, from phytoplankton to microbial plankton, zooplankton, and fish. The extent of primary productivity, biomass, and succession of the plankton community in the Gulf of Trieste exhibits a strong seasonal pattern related to allochthonous inputs of nutrients (Fonda Umani et al. 1992; Malej et al. 1995).

Seasonal cycles vary greatly from place to place, but in temperate climates there are usually two phytoplankton blooms per year. As water temperatures rise in the spring, water bodies stabilise and the light intensity penetrating the upper layers of water becomes higher. Calm weather favours water stabilisation and rapid and intense phytoplankton growth dominated by diatoms. Phytoplankton growth continues as long as nutrients are available. Reduced silicate content will limit diatom growth, and herbivores, usually zooplankton predators, may also reduce phytoplankton biomass. This will cause the diatom bloom to abort in the spring. Phytoplankton populations tend to be small in summer and dominated by dinoflagellates, who are able to migrate through the water column, implying that they are capable of the uptake of nutrients below the thermocline. In late summer and fall, when light intensity is still high and nutrients become available again due to mixing, a second, smaller bloom occurs, usually dominated again by diatoms. Due to strong winds that cause turbulence in late fall and winter, phytoplankton biomass production decreases dramatically due to limited light conditions. Complete mixing of the water column in winter provides high nutrient levels needed for the next spring bloom, while coccolithophores dominate in winter (Cullen 1985; Miller 2004; Reynolds et al. 2006; Hoppenrath et al. 2009). The dynamics described are also characteristic of the Gulf of Trieste, but are subject to great variations, mainly due to the geomorphological and hydrological characteristics of the bay and weather conditions (Mozetič et al. 2012; Panjan 2018).

About one third of all the microalgae identified in the Gulf of Trieste have also been identified in the Slovenian sea (Cabrini et al. 2018; Mozetič et al. 2019; Pagon 2019). In 2019, there were more than 80 known diatoms, about 100 known dinoflagellates and more than 10 known coccolithophorids in Slovenian sea (Francé et al. 2019), while Vascotto et al. (2021) reported a total of 130 taxa determined between 2005 and 2017 on LTER 000F. 53 of the taxa were diatoms, 50 dinoflagellates and 15 coccolithophores. The remaining 12 taxa were distributed among the classes Cryptophyceae, Chlorophyceae, Euglenophyceae, Prasinophyceae, Chrysophyceae, Dictyochophyceae and other undetermined nanoflagellates.

Potentially toxic phytoplankton are monitored regularly in all three Slovenian shellfish aquaculture sites – once a month through the year and twice a month or every week in summer, when the possibility of growing abundances of HAB species are bigger. This is important because toxic metabolites of HAB taxa can accumulate in shellfish and cause poisoning. Six different groups of poisonings are known worldwide, three of which are relevant to Slovenian seas: amnesic (ASP), paralytic (PSP) and diarrhetic shellfish poisoning (DSP), since the microalgae that cause them are regularly found in our seas. Toxins that are measured relatively regularly in our shellfish belong to the group of DSP and related lipophilic toxins that cause gastrointestinal symptoms (nausea, vomiting, diarrhoea). DSP is caused by certain species of dinoflagellates from the pelagic genera *Dinophysis* and *Phalacroma* and the benthic genus *Prorocentrum*. So far, about 20 species of the genera *Dinophysis* and *Phalacroma* have been identified in the Slovenian Sea, and toxins have already been detected in at least half of them. In addition to those already listed, we also find the causative agents of PSP (some species of dinoflagellates of the genus *Alexandrium*) and ASP (some species of diatoms of the genus *Pseudo-nitzschia*). Turk Dermastia et al. (2020) identified eight species from the genus *Pseudo-nitzschia* in Slovenian coastal sea. In Slovenian shellfish, the causative toxins have never exceeded the limits (Francé et al. 2013, Francé et al. 2020). We identified HAB species according to Lassus et al. (2016), where as many as 174 HAB taxa are listed and described.

As concerns the microalgal community in brackish environments, the first data in the area of the Port of Koper was obtained during the BALMAS (Ballast Water Management System for Adriatic Sea Protection) project (Kraus et al. 2018) and Pagon's master's thesis in 2019, which otherwise involved only seasonal sampling. A first study of phytoplankton in Škocjanski zatok Nature Reserve was conducted more than 20 years ago (Čermelj et al. 2000), while, to the best of our knowledge, there was no study of the microalgal community in Stjuža yet.

1.4 Objectives and hypotheses

The aim of the master's thesis is to provide a detailed overview of the biodiversity of microalgae, with emphasis on planktonic algae - phytoplankton, in the Port of Koper, Stjuža lagoon and Škocjanski zatok Nature Reserve (hereafter Stjuža and Škocjanski zatok, respectively) and to determine how the occurrence of species varies depending on the location and time of sampling. The focus of this study is also on non-indigenous species, (NIS) as highly modified and/or freshwater environments are an important recipient sites for NIS. Among the latter, as among native species, there may be those that cause harmful algal blooms (HAB) that can have significant negative impacts on the ecosystem and humans.

These are also being investigated in this study. By comparing the species diversity of phytoplankton in the Stjuža and in the sea, we will determine whether the so-called marinization (the transformation of brackish water into a marine environment due to rising water level and saltwater intrusion into lagoons) occurs in transitional ecosystems such as lagoons.

Hypotheses:

1. Brackish and heavily modified water bodies in transitional waters (Škocjanski zatok, Stjuža, Port of Koper) are similar in microalgae composition.
2. Species diversity of microalgae in brackish environments differs from that in the coastal sea.
3. More non-indigenous species are found in brackish waters than in marine environments.
4. Species indicating transformation of lagoons into marine environments can be observed in Škocjanski zatok and Stjuža.

2 MATERIALS AND METHODS

The master thesis was carried out at the Marine Biology Station Piran (National Institute of Biology) as part of the project "Monitoring the diversity and abundance of non-indigenous species in the Slovenian sea" commissioned by the Ministry of Agriculture, Forestry and Food and as part of the ARRS research program "Coastal Sea Research". The study focused on the inventory of microalgal flora in important recipient sites for non-indigenous species in the Slovenian sea (Gulf of Trieste), namely the Port of Koper, the Stjuža lagoon and the Škocjanski zatok Nature Reserve. The results were then compared with those of the marine coastal station 000F, which is designated as a marine LTER site "Gulf of Trieste" in the LTER-Slovenia network and represents near-natural, undisturbed conditions in the Gulf of Trieste (Figure 2).

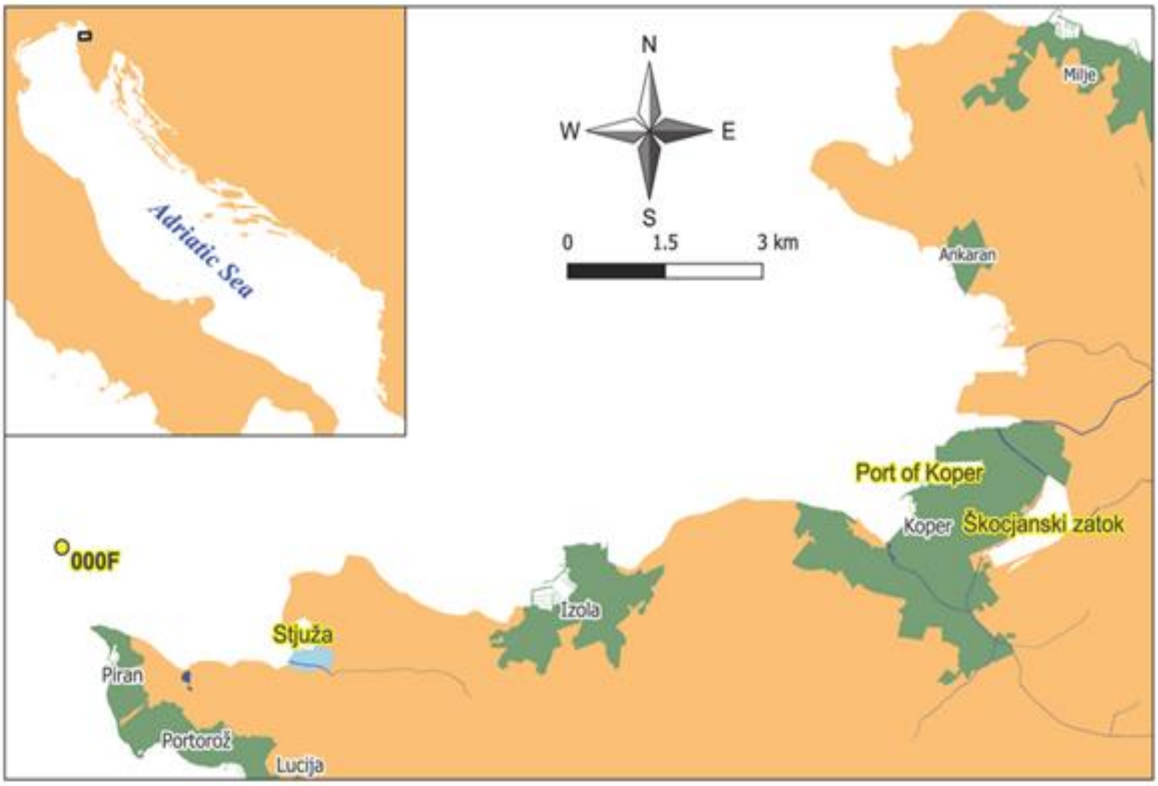

Figure 2: Map of Slovenian sea with marked sampling sites (yellow) (Drawing: Milijan Šiško)

2.1 Description of the study sites

The northern Adriatic is the northernmost protrusion of the Mediterranean and ends with the Gulf of Trieste. The entire area is heavily influenced by rivers, the Po being the largest river flowing into the northern Adriatic, and is home to large urban, industrial and agricultural settlements (Malone et al. 2021).

The Gulf of Trieste is a semi-enclosed basin bordered on the south by Cape Savudrija and on the west by the city of Grado. It is a shallow bay with a maximum depth of 25 m, while the water in a good fifth of the bay is not deeper than 10 m. The Gulf of Trieste is divided by three countries: Italy, Croatia and Slovenia (Orožen Adamič 2002). The Gulf of Trieste has a surface area of about 600 km2, volume of 9.5 km3 and average freshwater input of 150 m3 s-1, giving an average volume-specific discharge nearly 3 times higher than the Northern Adriatic as a whole (Malej et al. 1995).

The Gulf of Trieste is mainly continental, which is reflected in the high temperature variations of the seawater: In summer, temperatures reach 26°C or more, while in winter they can drop to 6°C. High variations in salinity are also characteristic, but only in the surface layer (between 28.0 and 38.5). The stability of the water column changes seasonally: in spring, due to the warming of the surface layer and the increase in freshwater inflows, a pycnocline begins to form, which is more intense in summer. In autumn, due to the cooling of the atmosphere and more intense winds, the water layers begin to mix and the stratification of the water column is interrupted (Malačič 1991). In the last decade, an increase in seawater temperature and salinity has been observed in the area, especially at the surface, and a decrease in nitrate and silicate has been recorded (Malačič et al. 2006; Mozetič et al. 2012). During the period 2008-2018, bottom temperature in the GoT increased at a rate of 0.25°C per year, while sea surface temperature increased at a rate of 0.11°C +/- 0.025°C per year, whereas during the period 1995-2015, sea surface temperature in the GoT increased at a rate of 0.08°C +/- 0.01°C. Preliminary analyses conducted by the Marine Biological Station in GoT in the period 2008-2018 also show a statistically significant positive trend in bottom salinities in the coastal waters of Slovenia, ranging from 0.04 to 0.08 (Boicourt et al. 2020).

Due to peculiar characteristics of the Gulf, the response of the ecosystem to different environmental changes can be rapid and therefore easier to detect (Malej et al. 1995). For example, the carbon cycle is strongly influenced by freshwater input, and by atmospheric influences, mainly by the strong East-North-East bora wind (Cibic et al. 2018).

2.1.1. Port of Koper

The Port of Koper (Fig. 2) is the only Slovenian international cargo port recognized as one of the core ports of the European Union due to its location (Koper lies on the axis of two major European transport corridors, the Adriatic-Baltic and the Mediterranean) (Luka Koper 2015). Since the port is located on the northernmost part of Adriatic Sea, it represents a natural waterway that penetrates deep into the European continent.. Port of Koper is connected to all major ports in the world by regular shipping lines (Twrdy & Hämäläinen 2017). The port is divided into three basins (indicated as PBS1, PBS2, and PBS3 in Figure 2) with two piers that provide specialized terminals for handling a variety of shipments and goods. Basins PBS1 and PBS2 have a maximum depth of about 14 m, while basin PBS3 has a depth of about 18 m. The anchorage for waiting vessels is located 2 to 5 km outside the harbour and has a depth of 17 to 19 m (indicated as sampling site PBS5 in Figure 2). The river Rižana, which is the main source of fresh water in the region, discharges into the second basin and carries treated municipal and industrial wastewaters (Mozetič et al. 2015). Samples were collected at sampling sites in basins PBS1 and PBS2 and at anchorage PBS5.
The original soil below the harbour consists of a deposit of soft marine clay up to 30 m thick, underlain by flysch rock or by medium-dense to dense sandy and silty gravel deposited there by the Rižana River (Logar 2012). Ports are often a catchment area for non-indigenous organisms due to the possibility of their introduction and colonisation with ballast water and sediments. The Port of Koper is primarily a transshipment port, which means that it can be both a receiving and donor environment.

Figure 3: Port of Koper with three port basins and the anchorage where sampling was carried out (except for the third basin PBS3). The outflow of the river Rižana in the second basin is marked with an arrow (Source: Google maps)

2.1.2. Stjuža lagoon

In addition to ports, lagoons, wetlands and mariculture are also important recipient environments for non-indigenous species. Due to their location between the marine and freshwater environments, lagoons have a special ecological role. They are very productive ecosystems due to nutrient input from the land, sea (tidal), atmosphere, and sediments (Šajna et al. 2005; Batelli and Gregorič 2020). Stjuža is the only Slovenian marine lagoon (Fig. 3) and belongs to the Natura 2000 network of protected areas as part of the Strunjan Stjuža Nature Reserve. It is a shallow, partially enclosed brackish lagoon divided into two parts: first, smaller, deeper part of the lagoon and a larger main lagoon formerly used for fish farming. After aquaculture in the area was abandoned, the lagoon was closed to fishing about 400 years ago and remained connected to the sea through three channels. This is where the name Stjuža comes from - from the Italian word 'chiusa', which means closed (Šajna et al. 2005; Batelli and Gregorič 2020; Park Strunjan 2022). The shoals were created by the deposition of sediments at the mouth of the Strunjan river. After the construction of the dam that artificially closed the bay and the lagoon remained connected to the sea only by current channels. The exchange of water between the lagoon and the sea depends only on the tides. Since the lagoon is on average only half a meter deep, the water in the lagoon warms up and cools down quickly (Park Strunjan 2022). This artificial lagoon is not only an important habitat for water birds, but is also characterized by a seagrass meadow of lesser Neptune grass (*Cymodocea nodosa*) and dwarf eelgrass (*Zostera noltei*), and halophytic vegetation.

Figure 4: Stjuža lagoon with marked sampling area (left) and marked sampling sites ss1 and ss2 (right) (Source: Google maps)

2.1.3. Škocjanski zatok Nature Reserve

The Škocjanski zatok Nature Reserve is largest semi-saline wetland (Fig. 5) in southwestern Slovenia, covering 122.7 ha and is a remnant of a sea bay where the rivers Rižana and Badaševica flowed into the sea. It consists of two parts - the freshwater part of the reserve, called Bertoška bonifika, which is characterised by marshy meadows and water areas of varying depths surrounded by reeds and thermophilic shrubs, and the brackish lagoon with shallow and muddy bottom influenced by tides and covered with various halophytes (Šalaja and Mozetič 2007; Mozetič et al. 2010). In Slovenia there are no natural lagoons, therefore the artificial lagoon (i.e. of anthropogenic origin) Škocjanski zatok represents a semi-enclosed euryhaline and eurythermal habitat (Lipej et al. 2020).

Škocjanski zatok is surrounded by the coastal town of Koper, mainly by the portual ara, highway and railroad, and other suburban areas. It can therefore be considered an urban wetland according to the Ramsar classification. As it is located near the city of Koper, it belongs to the coastal region of Koper and is also a part of the Bay of Koper and the Gulf of Trieste (Učakar 2009; Lipej et al. 2020).

Figure 5: Škocjanski zatok with marked sampling area (Source: Google maps)

2.2 Sampling and field measurements

Phytoplankton was sampled with phytoplankton net with mesh size of 20 μm (KC Denmark) between April 2018 and May 2021 in three sampling areas: the Port of Koper, Stjuža and Škocjanski zatok (dates in Table 1). In the Port of Koper, samples were collected once per month from April 2018 to March 2019 in three sampling sites (Fig. 3): two were located in port’s basins with active berths (PBS1 and PBS2) and one in front of the two basins (PBS5). From October 2020 to April 2021, samples were again collected once a month, but this time only in PBS2. At stations PBS1 and PBS2 phytoplankton was collected by vertical net tows (two tows 10 to 15 m apart) (Fig. 6), while at station PBS5 horizontal tows were done at the depth of approx. 2 m along a 120 m long transect.

In the Škocjanski zatok, sampling station was located in the northern part of the brackish lagoon, where seawater from the Port of Koper flows through a sluice system (Fig. 7). Because of the difficult access to the lagoon and its shallowness (1m depth), we took the samples by canoe in the deepest part of the channel (Fig. 5). Samples were taken by two horizontal net hauls just below the water surface, each for about one minute in one direction, avoiding sampling the same transect both times. Samples were collected every other month from April 2019 to January 2020 and in certain months between August 2020 and February 2021 to fill in missing gaps, i.e., seasons in the sampling scheme (Table 1).

In Stjuža, we sampled only twice: on 25.8.2020 and on 4.11.2020. Two sampling sites were selected: one in the first, deeper part of the lagoon, which is connected to the sea (ss1), where a horizontal net tow was done from the boat along a 50 m long transect, and one in the main lagoon (ss2), where several vertical tows were performed manually from an old wall (Fig. 4). At both sites, we also measured temperature and salinity at the water surface with a WTW Multi 3620 IDS probe. We started measuring temperature and salinity in August 2020. Starting in October 2020, measurements were made at the surface and at 1 m depth. In December 2020, only surface measurements were performed. In the Port of Koper, we only measured the parameters in basin PBS2, which is the representative sampling site for a brackish environment, since river Rižana flows into it. In Stjuža we only took measurements on the surface. In the nature reserve Škocjanski zatok, no measurements were performed due to the difficult conditions for sampling.

In the same weeks that the sampling was carried out in Stjuža, we also took samples in the port of Koper and Škocjanski zatok, so that we could compare the microalgae communities in all three areas considered. Samples were stored in dark bottles with a volume of 0.5 l.

They were fixed with 4% formaldehyde (previously neutralized with hexamine) upon arrival from the field and stored until examination.

Table 1: Dates of sampling in three areas considered.

Port of Koper	Škocjanski zatok	Stjuža
6.04.2018		
7.05.2018		
5.06.2018		
3.07.2018		
1.08.2018		
13.09.2018		
5.10.2018		
7.11.2018		
3.12.2018		
9.01.2019		
7.02.2019		
5.03.2019		
	25.04.2019	
	20.06.2019	
	29.08.2019	
	15.01.2020	
27.08.2020	28.08.2020	25.08.2020
8.10.2020		
4.11.2020	3.11.2020	4.11.2020
27.11.2020	28.11.2020	
16.12.2020		
28.01.2021	15.01.2021	
19.02.2021	22.02.2021	
26.03.2021		
29.04.2021		
26.05.2021		

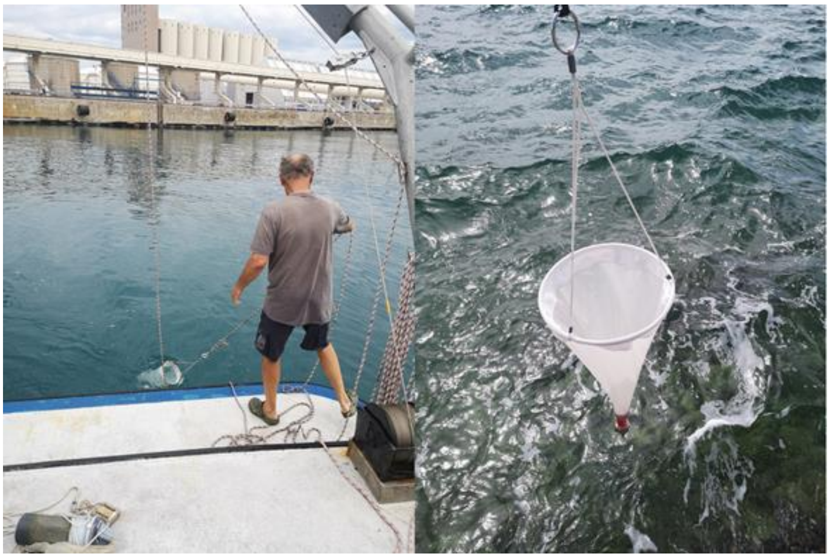

Figure 6: Sampling in Port of Koper (left) and phytoplankton net (right).

Figure 7: Sampling in Škocjanski zatok.

2.3 Laboratory analysis

Before examination under the microscope, the samples were mixed well and 2.5 ml was poured into a sedimentation chamber where it was allowed to settle overnight or for at least two hours. An Axio Observer Z1 (ZEISS) inverted epifluorescence microscope with an AxioCam Mrc5 (ZEISS) integrated digital camera was used to examine the samples. To analyze the sample as accurately as possible, the entire bottom of the chamber was examined, mostly at 200x magnification, but also at 400x for smaller species. Some smaller and/or more difficult-to-identify species were also examined at 1000x magnification with immersion oil. Some armoured dinoflagellates were stained with the dye Calcofluor white M2R (Fritz and Triemer 1985), which allowed us to view dinoflagellate plates whose distribution is an important taxonomic feature. In cases where difficult i.e., challenging organisms could not be identified even with SEM, other sample preparation protocols were used. These organisms were isolated from fresh unfixed samples. In this way, we had concentrated samples that facilitated identification.

The scanning electron microscope (TESCAN MIRA; SEM) has been used to identify certain specimens that could not be identified under the light microscope (LM). Most of the samples were prepared for the SEM following the protocol we developed and worked best: 2 mL of the sample prefixed with 4% formaldehyde was filtered onto a 10 μm polycarbonate filter and dehydrated with alcohol (20%, 40%, 60%, 80%, 90%, 99%, abs. EtOH). After adding a drop of hexamethyldisilazane (HMDS), the sample was air dried for approximately 12 hours. The filter containing dried organisms was then imprinted on an electron microscopy stub equipped with an adhesive carbon disk. Prior to examination, we coated the samples on the stub with a 5 nm thick layer of Au-Pd using a Q150R S/E/ES Plus sputter (Quorum).

Sputter coatings are used in scanning electron microscopy to create an electrically conductive thin film needed for studying surface topography of the sample. Such films prevent "charging", reduce thermal damage, and improve secondary electron emission.

In our case, the method that best worked for diatoms is provided by Trobajo and Mann (2019), where diatom samples are prepared and cleaned directly on coverslips. Because marine, brackish, and hypersaline samples must be prewashed to remove most salts, which otherwise prevent the cells from adhering to the coverslips, we first fixed the sample with ethanol (final concentration 30%) in a tube. This fixes the diatom cells, promotes their sedimentation, and also dilutes the salts. After allowing the cells to sediment (we let them sit overnight), we removed the supernatant and added deionized water using a Pasteur pipette. These steps were repeated two more times. We then removed a small volume of the sample (the pellet) with a pipette and placed it on a clean, dry coverslip. We allowed the coverslips containing the sample to dry undisturbed overnight and avoided dust contamination by covering the samples lightly with aluminium foil. Once the coverslips were completely dry, we placed them on a ceramic hot plate in a fume hood using fine forceps with the diatoms facing up. We left enough space between the coverslips to avoid the possibility of material splashing from one coverslip to the other and causing cross-contamination. We heated the hotplate to about 90°C and added the oxidant (65-70% HNO3), which was previously added to a beaker with a Pasteur pipette to obtain a domed meniscus. We allowed the acid to evaporate, which took about 10-15 minutes. We added the acid two more times until there was no coloured residue and all visible residue (the diatoms) was white. After the last acid treatment, we allowed all the acid to evaporate. We then transferred the coverslips to the paper towel with tweezers, taking care to place them in known positions with the diatoms facing up and noting the positions. Using a Pasteur pipette, we carefully added deionized water to form a fully domed meniscus that covered the entire coverslip. After 10 minutes, we drained the coverslip by tilting it about 80° to the horizontal, then returning it to the horizontal and repeating this step again. In the end, we drained and completely air dried the coverslip, then placed it on a stub and coated it with a 5nm thick Au-Pd layer and examined with SEM.

All samples were examined in high vacuum, usually at an energy of 2 (or sometimes 5) keV, a beam current of 100 pA, and using a secondary electron (SE) or In-Beam SE detector.
The organisms observed were identified to the smallest possible taxon using various phytoplankton identification keys and literature: Hustedt 1930, Rampi and Bernhard 1980, Dodge 1982, Tomas 1996, Faust and Gulledge 2002, Viličić 2002, Pompei et al. 2003a, Pompei et al. 2003b, McDermott and Raine 2006, Kraberg et al. 2010, Larink in Westheide 2011, as well as various online sources and scientific papers.

2.4 Statistical analyses

Statistical analyses were carried out using the PRIMER-7 software package to find similarities among years, seasons and sampling areas and to estimate biodiversity in each sample (Clarke and Gorley 2015). Similarities between different samples in terms of taxonomic composition of microalgal communities were estimated with the Sørensen similarity, which is Bray-Curtis similarity calculated on presence/absence data. The Sørensen similarity between any two samples is calculated with the following equation:

$$\mathbf{S8 = 100 * 2a / (2a + b + c)}$$

where a is the number of taxa present in both samples; b is the number of taxa present in the first sample but absent from the second sample; and c is the number absent in the first sample but present in the second sample (Clarke and Gorley 2015). These similarities were graphically represented using non-metric multidimensional scaling (nMDS).

First, we compared different sampling sites in brackish habitats and the coastal waters. For the latter, we took into account phytoplankton data from the LTER site (LTER 000F, data from MBP-NIB database), the reference station for national monitoring of the ecological status of the sea of the Slovenian Environment Agency (ARSO). For this analysis, we used just the data from August and November 2020 in which all sampling sites were sampled. Second, we explored similarities within and between seasons for Port of Koper and LTER 000F in the period 2018-2021. The samples were grouped into four seasons as follows: Spring (April - June), Summer (July - September), Autumn (October - December), and Winter (January - March) (after Grilli et al. 2020).

In order to identify the species that contribute the most to the similarities within and between groups of samples (i.e., sampling sites/habitats or seasons) we performed the SIMPER analysis (Clarke and Gorley 2015), which was calculated from Bray-Curtis similarity. To test the statistical significance of the similarities within and between groups of samples, we used PERMANOVA (Permutational MANOVA).

3 RESULTS

3.1 Environmental characteristics of the brackish environments on the Slovenian coast

This chapter presents the temperature and salinity data in the sampling areas. Water temperature in Port of Koper (station PBS2) ranged from 9.3°C in February to 25.4°C in August and was in general similar between surface and 1 m depth (Table 2). On the contrary, salinity difference between surface and 1 m was at times very large, indicating the influence of the river Rižana discharge in the surface layer (Table 2). The lowest salinity (12.0) was measured in November 2020.

Table 2: Water temperature and salinity in Port of Koper (PBS2).

Date	27.08. 2020	8.10. 2020	4.11. 2020	27.11. 2020	16.12. 2020	28.01. 2021	19.02. 2021	26.03. 2021	29.04. 2021	26.05. 2021
T surface	25.4	15.5	18.2	12.1	10.6	10.1	9.4	11.6	13.9	14.9
T 1m	/	20.6	18.6	14.3	/	10.8	9.3	11.1	13.4	16.5
salinity surface	36.5	15.8	36.1	12.0	15.1	31.0	29.0	35.4	12.9	19.0
salinity 1m	/	35.6	37.2	36.7	/	38.0	37.2	37.2	36.6	35.7

In Stjuža (Table 3), the water temperature ranged from 17.7°C in November to 24.6°C in Avgust 2020. Temperature was similar in both sampling sites (ss1 and ss2), while salinity was exactly the same in both sites. The salinity reported in the results is typical of the marine environment

Table 3: Water temperature and salinity in Stjuža.

Date	**T $_{ss1}$**	**T $_{ss2}$**	**Salinity $_{ss1}$**	**Salinity $_{ss2}$**
25.8.2020	23.7	24.6	38.4	38.4
4.11.2020	18.0	17.7	37.2	37.2

3.2 Diversity of microalgae in the brackish environments on the Slovenian coast

The taxa from the individual sampling sites of Port of Koper and Stjuža were pooled and are presented together for the respective brackish water environment. At all three sampling sites combined, we identified 284 taxa (Appendix A). The highest number of taxa belonged to the Dinophyceae (126) and Bacillariophyceae (125). A substantially lower number of taxa was assigned to other groups: 15 to the Coccolithophyceae, 4 to the Dictyochophyceae, one to the Ebriida, two to the Euglenophyceae, three to the Chlorophyta, two to the Ochrophyta, and 6 to the Cyanophyceae. Most of the taxa observed were planktonic marine organisms, but some were brackish or even freshwater organisms and some of them were benthic.

Because of the mesh size of the phytoplankton net, most of the taxa found belong to the microphytoplankton size class (> 20 µm). Smaller organisms belonging to the nanophytoplankton size class (2 to 20 µm) were caught incidentally, for example Chrysochromulina spp.

The highest number of taxa (249) was determined in the port of Koper, but we must emphasize that most of the sampling was done here. In Škocjanski zatok we identified 151 taxa and in Stjuža 101 taxa.

The highest share of taxa in the Port of Koper (Fig. 8) was represented by dinoflagellates (47%) with 118 taxa determined, followed by diatoms (42%) with 105 taxa determined. Coccolithophores represented a much smaller share (6%) with 14 taxa. Taxa from other groups together accounted for 5% (7 taxa).

An inverse situation was observed in Škocjanski zatok (Fig. 9), where the majority of taxa (52%, 29 taxa) belonged to diatoms, followed by dinoflagellates (36%, 54 taxa). A small share of 4% (6 taxa) belonged to coccolithophores and taxa from other groups together accounted for 8% (5 taxa).

In Stjuža (Fig. 10), the share of dinoflagellates and diatoms was equal (47%, 47 taxa). Coccolithophores represented 4 % (4 taxa) and all other groups together accounted for 3% (3 taxa)

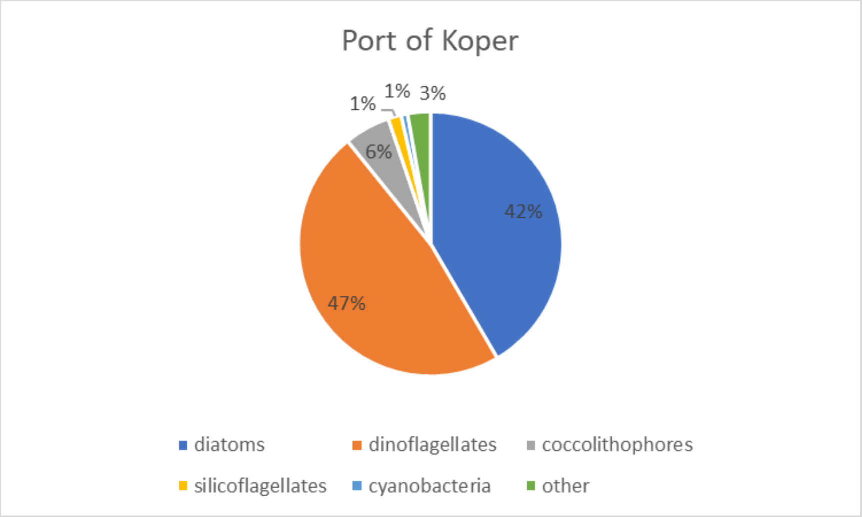

Figure 8: Structure of microalgal community in Port of Koper in the period 2018-2021.

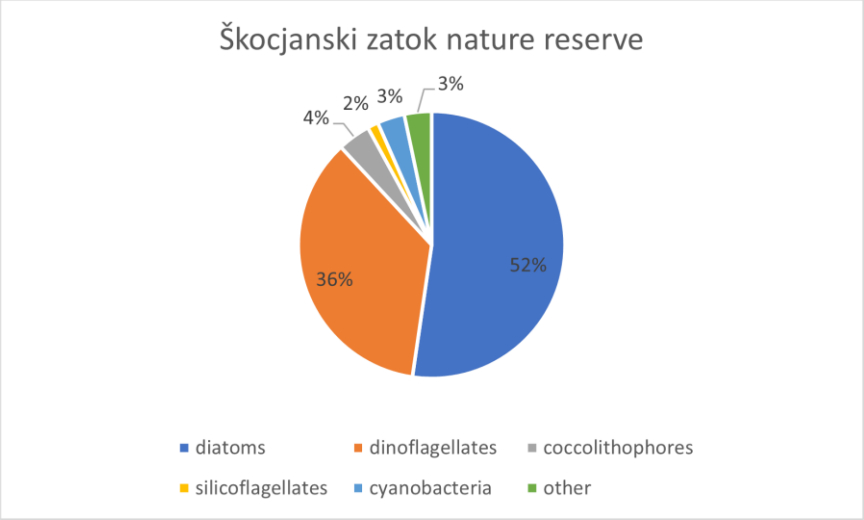

Figure 9: Structure of microalgal community in Škocjanski zatok in the period 2018-2021.

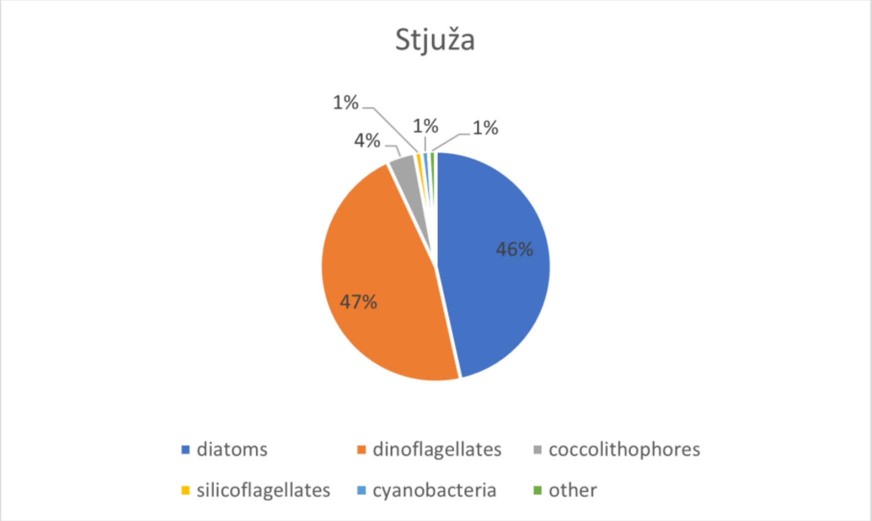

Figure 10: Structure of microalgal community in Stjuža in the period 2018-2021.

Different microalgal taxa prefer different aquatic environments (Fig. 12). The majority of the identified taxa are common in marine environments (235 taxa), with some of them occurring in both marine and freshwater (18 taxa), or even in marine, freshwater, brackish and terrestrial environments (15 taxa). Also exclusively brackish (3) and freshwater (7) taxa were found, especially in the Škocjanski zatok. Prefered environment of taxa found was determined according to the World register of marine species (WoRMS 2022).

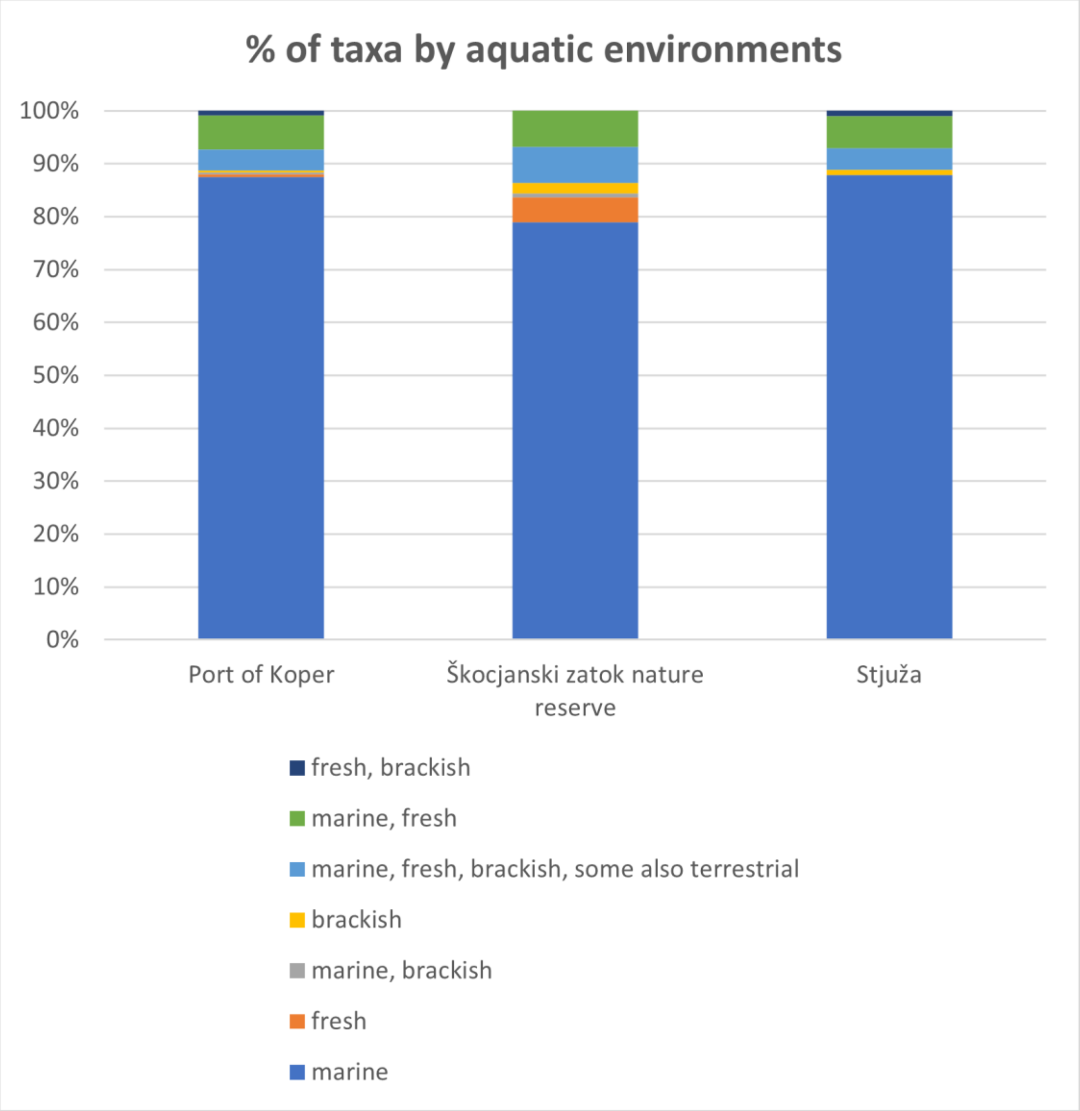

Figure 11: Procentage of taxa by aquatic environments.

3.3 First observed, NIS and HAB species in brackish environments

In this thesis, in addition to first observed species, we also identified NIS, HAB and cryptogenic species. This is also the first inventory of the biodiversity of microalgae in the brackish environments in Slovenian coastal sea.

Table 4 First observed, NIS, HAB, and cryptogenic species from brackish environments ("+" - applies to this taxa, "(+)" - applies to some species of this genus, "?" - not confirmed); numbers show where the taxa was found: 1 - Port of Koper, 2 - Škocjanski zatok, 3 – Stjuža.

	First observed	NIS	Cryptogenic	HAB
DIATOMS / Bacillariophyceae				
Actinocyclus sp.$_{1,2}$	+			
Asteromphalus cf. *parvulus*$_{2}$	+			
cf. *Craticula cuspidata*$_{2}$	+			
cf. *Diatoma vulgaris*$_{1,3}$	+			
Chaetoceros cf. *subtilis*$_{1}$	+			
Cocconeis cf. *sawensis*$_{2}$	+			
Corethron sp.$_{2}$	+			
Cymbella spp.$_{1}$	+			
Eupyxidicula turris$_{2}$	+			
Gomphonema cf. *acuminatum*$_{1}$	+			
Gyrosigma fasciola$_{1,2}$	+			
Navicula cf. *subrostellata*$_{1}$	+			
Nitzschia cf. *sigmoidea*$_{2}$	+			
Odontella aurita$_{1}$	+			
Pseudo-nitzschia multistriata$_{1}$		+		+
Pseudo-nitzschia spp.$_{1,3}$				+
Tryblionella sp.$_{3}$	+			
DINOFLAGELLATES / Dinophyceae				
Akashiwo sanguinea$_{1,2,3}$				+
Alexandrium insuetum$_{1,2}$				+
Alexandrium cf. *minutum*$_{1,2,3}$				+
Alexandrium cf. *tamarense*$_{1}$				+
Alexandrium pseudogonyaulax$_{1,2}$				+
Alexandrium spp.$_{1,2,3}$				+
Azadinium caudatum var. *margalefii*$_{1,2}$	+		+	
Coolia monotis$_{2}$	+			+
Corythodinium tesselatum$_{3}$	+			
Dinophysis acuminata$_{1}$				+

	First observed	NIS	Cryptogenic	HAB
Dinophysis acuta[3]				+
Dinophysis caudata[1,2]				+
Dinophysis fortii[1,2,3]				+
Dinophysis ovum[1]				+
Dinophysis parva[1]				?
Dinophysis sacculus[1,2,3]				+
Dinophysis spp.[1]				+
Dinophysis tripos[1,3]				+
Gonyaulax polygramma[1,3]				+
Gonyaulax spinifera[1]				+
Gymnodinium cf. *fuscum*[1]	+			
Heterocapsa group[1,2,3]				+
Lingulodinium polyedra				+
Pentapharsodinium cf. *dalei*[1]	+			
Peridinium quadridentatum[1,2]	+			
Phalacroma mitra[1,2]				+
Phalacroma rotundatum[1,2,3]				+
Prorocentrum cf. *formosum*[1,3]	+		?	
Prorocentrum lima[1,2,3]				+
Protoceratium reticulatum[1]				+
Scaphodinium mirabile[1,2]	+			
Tripos teres[1]	+			
COCCOLITHOPHORES / Coccolithophyceae				
Calcidiscus leptoporus[1]	+			
Helicosphaera cf. *carteri*[1]	+			
SILICOFLAGELLATES / Dictyochophyceae				
Dictyocha crux[1]	+			
Chlorophyta				
Pediastrum sp.[2]	+			
Scenedesmus sp.[1,2]	+			
CIANOBACTERIA / Cyanophyceae				
cf. *Anabaena* sp.[1]	+			(+)
Lyngbya sp.[2]	+			(+)
Nostoc sp.[2]	+			
Merismopedia sp.[2,3]	+		+	
Oscillatoria sp.[1,2]	+			
Synechococcus sp.[2]	+			

In this work, we identified 24 species of dinoflagellates and two diatom taxa that can cause harmful blooms, as well as two species of cyanobacteria (cf. *Anabaena* sp. and *Lyngbya* sp.) also potentially harmful, for a total of 28 potentially HAB taxa (Table 4).

One diatom species, *Pseudo-nitzschia multistriata* (Fig. 12), has been identified as NIS, and two taxa have been identified as cryptogenic, meaning that their origin is not certain (Table 4). These are a dinoflagellate species *Azadinium caudatum* var. *margalefii* and cyanobacteria from the genus *Merismopedia*.

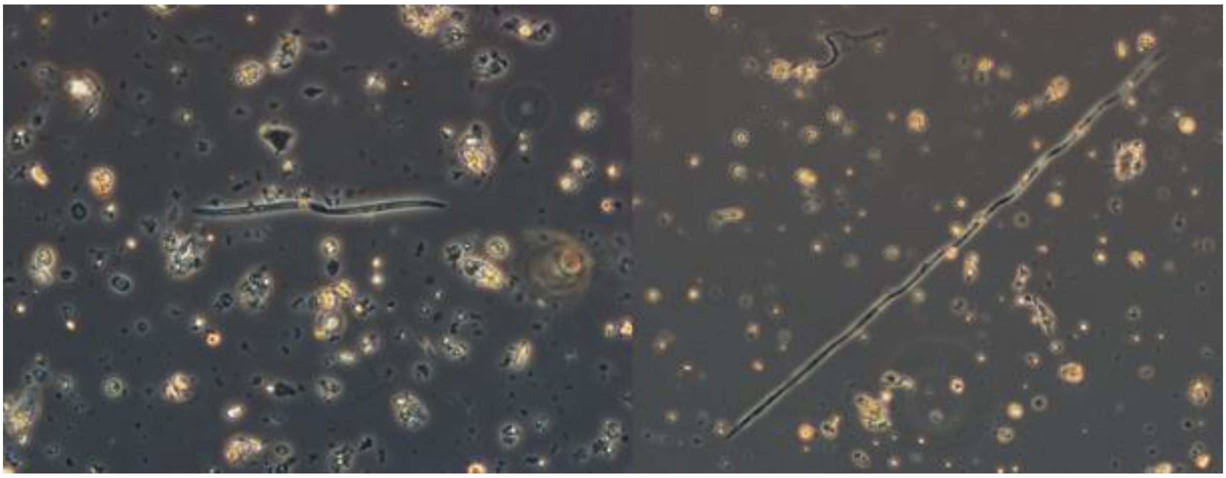

Figure 12: The only NIS species found *Pseudo-nitzschia multistriata* (photo: Janja Francé).

35 of the 284 taxa found were observed for the very first time in the Slovenian sea (Table 4). 19 of them were diatoms, 9 dinoflagellates, two coccolithophores, one silicoflagellate, two chlorophytes and 6 belonging to cyanobacteria. Fig. 13 represents some of such species found in Port of Koper and of which we have photographic material under the light microscope: *Gomphonema* cf. *acuminatum*, *Odontella aurita*, *Helicosphaera carteri* and *Dictyocha crux*. *Gomphonema* cf. *acuminatum* was found in April 2021 and was seen only once. Odontella aurita was observed in samples from January of the same year. Coccolithophore Helicosphaera carteri was observed on two different occasions: first in October 2018 and again in December 2018. Dictyocha crux was also observed only once, in May 2021.

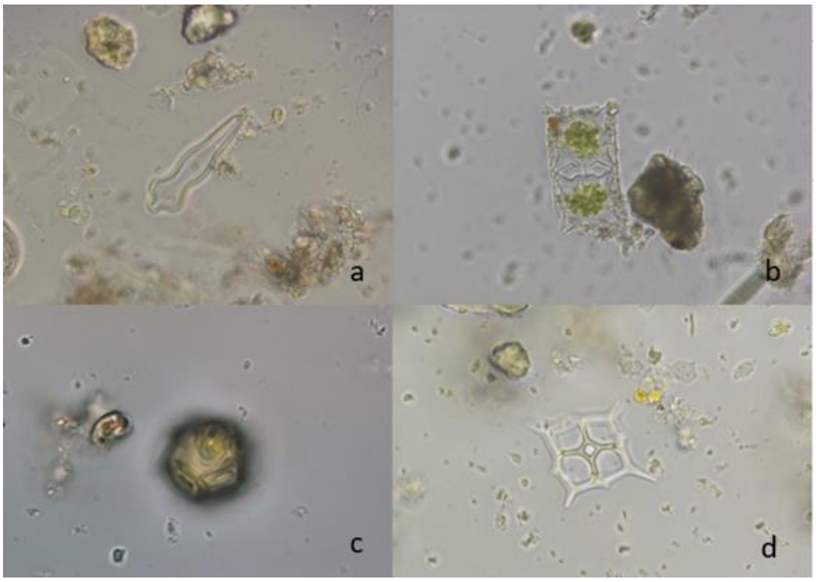

Figure 13: Some of the first observed microalgae: diatoms *Gomphonema* cf. *acuminatum* (a) and *Odontella aurita* (b), coccolithophore *Helicosphaera carteri* (c) and silicoflagellate *Dictyocha crux* (d).

From the first identified dinoflagellates (Fig. 14a) *Azadinium caudatum* var. *margalefii*, which was found in Port of Koper and Škocjanski zatok, was also identified as cryptogenic. First identified freshwater species *Gymnodinium* cf. *fuscum* (Fig. 14b) was found in the PBS2 in Port of Koper, into where Rižana flows in October 2018. Coccolithophore *Helicosphaera carteri* (Fig. 14c) was found in Port of Koper on multiple occasions: in October 2018, November 2018, December 2018 and January 2019. Silicoflagellate *Dictyocha crux* was also found in the Port of Koper, but only in May 2021.

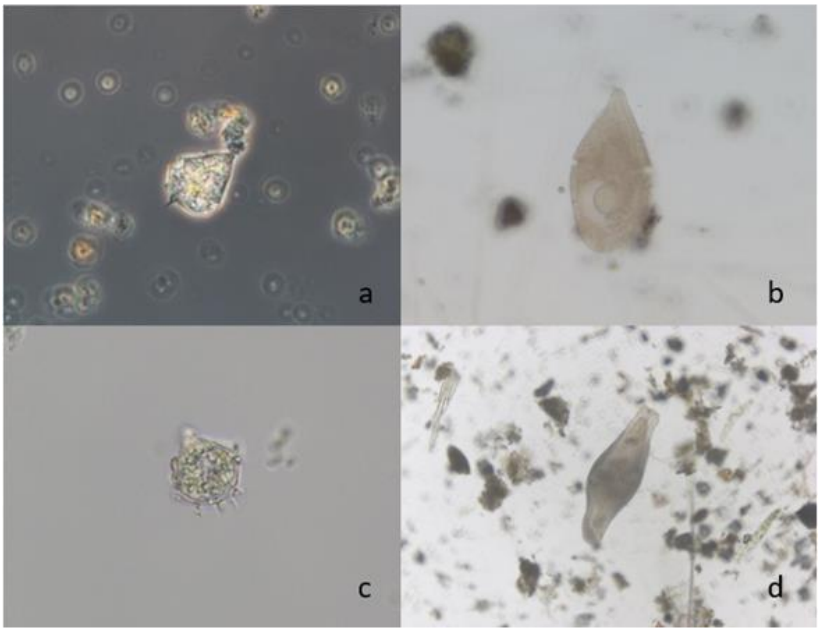

Figure 14: Some of the first observed dinoflagellates: *Azadinium caudatum* var. *margalefii* (a), *Gymnodinium* cf. *fuscum* (b), *Peridinium quadridentatum* (c), *Scaphodinium mirabile* (d).

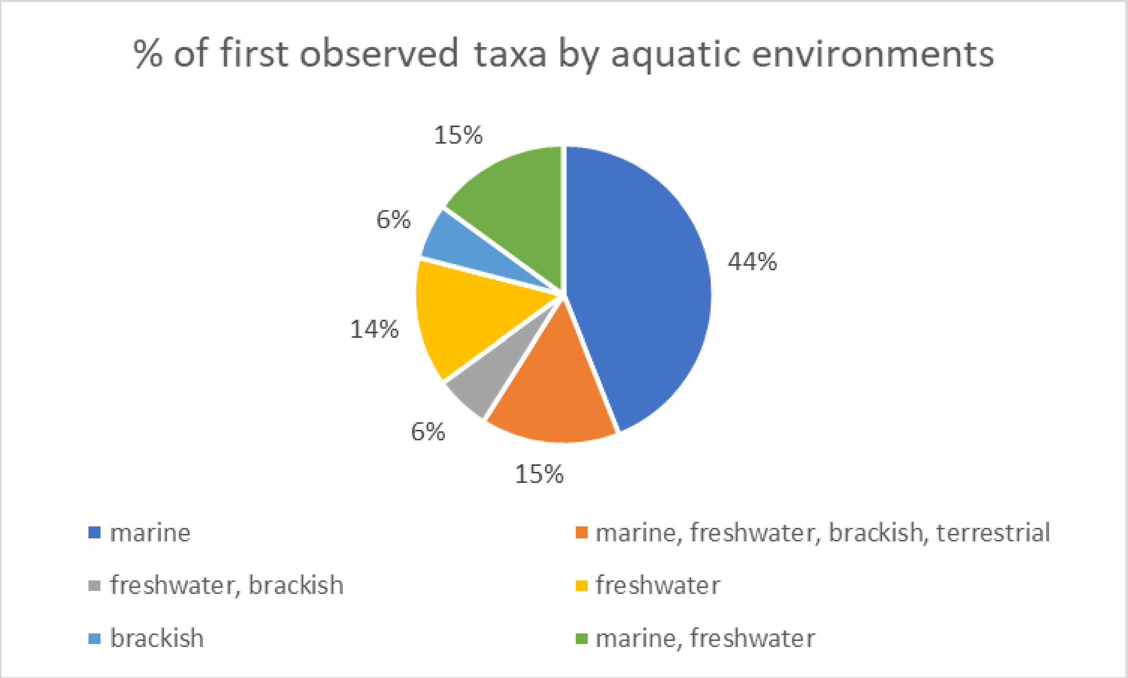

Figure 15: % of first observed species by aquatic environments.

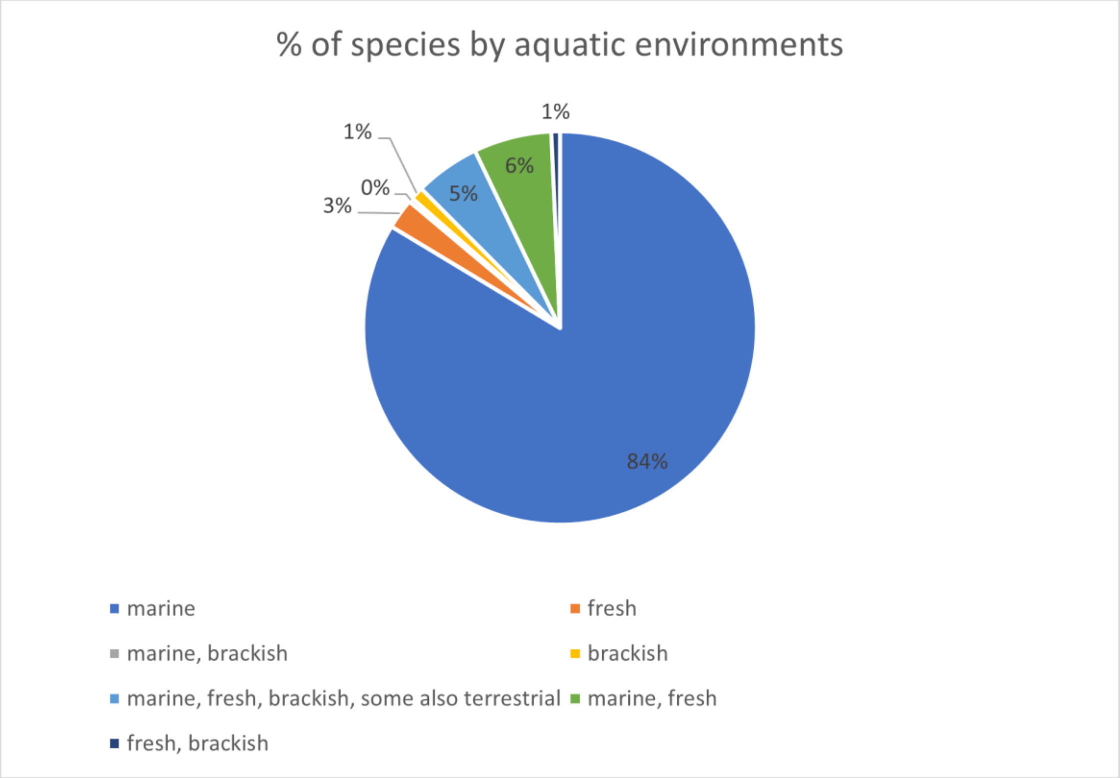

Figure 16: % of species by aquatic environments.

Most of the first identified taxa in all sampling areas together (Fig. 15) were marine (44%), even though we found them in brackish environments. 15% of them can live in both marine and freshwater (FW) environments and another 15% have large salinity tolerance, being able to colonise a wide range of environments, from marine to FW, brackish water, and terrestrial environments. 14% of first identified taxa was exclusively FW, 6% tolerate both FW and brackish water environments and another 6% was exclusively brackish. In comparison, of all the taxa found during the study (Fig. 16), 83.6% were marine, 6.4% can live in marine and FW environments and 5.3% tolerated marine, FW, brackish and some also terrestrial habitats.

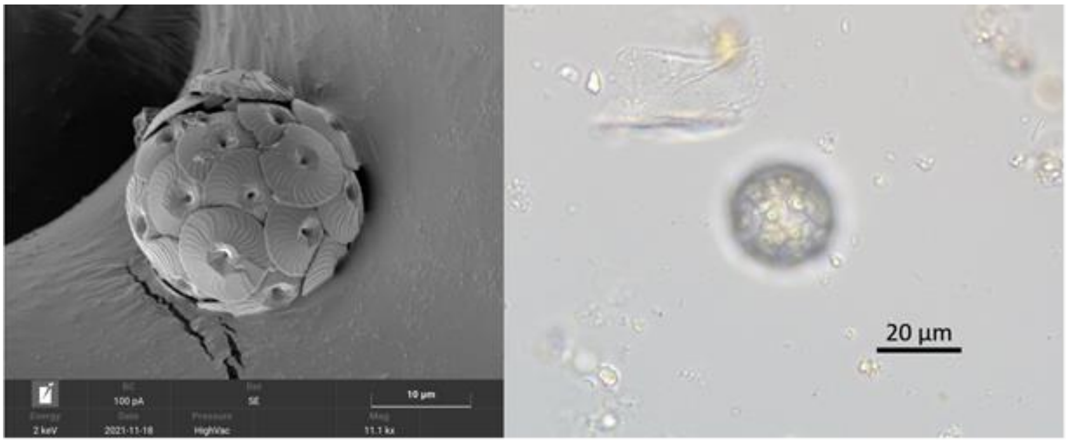

Figure 17: *Calcidiscus leptoporus* photographed with SEM (left) and inverted LM (right).

We highlight those species observed for the first time, except for *Alexandrium insuetum*, that were difficult to identify under the light microscope and required analysis at SEM. These are the coccolithophore *Calcidiscus leptoporus* (Fig. 17), the diatoms *Actinocyclus* sp. (Fig. 18) and *Navicula* cf. *subrostellata* (Fig. 19), and dinoflagellates *Alexandrium insuetum* (Fig. 20), *Pentapharsodinium* cf. *dalei* (Fig. 21) and *Coolia monotis* (Fig. 22).

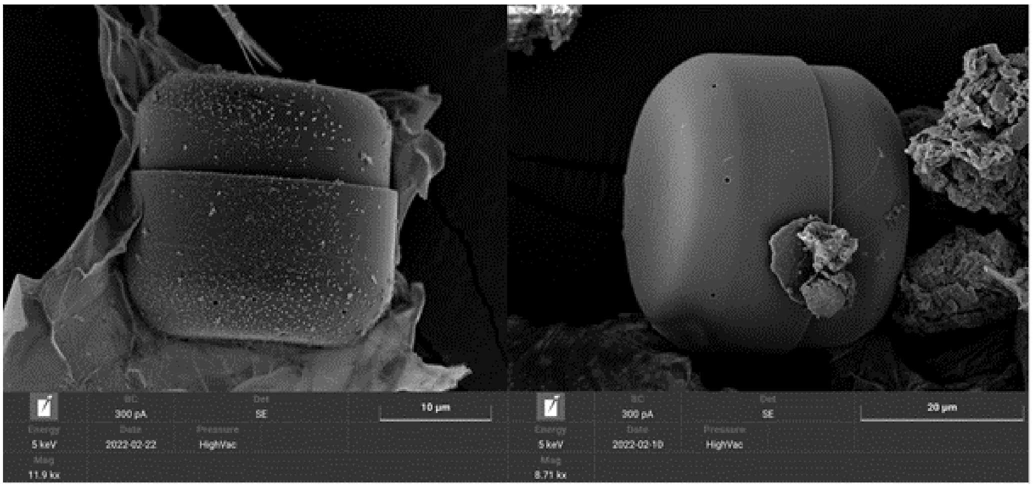

Figure 18: First identified diatom *Actinocyclus* sp.

Actinocyclus sp. (Fig. 18) is a quite large diatom genus found in both marine and freshwater habitats. It was found in port of Koper and Škocjanski zatok and was erroneously identified as *Cyclotella* sp. in culture (I. Vascotto, pers. Comm.).

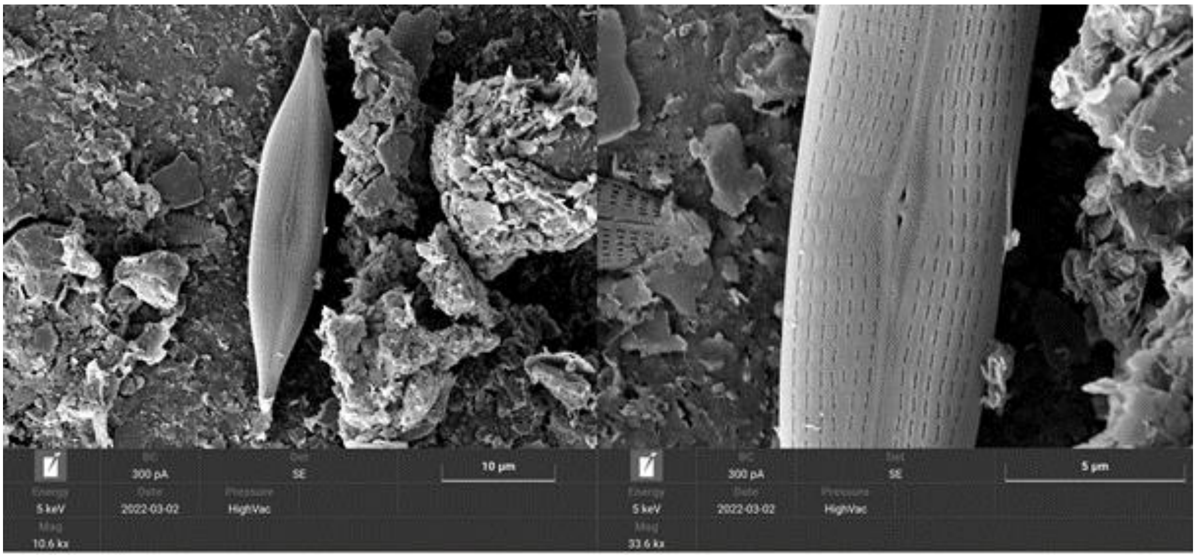

Figure 19: First identified diatom *Navicula* cf. *subrostellata*.

The brackish water diatom *Navicula* cf. *subrostellata* (Fig. 19) belongs to a genus whose species are usually difficult to identify under LM. It was found in Škocjanski zatok.

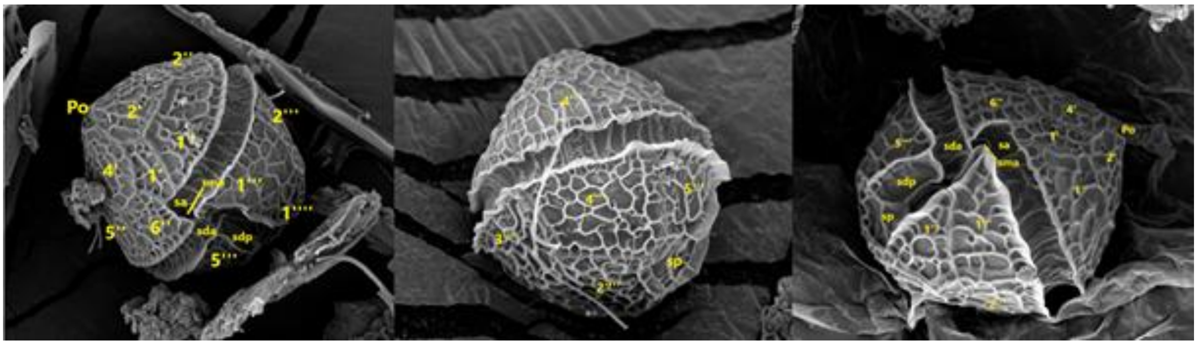

Figure 20: *Alexandrium insuetum* with marked thecal plates after Kim et al. (2016).

Although the species *Alexandrium insuetum* (Fig. 20) occurs regularly in the Slovenian sea, until recently it was not possible to identify it reliably as such because of its close resemblance to *Protoceratium reticulatum* under LM. It also occured regularly in the Port of Koper and Škocjanski zatok. With SEM we were able to see the plates of theca, which was crucial for further detailed identification.

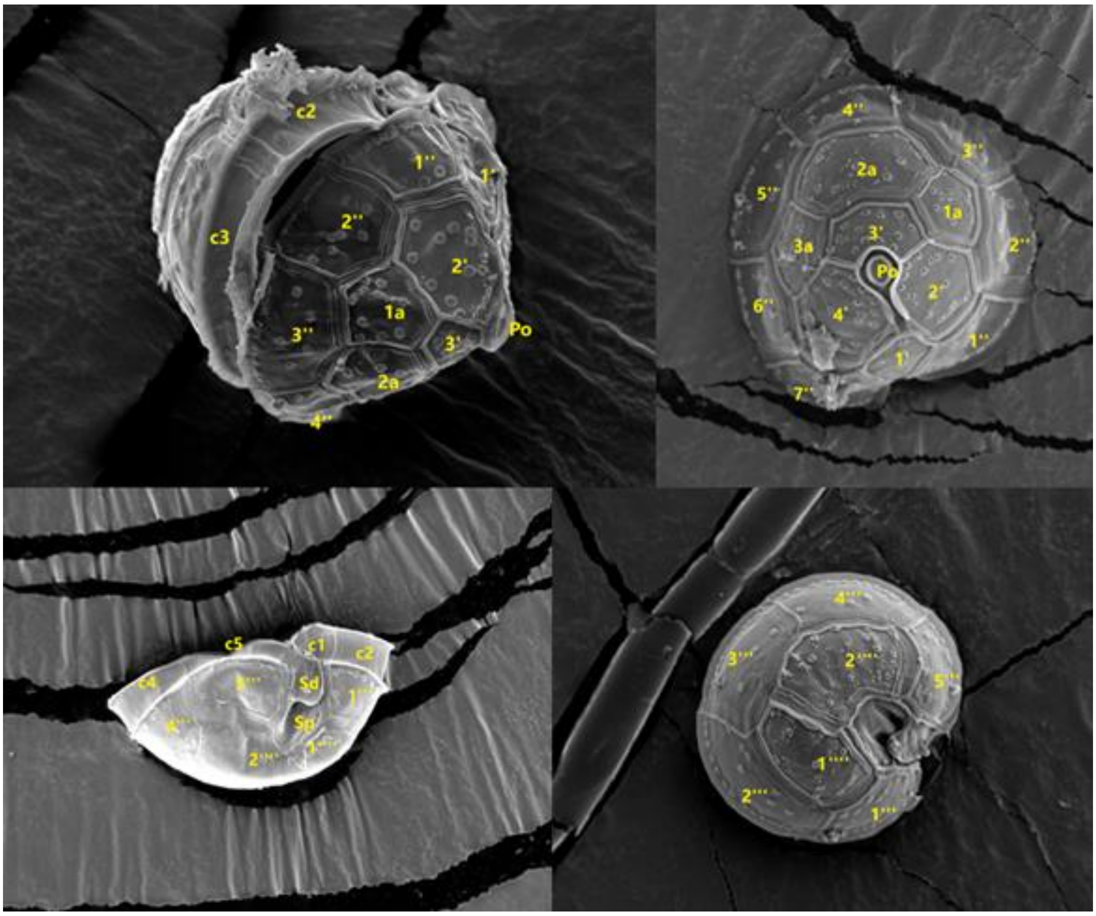

Figure 21: *Pentapharsodinium* cf. *dalei* with marked thecal plates after Gu et al. 2013.

It was similar with the species *Pentapharsodinium* cf. *dalei* (Fig. 21), which is also quite common, but we were unable to identify it to species level due to its small size. With the help of SEM we were finally able to identify it as *Pentapharsodinium* cf. *dalei* using the theca plate tabulation shown in. P. cf. *dalei* was found only in Škocjanski zatok.

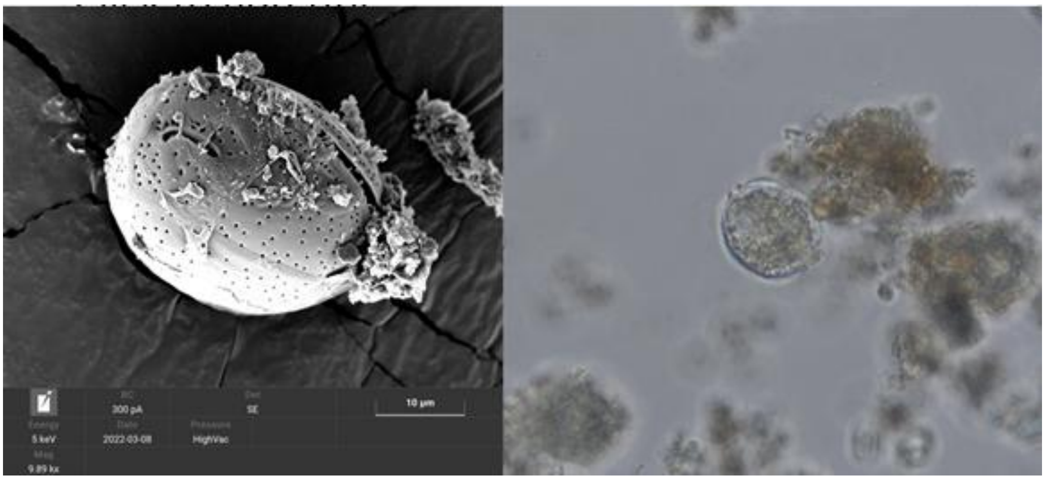

Figure 22: *Coolia monotis* photographed with SEM (left) and inverted LM (right).

Of the 35 newly identified species for Slovenian coastal ecosystems, 13 were planktonic, 12 were primarily benthic, and 10 species occurred in both benthos and the water column and are referred to as tychopelagic species.

In Port of Koper, most of the first identified algal species were planktonic (57%), 29% were benthic, and only 14% occur in both pelagic and benthic environments (Fig. 23). In Škocjanski zatok, mainly benthic species were recorded for the first time (47%), followed by planktonic taxa (37%) and those (16%) occurring either in plankton or benthos (Fig. 23). In Stjuža it was found that among the species observed for the first time, benthic species predominated (57%), followed by planktonic (36 %) and tychopelagic (7 %) (Fig. 23).

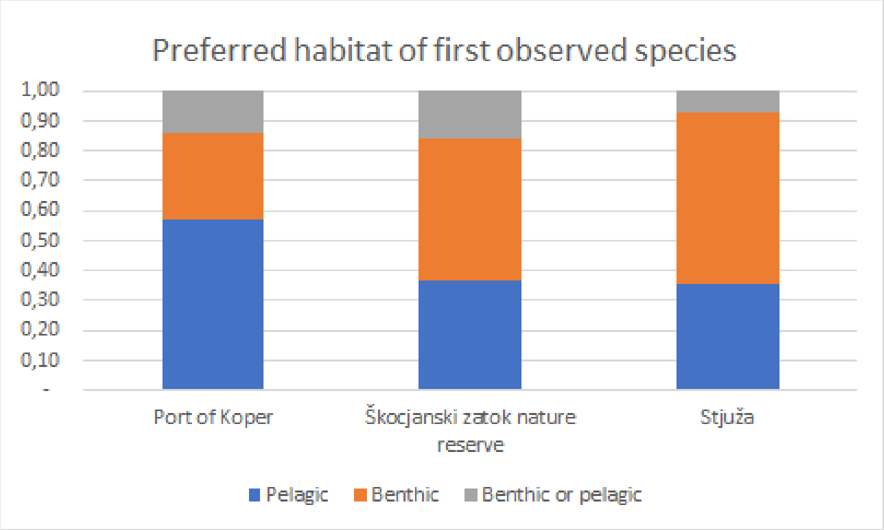

Figure 23: Preferred habitat of first identified species.

3.4 Comparison of biodiversity and seasonal dynamics of microalgae in brackish environments with coastal sea

For comparison of microalgal biodiversity in brackish environments (Port of Koper, Škocjanski zatok and Stjuža) and coastal sea (LTER 000F), we used data from summer (August) and autumn (November) 2020, as this is the only time we sampled at all sampling sites. In the nMDS plot of Sørensen similarity (Figure 24), we see that microalgal biodiversity at LTER 000F is most different from that in brackish water environments. While both samples from LTER 000F group close together, samples from the brackish environments form seasonal groups. Sampling sites ss1 and ss2 in Stjuža are very similar in both seasons, and the summer samples from Stjuža also have 40% similarity with the summer samples from Port of Koper. The autumn sample from the Škocjanski zatok is also 40% similar to the one from Port of Koper, while the summer samples from the Škocjanski zatok differ from all other samples.

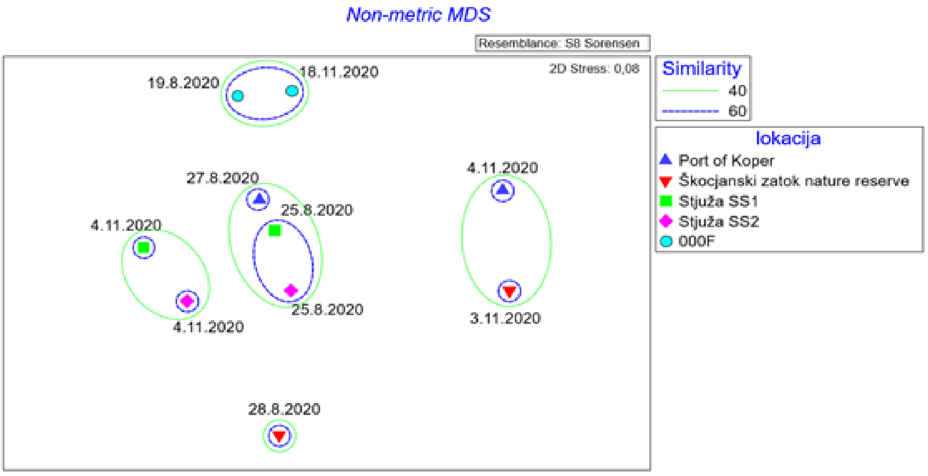

Figure 24: Comparison of microalgal biodiversity of brackish environments and coastal sea in August and November 2020.

According to SIMPER analysis (Table 5), the similarity between different sampling sites in terms of microalgal biodiversity was between 19% (between Škocjanski zatok and LTER 000F) and 48% (between ss1 and ss2). While the location was a statistically significant source of variation (PERMANOVA, Pseudo-F=1.67, df=4, P(perm)=0.0349), the pairwise comparison did not show significant differences between any pair of sampling site (P(perm)>0.05). In line with the grouping in the nMDS, the similarity within the sampling sites was quite low except at station 000F (71.8%), reflecting the differences between the two seasons.

Several species that contributed to dissimilarity between brackish environments and coastal sea were benthic diatoms, for example *Achnanthes* spp. and *Licmophora* sp., which were found only in all brackish environments, *Entomoneis decussata*, which was found only in the Port of Koper and Škocjanski zatok and *Bacillaria paxillifera*, which was found only in Stjuža. Among benthic dinoflagellates, *Prorocentrum lima* was found only in both lagoons. The same is true for the cyanobacteria species from the genus *Merismopedia*.

Table 5: Similarity (%) of microalgal community within sampling sites in brackish environment and coastal sea and between them in August and November 2020.

Average Similarity between/within groups	Port of Koper	Škocjanski zatok nature reserve	Stjuža SS1	Stjuža SS2	000F
Port of Koper	29.5				
Škocjanski zatok	26.3	22.0			
Stjuža SS1	32.2	23.8	39.3		
Stjuža SS2	31.9	29.8	48.1	43.6	
000F	29.2	18.9	32.6	27.5	71.8

Seasonality of the microalgal community in brackish environments was explored only in the Port of Koper with the longest time-series of data (2018-2021) and compared to the seasonality in the coastal sea sampling site (000F) in the same period.

The nMDS plots in Figures 25 and 26 show similarities between seasons for the samples from the port of Koper and LTER 000F, respectively. In the port of Koper (Fig. 25), seasons are not well separated. One group at 40% similarity is formed by most of the autumn and winter samples, but the same samples belong also to a 40% similarity group with some samples from all other seasons. Spring and summer samples were more scattered. One spring and one autumn sample differed strongly from the others. Just a few couples of samples were similar at 60%.

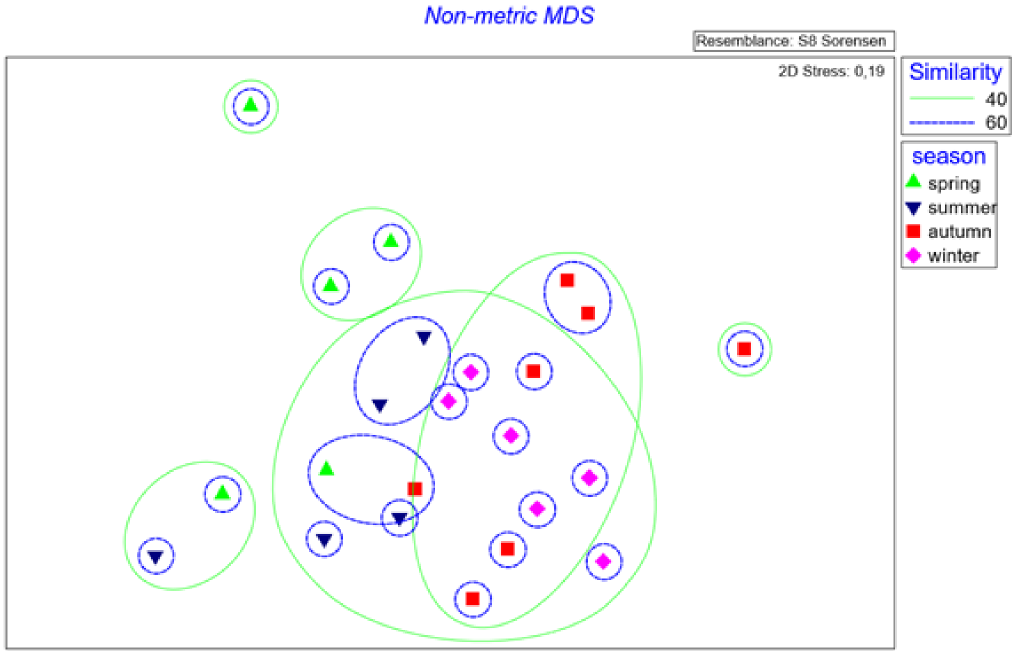

Figure 25: Grouping of samples by season based on species composition of microalgae in the Port of Koper

The SIMPER analysis (Table 6) revealed that the similarity of microalgal communities between different seasons was only between 31.4% (between spring and autumn) and 41% (between autumn and winter). The season was therefore a statistically significant source of variation (PERMANOVA, Pseudo-F=2.22, df=3, P(perm)=0.0001). Differences between all pairs of seasons were also statistically significant (P(perm)<0.05), except for the spring and summer. Relatively low similarities within seasons (33.9% - 47.5%) support the nMDS representation.

Table 6: Similarity (%) of microalgal community in the Port of Koper within and between seasons in the period 2018-2021.

Average Similarity between/within groups	spring	summer	autumn	winter
spring	33.9			
summer	37.2	44.4		
autumn	31.4	37.2	43.7	
winter	33.9	37.9	41.0	47.5

Microalgal taxa that were typical for the seasons in the Port of Koper as revealed by SIMPER analysis are presented in Table 7. Three species of dinoflagellates that were typical for the spring occurred also during summer along with some centric diatom species. Centric diatoms were typical also for autumn along with a pennate genus Pseudo-nitzschia, while a mixed community of diatom and dinoflagellate taxa was typical for winter. Diatom Proboscia alata was typical for both summer and autumn.

Table 7: Microalgal taxa typical for the seasons in the Port of Koper in the period 2018-2021.

spring	**summer**	**autumn**	**winter**
Prorocentrum micans	*Hemiaulus hauckii*	*Guinardia striata*	*Eucampia zodiacus*
Tripos furca	*Leptocylindrus danicus*	*Paralia sulcata*	*Guinardia flaccida*
Tripos fusus	*Proboscia alata*	*Proboscia alata*	*Licmophora* sp.
	Thalassiosira sp.	*Pseudo-nitzschia* sp.	*Nitzschia longissima*
	Prorocentrum micans	*Tripos lineatus*	*Skeletonema costatum s.l.*
	Tripos furca		*Striatella unipunctata*
	Tripos fusus		*Diplopsalis group*
			Tripos fusus
			Tripos horridus

At LTER 000F, which represents the reference conditions of the Slovenian coastal waters, samples from the same season showed a higher similarity than those in the Port of Koper, as inferred from the nMDS plot (Fig. 26). Nevertheless, all samples fall in one 40% similarity group at the same time. Approximately half of the autumn and winter samples formed a group at 60% similarity, while three winter samples differed and formed another group. Spring and summer samples, along with some autumn samples formed a third 60% similarity group. One spring and one autumn sample were different from the others.

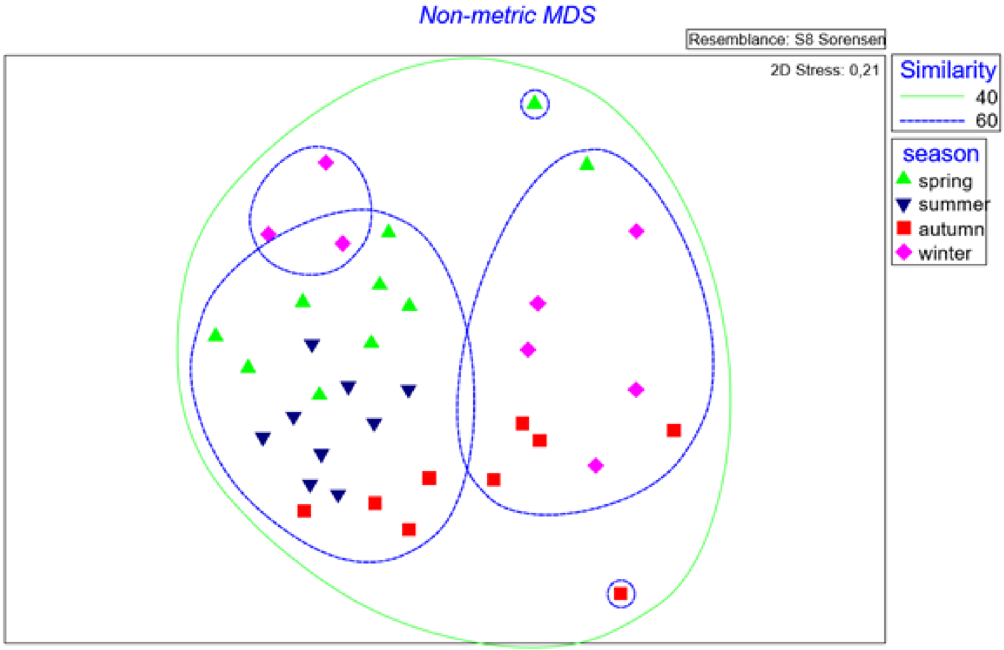

Figure 26: Grouping of samples by season based on species composition of microalgae at LTER 000F.

Also the SIMPER analysis confirmed a higher similarity between and within seasons at the LTER 000F (Table 8) as compared to those at Port of Koper. The similarity of microalgal community between different seasons was between 54.1% (between spring and autumn) and 41% (between spring and summer), but the seasons were still a statistically significant source of variation (PERMANOVA, Pseudo-F=3.641, df=3, P(perm)=0.0001). Differences between all pairs of seasons were statistically significant (P(perm)<0.05). Also the similarities within seasons were quite high, ranging from 57.9% in winter to 68.7% in summer) supporting the nMDS representation.

Table 8: Similarity (%) of microalgal community in LTER 000F within and between seasons in the period 2018-2021

Average Similarity between/within groups	spring	summer	autumn	winter
spring	62.7			
summer	61.8	68.7		
autumn	54.1	60.3	61.4	
winter	56.3	55.9	56.0	57.9

Table 9: Microalgal taxa typical for the seasons in LTER 000F in the period 2018-2021.

spring	**summer**	**autumn**	**winter**
Cyclotella sp.	*Cylindrotheca closterium*	*Cylindrotheca closterium*	*Gymnodinium* sp.
Gymnodinium sp.	*Dactyliosolen fragilissimus*	*Nitzschia sp.*	*Gyrodinium* sp.
Gyrodinium sp.	*Leptocylindrus danicus*	*Thalassionema nitzschioides*	*Heterocapsa group*
Heterocapsa group	*Nitzschia* sp.	*Gymnodinium* sp.	*Emiliania huxleyi*
Prorocentrum cordatum	*Proboscia alata*	*Gyrodinium* sp.	
Emiliania huxleyi	*Pseudo-nitzschia calliantha*	*Emiliania huxleyi*	
	Thalassionema nitzschioides		
	Gymnodinium sp.		
	Gyrodinium sp.		
	Heterocapsa group		
	Prorocentrum cordatum		
	Emiliania huxleyi		

Table 9 presents microalgal taxa typical for each season in LTER 000F. Most of the taxa appeared as typical in more than one season (e.g. dinoflagellate *Prorocentrum cordatum* for spring and summer, diatoms *Cylindrotheca closterium*, *Thalassionema nitzschioides* and *Nitzschia* sp. for summer and autumn). Undetermined naked dinoflagellates marked as *Gymnodinium* spp. and *Gyrodinium* spp. were revealed as typical for the whole year. This was also true for coccolithophore *Emiliania huxleyi*. Just two species revealed as typical for the summer in the Port of Koper, diatoms *Proboscia alata* and *Leptocylindrus danicus*, were also confirmed as typical in LTER 000F.

Microalgal community in the Škocjanski zatok did not show any obvious pattern attributable to seasonal dynamics, so we show just the results of the clustering analysis (Fig. 27).

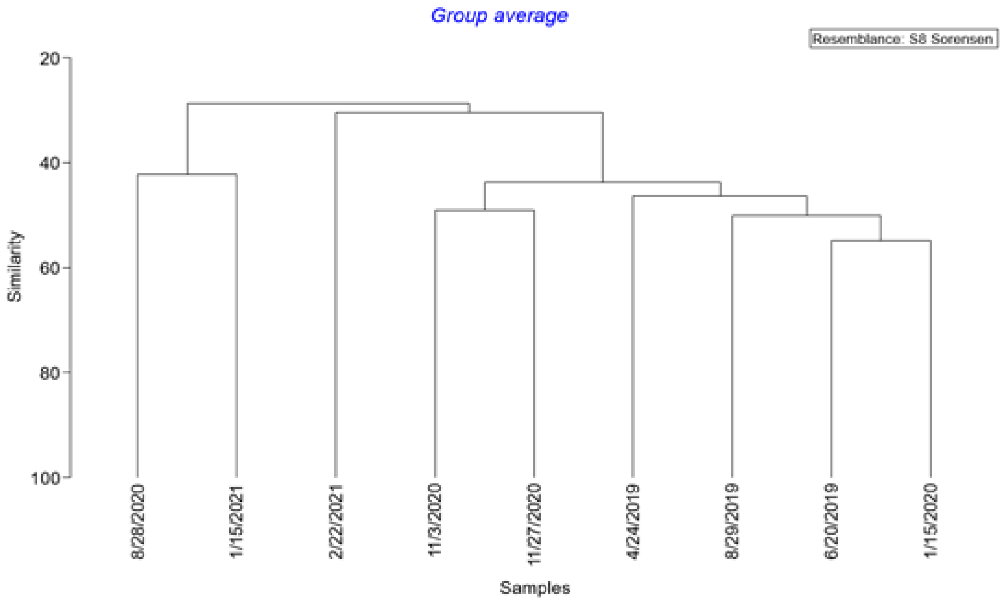

Figure 27: Cluster analysis of samples from Škocjanski zatok.

4 DISCUSSION

The aim of this master's thesis was to provide a detailed overview of the biodiversity of microalgae, with emphasis on phytoplankton, in brackish environments in the Port of Koper, Škocjanski zatok and Stjuža and to determine how the presence of taxa varies depending on the location and time of sampling. The results were compared with results from the LTER site in the coastal sea.

The results are also important as a baseline study for the assessment of the environmental status in the framework of Descriptor 2 - Non-indigeous species of MSFD.
At the beginning, we proposed four hypotheses:

1. Brackish or anthropogenically modified water bodies (Škocjanski zatok Nature Reserve, Stjuža lagoon, Port of Koper) are similar in microalgae composition.
2. Species diversity of microalgae in brackish environments differs from that in the coastal sea.
3. More non-indigenous species are found in brackish environments than in the coastal sea.
4. Species indicating transformation of lagoons into marine environments can be observed in Škocjanski zatok and Stjuža.

4.1 Methodological remarks

In studying the biodiversity of an area, the first and very important step is field sampling. Sampling brackish water environments proved to be anything but easy. We faced problems not only because of the weather conditions (not being able to perform fieldwork as planned because of rain or bora wind), but also due to very shallow waters of Škocjanski zatok and Stjuža, the inaccessibility of the main lagoon in Stjuža by boat and lockdowns because of Covid- 19 pandemic.

Most of the sampling was done in the port of Koper, first in three different basins: PBS1, PBS2 and PBS5 and later only in PBS2. In Škocjanski zatok we sampled 9 times, but with intermediate breaks due to weather conditions and Covid-19. In Stjuža we sampled only twice in 2018-2021. For better and more comprehensive results, sampling should be done more regularly and preferably at all sites at about the same time. Regular sampling with at least monthly frequency would give us a better picture of seasonal dynamics in brackish water habitats. Since monitoring program at LTER site (station 000F) is also carried out once per month, we would be able to compare biodiversity and seasonal dynamics of microalgae at all sampling sites. It would also be good to always measure abiotic parameters such as temperature and salinity, especially because these are habitats where greater fluctuations in salinity and temperature are expected.

The lack of data on abiotic parameters is one of the weaknesses of this work that should be addressed in future studies. Consistent measurements of salinity and temperature will make it possible to characterise the brackish water bodies on the Slovenian coast (average values and range of variation, fluctuations), and at the same time the measurements would provide information on whether the coastal lagoons such as the Stjuža are under the influence of the rising seawater level, thus turning into a real marine environment.

Species identification using light and electron (SEM) microscopy was time consuming but effective. For statistical data processing, it would be more optimal if we estimated taxa abundance instead of using presence/absence data only. In the future, the five-level semiquantitative abundance scale (Table 10) from the Guidelines for monitoring of phytoplankton species composition, abundance and biomass (HELCOM, 2021) should be used.

Table 10: Semiquantative abundance scale.

Level of scale	Description
1 Very sparse	One or a few (less than five of the >20 µm fraction) cells or units in the analysed area, i.e. in the sedimented sample.
2 Sparse	Slightly more cells or units in the analysed area.
3 Scattered	Irrespective of the magnification several cells or units in many fields of view.
4 Abundant	Irrespective of the magnification several cells or units in most fields of view.
5 Dominant*	Irrespective of the magnification many cells or units in every field of view.
	**In terms of abundance, not biomass. Large sized taxa may be dominant in terms of biomass even if not dominant in terms of abundance.*

To extend the limits of possible statistical analysis and increase the precision of our controls, we could also examine multiple parallels of the same sample. To avoid possible error due to multiple observers, it would be best if all samples were examined by the same person or team.

If similar studies are conducted in the future, it would be interesting to investigate community structure using metabarcoding and/or metagenomics as well.

4.2 Physical and chemical characteristics of the areas considered

Since the diversity of microalgae and their seasonal patterns also depend on the variability of abiotic factors, we wanted to measure the most important physical and chemical factors in Slovenian brackish waters at the transition between freshwater and marine ecosystems, i.e. transitional waters. Following the Water Framework Directive, which introduced the term "transitional waters" in the European water policy, the most important factors for the typology of TW are salinity and tidal regime, besides water temperature as one of the several optional factors (WFD CIS Guidance Document No. 5 2003).

However, due to the sampling difficulties described in chapter 4.1, we managed to satisfactorily describe the environmental characteristics only in the plume of the Rižana River, which flows into the second basin of the Port of Koper (sampling site PBS2). Here, a very strong salinity gradient developed between the uppermost surface layer and subsurface layer at a depth of 1 m, which already resembled seawater conditions. This gradient was particularly strong in autumn (October, November 2020) and spring (April, May 2021), probably due to heavy precipitation and high river flow or because we sampled during low tide. The strong river influence in October 2020 was also evident in the temperature values (5^{o} C difference), which, unlike salinity, showed no such variation during other samplings. If the parameters had been measured in PBS1 and PBS5 where we also took samples in 2018, we probably would not have found such fluctuations.

The two measurements from Stjuža alone are far too few for a constructive analysis. In any case, the salinity values from two sampling sites near channels connecting the lagoon to the sea indicate a typical marine environment. We suspect that different conditions would prevail in the central part of the lagoon, but unfortunately this part was not accessible for sampling. The same is probably true for Škocjanski zatok, for which we did not measure temperature and salinity as well. We assume that due to the shallow depth of the lagoon, the absence of a thermocline and thus a greater influence of precipitation compared to the coastal sea, this body of water is also characterised by strong temperature and salinity fluctuations; in particular, water temperatures can reach higher values compared to the coastal sea.

4.3 Biodiversity of microalgae in brackish and marine coastal ecosystems

The most important result of this master thesis is the list of taxa occurring in brackish and heavily modified water bodies. We expect this list to grow in the future, as some of the taxa found have not yet been identified. It will serve as a checklist of taxa identified in Slovenian brackish waters. With such a checklist, we can pay attention to the possible occurrence of identified taxa in the future, especially non-indigenous and potentially harmful species.

Comparison of the results of this study with the results of the BALMAS project on the implementation of the Port Baseline Survey (PBS) in 2014-2015 and the establishment of a monitoring program for harmful aquatic organisms and pathogens in the ports of the Adriatic Sea, including the port of Koper (Mozetič et al. 2019) showed a substantial improvement in the number of identified microalgal taxa during this study. Within BALMAS, 184 taxa were identified in the Port of Koper. More than half of the species were diatoms (94), followed by 78 species of dinoflagellates, 6 coccolithophorids, 3 silicoflagellates, two species from the group of heterotrophic flagellates and one species from the class Xanthophyceae (Mozetič et al. 2019; Pagon 2019), while we identified 250 taxa in Port of Koper. Most of them were dinoflagellates (118), followed by diatoms (105), 14 coccolithophorids, 2 cyanobacteria and 7 belonging to other taxonomic groups (Euglenophyceae, Chlorophyta, Ochrophyta and Ebriida). In Port of Koper, we also observed 20 first identified taxa from different taxonomic groups, while within BALMAS, 5 diatom taxa were first identified (Mozetič et al. 2019; Pagon 2019). Newly observed species from this study are most likely due to more frequent sampling that included all months (Mavrič et al. 2021) and also because of more advanced methods, including electron microscopy.

As regards the first identified species in all of the brackish environments together, most are marine species or species that can live in marine environments (among others, for example marine and FW, or marine and brackish waters), but 26% of the newly identified taxa are exclusively adapted to fresh and/or brackish water. This was expected, as the biodiversity of brackish water habitats on the Slovenian coast, with the exception of the port of Koper, has not been studied to this extent before. The exclusively freshwater and/or brackish water organisms found in port of Koper (*Cymbella* sp., *Gomphonema* cf. *acuminatum*, *Gymnodinium* cf. *fuscum*) were probably brought in by the inflow from the Rižana River.

Gomphonema is a relatively large genus, but species belonging to it cannot be identified based solely on LM studies. Because the characteristics of some species overlap, SEM studies are needed to distinguish these taxa (Wojtal 2003). The marine diatom *Odontella* *aurita* (Fig. 15b) is a widespread species and has already been recorded in the northern Adriatic (Viličić et al. 2002). *Helicosphaera carteri* (Fig. 15c) is a coccolithophore that had also not been found in the Slovenian sea prior to this study, but is present in oligotrophic Mediterranean seas (Dimiza et al. 2014). Silicoflagellate *Dictyocha crux* (Fig. 15d) has previously been recorded in the Adriatic Sea (Totti et al. 2000).

More first observed taxa were found in the Škocjanski zatok: the freshwater diatoms *Craticula cuspidata, Cymbella* sp. and *Nitzschia* cf. *sigmoidea*, the cyanobacteria *Pediastrum* sp. and *Nostoc* sp. and the brackish water diatoms *Cocconeis* cf. *sawensis* and *Navicula* cf. *subrostellata*. Diatom cf. *Diatoma vulgaris,* which can live in both fresh and brackish water, was found not only in the port of Koper, but also in Stjuža. Thus, this is the only non-marine taxa from Stjuža that was observed for the first time. On this point, it should be emphasized that many of the taxa first identified only to the genus level are also not exclusively marine, such as the genera *Actinocyclus, Pinnularia, Scenedesmus*, and *Synechoccus,* which have both freshwater and marine representatives. Species in the genera *Tryblionella, Anabaena, Lyngbya,* and *Merismopedia,* also identified for the first time, can live in marine, freshwater, brackish water, or even terrestrial habitats.

Among the dinoflagellates identified for the first time, we found *Azadinum caudatum* var. *margalefii* (Fig. 16a). The species was classified as cryptogenic by Mozetič et al. (2019), as it was found in the Italian port of Ancona during the BALMAS project. We also identified *Gymnodinium* cf. *fuscum* (Fig. 16b). This is a widespread species in fresh and brackish waters, also found in the Mediterranean (Gómez 2003). The first identified dinoflagellate *Peridinium quadridentatum* (Fig. 16c) was recognized as a species causing red tides (high biomass blooms) by Rodríguez-Gómez et al. (2019), but is not included in the species list of HAB. It is a common dinoflagellate distributed worldwide in tropical and subtropical areas and also in most of the Mediterranean Sea, including the Adriatic Sea (Gomez 2003; Rodríguez-Gómez et al. 2019). *Scaphodinium mirabile* (Fig. 16d) is a heterotrophic dinoflagellate species that has most likely been overlooked until now because it does not resemble related species. It is widespread in the Mediterranean, including the Croatian coast, and is described as a species that is spreading due to climate change (Pećarević et al. 2013; Mavrič et al. 2021).

Another species that probably occur regularly in our sea but that was overlooked is *Coolia monotis* (Fig. 22), which we discovered by chance in March 2022 when we examined the 2019 samples from the Škocjanski zatok under the scanning electron microscope. This means that it was present for at least three years before it was identified, probably due to the

difficulty of identification with a light microscope. *C. monotis* is an epiphytic dinoflagellate believed to produce hemolytic substances and is potentially toxic (Lassus et al. 2016).
The coccolithophore *Calcidiscus leptoporus* (Fig. 17) is a cosmopolitan coccolithophore species with one of the longest fossil records (Renaud and Klaas 2001). It is the only coccolithophore, along with *Helicosphaera carteri*, to be identified for the first time in this work.

Due to the shallow waters in the Škocjanski zatok and Stjuža, we expected that the species first observed would be predominantly benthic, while in the Port of Koper the new species would be pelagic. The water depth of Port's basins is about 14 m (Pagon 2019). This expectation was confirmed (see Fig. 23), as most of the new taxa from Port of Koper was truly pelagic (8), followed by 4 benthic species and 2 tychopelagic. In contrast, in the Škocjanski zatok, most of the taxa detected for the first time were benthic (9), 3 species were tychopelagic (can live in both plankton and benthos), and 7 species were purely pelagic. A similar situation was observed in Stjuža, where benthic species predominated among the newly observed species (8), in contrast to 5 pelagic and 1 tychopelagic species.

Ports often provide a recipient environment for NIS, because port and ship activities serve as vectors for the spread of NIS (e.g., via ballast water and sediments from ship ballast tanks) and because a highly altered and degraded environment allows allochthonous and more resilient species to occupy empty niches and establish permanent populations (Corriero et al. 2016; Kraus et al. 2019). The same applies to lagoons and wetlands, and to mariculture areas characterised by "unstable," fluctuating conditions or where marine organisms introduced from elsewhere are cultured. However, during this study we found only one NIS species, the diatom *Pseudo-nitzschia multistriata*. It was last seen in January 2019 and was found only in the Port of Koper. We have not seen it since, although it would be difficult to miss due to the specific convoluted shape of the cells. This species was first detected on the Italian side of the Gulf of Trieste in the 2000s (Cabrini et al. 2012), while a few years later, in 2015, it was discovered on the Slovenian side of the Gulf (Mozetič et al. 2019), when it was also found in ballast water tanks of ships arriving in the ports of Koper and Venice (Cabrini et al. 2019). We also found three potentially cryptogenic taxa: *Azadinium caudatum* var. *margalefii*, *Merismopedia* sp. and *Prorocentrum* cf. *formosum*. *Azadinum caudatum* var. *margalefii* and species of the genus *Merismopedia* were found in the Italian ports of Bari and Ancona during the BALMAS project and classified as cryptogenic by Mozetič et al. (2019). Species *Prorocentrum* cf. *formosum* is probably cryptogenic, since it has been described from mangroves in the Caribbean (Faust 1993), but further confirmation of its identification by molecular methods or electron microscopy is needed.

We classified 28 taxa as potentially harmful. Not all organisms classified as potentially harmful are known to cause harmful effects. Some genera contain both harmful as well as harmless species, but if we do not have species-level identification, it is better to take precautions and classify taxa as potentially harmful. Dinoflagellates of the genus *Alexandrium*, which can cause paralytic shellfish poisoning (PSP), occur regularly in the Slovenian sea. They are most abundant from April to June, although their abundance rarely exceeds a few 100 cells/l. Toxins produced by this genus have not yet been detected in Slovenian shellfish. To date, only one case of PSP and a resulting ban on hellfish harvesting has been reported from the northern Adriatic in the mid-1990s. (Honsell et al. 1996). Some *Alexandrium* species are difficult to identify with LM, and the identification of *Alexandrium insuetum* was confirmed in this study for the first time with SEM. Dinoflagellates from the genera *Dinophysis, Phalacroma* and *Prorocentrum* release lipophilic toxins that cause mainly digestive disorders with diarrhoea in humans, which is called diarrhetic shellfish poisoning (DSP). The species of the above genera are typical representatives of the phytoplankton of the Slovenian sea, usually present in low numbers (a few 10s to 100s cells/l). The dinoflagellates *Lingulodinium polyedra*, *Protoceratium reticulatum* and *Gonyaulax spinifera*, which produce yessotoxins and which we have found in the area of the Port of Koper, are also typical representatives of the phytoplankton community of the Slovenian Sea. *Gonyaulax polygramma* was included in the list HAB because it can cause high biomass blooms or red tides. Since the genus *Heterocapsa* also contains harmful species, we have also included it in the list HAB. *Coolia monotis* is a benthic dinoflagellate, that has been seen in the Slovenian sea and identified for the first time in this study. It has been shown to be toxic in culture and is reported to produce haemolytic substances (Lassus et al. 2016). An Australian strain of *C. monotis* produces cooliatoxin, a monosulfated polyether that is believed to be a yessotoxin (YTX) derivative. The injection of lethal doses of cooliatoxin to mice causes symptoms similar to those described for yessotoxin. However, this species may actually be *C. tropicalis*, and the actual toxicity of *C. monotis* remains unclear (Lassus et al. 2016). The first identified dinoflagellate in this study *Peridinium quadridentatum* is not included in the IOC-UNESCO taxonomic reference list (Lundholm et al. 2009 onwards), but can cause red tides (Rodríguez-Gómez et al. 2019). *Pseudo-nitzschia* is the only diatom genus we identified as potentially harmful. Some of the species from the genus produce domoic acid and are responsible for the amnesic shellfish poisoning (ASP). For the vast majority of species in this genus, accurate identification is possible only with the aid of an electron microscope or genetic analysis, so all specimens found were classified as *Pseudo-nitzschia* spp. with an exception that was already mentioned - *Pseudo-nitzschia multistriata*. The latter is the only easily recognizable species of this genus due to its characteristic shape. In a recent work, Turk Dermastia et al. (2020) identified 8 species of the genus in the Gulf of Trieste using genetic analysis and transmission electron microscopy:

P. multistirata, P. calliantha, P. fraudulenta, P. delicatissima, P. galaxiae, P. mannii, P. pungens in P. subfraudulenta. However, because this approach cannot be routinely applied, the genus Pseudo-nitzschia, with some morphologically distinct exceptions (*P. multistriata*, *P. galaxiae*), will continue to be considered as a whole in future studies based on LM unless novel genetic or hybridization methods are introduced for specific species in environmental monitoring programs. The cyanobacteria *Anabaena* and *Lyngbya* have also been classified as potentially harmful species. Some species of the genus *Anabaena* produce the toxins microcystin and cilindrospermopsin. One species of the genus *Lyngbya* produces dermatotoxin (Lopes and Vasconcelos 2011).

On first consideration, one could conclude that the diversity of microalgae in the Port of Koper is the highest of all three brackish environments, but this is also the location where the most samples (23) were taken. In contrast, 9 samples were taken in Škocjanski zatok and only two at each sampling site in Stjuža. Since most of the taxa in Port of Koper were marine phytoplankton, the species were also easier to identify since we see them frequently and we are more familiar with them. We also had the checklist and some photos of organisms from the BALMAS project to assist us as well. There is also more literature for marine phytoplankton than for brackish (and fresh) water microalgae, especially from Slovenian coastal ecosystems. Identification of taxa from the Škocjanski zatok and Stjuža has been a lot more challenging due to lack of literature and experience with benthic microalgae, the taxa often being very small with the morphological features required for identification not being visible with LM and larger amounts of sediment in the sample, that made organisms harder to detect. However, with the help of SEM, we were able to identify some species that we previously thought would remain unidentified. A good example of this is *Coolia monotis* (Fig. 22). We found it by chance with SEM in a sample from the Škocjanski zatok on 8.3.2022, when we were actually looking for a previously unidentified diatom. The sample in which *C. monotis* was found was from April 2019, which means that *C. monotis* could be present for at least 3 years before it was identified. After finding it with SEM, we examined the sample from April 2019 again with LM. This time we found it with LM and noted that it was very easy to miss because of its simple, round shape with few visible theca features. As mentioned earlier, there was also quite a bit of sediment in this sample.

Because the number of samples varied greatly in each area considered, meaningful statistical analysis was only possible for the periods when sampling was conducted (almost) simultaneously in all three brackish water areas and at LTER 000F in the coastal region, i.e., August and November 2020 (Fig. 28). As expected, biodiversity of microalgae from station LTER 000F was different from biodiversity in the other samples and it will be discussed more in detail in the next chapter (4.4). Part of this is likely due to the more stable environment in the coastal sea with less fluctuations in temperature and salinity. On the other hand, the phytoplankton data was obtained from a different investigator. This is another aspect to consider; if different people are examining the samples, it is more likely that their way of examining and identifying species, as well as their taxonomic knowledge, will be different. The third possible reason is that in brackish water environments (especially Škocjanski zatok and Stjuža) we found many benthic and some FW and brackish water taxa, while in LTER 000F we found only marine taxa. A sample that was clearly different from the rest of the groups is the August sample from the Škocjanski zatok. It was practically, with few and less common species present in small numbers, such as *Psammodictyon panduriforme*, *Azadinium caudatum* var. *margalefii*. If the number of species in the sample is small, they have a greater impact on biodiversity if the sample also contains many common species that would be found in other samples. The two sampling sites of Stjuža seem to be quite similar in both seasons, but in August the similarity is 60%, while in November it is only 40%. It was expected that sampling sites ss1 and ss2 would be similar, as they are only about 40 m apart. Since we sampled only twice, it is difficult to say why this is so, but the reason could be in the natural cycle of phytoplankton communities (which includes seasonal changes in abiotic factors), input of additional nutrients from the land, anthropogenic inputs/disturbances, or simply coincidence. The reasons for the lower similarity between sites in November and, conversely, the higher similarity in August could be many (e.g., changes in abiotic and biotic factors in the microenvironment or simply coincidence), but we cannot draw conclusions from only two sampling events. The main difference between the sites is that ss1 is located in the first, deeper part of the lagoon, which is more dynamic than the main lagoon where ss2 is located. Water flow at both sites depends only on tides, while there is no water movement caused by currents or waves in the main lagoon (Park Strunjan 2022). Nevertheless, site ss1 may be more disturbed than ss2 due to manipulation by small boats anchored in the shallow waters of the first, deeper part of the lagoon. It is interesting to note that summer samples from Stjuža have 40% similarity with the summer samples from the Port of Koper , while the autumn samples from the port of Koper have 40% similarity with the autumn samples from Škocjanski zatok. This could mean that some seasonal differences were detected, but only two samplings are not enough to confirm seasonality.

As one of the hypotheses we have highlighted the possible marinization of Stjuža and the Škocjanski zatok. It is difficult to draw conclusions here, as both are dynamic environments dominated by tidal currents in Stjuža or regulated by the flow of sea water in Škocjanski zatok. In Stjuža, the salinity is close to the sea salinity, but the species diversity in two samples is different from that in the sea. Due to the lack of current data (two samples are far too few data), but also data from the past, we cannot answer this question, although most of the taxa found are indeed marine.

4.4 Seasonal dynamics of microalgae in brackish environments and coastal sea

To observe the seasonal dynamics in brackish waters, we chose samples from the port of Koper, as this is the only place where we collected enough samples. We also wanted to see if the analysis would be possible with samples from the Škocjanski zatok, but the cluster analysis did not show any seasonality probably due to the small number of data and the large gaps between samplings.

While the results show differences in seasonality between Port of Koper and 000F, these differences are largely due to the method of sampling and examination under the microscope. Data from 000F are less accurate because many taxa are determined only to genus and many rarer species are missing because only 50 fields are examined, whereas samples from Port of Koper were taken with a phytoplankton net and the whole chamber bottom was examined. In addition, in 000F are included groups of nanoflagellates that are always present. It should be noted that this analysis is performed only on the basis of presence/absence data. Therefore, the differences between individual samples are smaller. We should still emphasize that Port of Koper, especially PBS2 is a dynamic environment characterised by freshwater inputs from the Rižana River and port operations. The fluctuation of salinity in the thin surface layer is much larger in Port of Koper (PBS2) than at 000F, indicating more unstable environmental conditions in PBS2 than in the open sea.

The typical species for the seasons in Port of Koper are mostly similar to the typical seasonal species for the marine environments in GoT, with benthic and freshwater species making a bigger difference.

In Port of Koper, the species that were found most frequently throughout all seasons were *Chaetoceros decipiens/lorenzianus, Tripos furca, Tripos fusus, Prorocentrum micans,* and *Protoperidinium steinii.* The most common taxa found in 000F (throughout all seasons) were diatoms *Amphora* spp., *Cerataulina pelagica, Chaetoceros affinis, Chaetoceros* spp., *Cyclotella* spp., *Cylindrotheca closterium, Dactyliosolen fragilissimus, Diploneis crabro, Leptocylindrus danicus, Nitzschia longissima, Nitzschia* spp. and *Thalassionema nitzschioides*, dinoflagellates *Gymnodinium* spp., *Gyrodinium* spp., *Heterocapsa* group, *Prorocentrum cordatum* and coccolithophores *Emiliania huxleyi* and *Ophiaster hydroideus.* Most of them are planktonic marine taxa. In every sample there was at least one taxa that could not be determined to the genus level, unlike in the Port of Koper where, except for two cases, we were always able to determine the organisms at least to the genus level.

Leptocylindrus danicus and *Proboscia alata* were only species typical for both Port of Koper and 000F. They were both typical for summer, but *P. alata* also appeared in autumn. Similarly is reported in Brush et al. (2021), where P. alata is shown to be typical for July, Vascotto et al (2021), where *P. alata* is one of the three diatoms, characteristic for summer and Cerino et al. (2019), where summer season appeared to be characterised by (among other taxa) a mixture of large diatoms, including *P. alata. Guinardia striata*, which was typical for autumn in Port of Koper, was shown to be dominating late summer to early autumn community by Brush et al. (2021), but was not among typical autumn taxa in 000F. *Skeletonema costatum s.l.*, a diatom typical for winter season (Cerino et al. 2019; Brush et al. 2021; Vascotto et al. 2021) was characteristic for winter in Port of Koper, among other taxa, including some benthic species (e.g. *Striatella unipunctata, Licmophora* sp.), which was probably due to the water column being mixed because of low winter temperatures. In spring, among the taxa that contributed most to the within season similarities were only dinoflagellates, while in 000F some diatoms were found. For example Cyclotella sp., which was typical for 000F in spring, was also recognized as typical for the mentioned season by Brush et al. (2021), Cerino et al. (2019) and Vascotto et al. (2021).

Based on data from the literature (Cullen 1985; Miller 2004; Reynolds et al. 2006; Hoppenrath et al. 2009), we expected two seasonal peaks of diatoms and a dominance of dinoflagellates in summer by examining the species that contributed most to the within-season similarities. In the Port of Koper, dinoflagellates indeed predominate in summer and diatoms in autumn, with a ratio of about 50:50 in spring. In winter, diatoms also predominate, but we must emphasise that we are only looking at presence/absence data and not biomass or abundance. At LTER 000F, we found diatom species to be predominant in summer and fall and spring and winter, with a ratio of diatoms to dinoflagellates of about 50:50. But as mentioned earlier, these are only the species that contributed most to the similarity (were identified most frequently during a season), not their abundance or biomass.

5 CONCLUSION

We studied the biodiversity of microalgae in Slovenian brackish waters, more specifically in the Port of Koper, Škocjanski zatok nature reserve and Stjuža from April 2018 to May 2021, comparing species diversity and seasonality with that of LTER site in the coastal sea. In the three brackish water environments, we identified a total of 284 taxa, most of which were determined at the lowest possible level, i.e., species level. We have found that certain taxa occur in all areas considered, including the true marine environment, while many species (especially brackish water and benthic species) are found exclusively in brackish water environments.

Based on our results, we give answers to the hypotheses we made at the beginning.

1. Brackish and heavily modified water bodies in transitional waters (Škocjanski zatok, Stjuža, Port of Koper) are similar in microalgae composition.
    - ➢ **Confirmed.** The brackish water environments are similar at the level of the main microalgae groups (diatoms, dinoflagellates, coccolithophores, silicoflagellates, cyanobacteria and others), which is especially true for Port of Koper and Stjuža. The two water bodies are also similar in terms of the number of species living in different environments, i.e., marine, brackish, freshwater and terrestrial. Compared to Škocjanski zatok, there are more microalgae adapted to the marine environment and few freshwater or brackish water taxa in the Port of Koper and Stjuža, while we found more of them in Škocjanski zatok. As for the habitat of the taxa found, i.e. whether the first identified taxa prefer pelagic or benthic environments, the composition of Škocjanski zatok and Stjuža is more similar, as most of the first identified taxa are benthic due to the very shallow water, while in Port of Koper, they are mostly pelagic.

2. Species diversity of microalgae in brackish water environments differs from that in the coastal sea.
    - ➢ **Partly confirmed.** Statistical results show, that the brackish environments indeed differ in species diversity from the coastal sea, but we cannot wholly confirm or refute this hypothesis, because we only have two samplings and the method and the person who examined the samples were not the same, and as Viličič (2009) noted, the comparison is therefore questionable.

3. More non-indigenous species are found in brackish water habitats than in the coastal sea.
    - ➢ **Not confirmed**. Only one species was found - *Pseudo-nitzschia multistriata*, which was previously recorded in the Gulf of Trieste. Although harbours, lagoons, and

other anthropogenically modified habitats have the potential to serve as habitat for NIS (Kraus et al. 2019), this trend has not been detected during this study.

4. Species indicating transformation of lagoons into marine environments can be observed in Škocjanski zatok and Stjuža.
   - ➢ We can **neither reject nor confirm** this hypothesis with certainty, since these are very dynamic environments dominated by tidal currents (Stjuža) or regulated by the flow of sea water (Škocjanski zatok). This prevents the establishment of a stable environment in which species characteristic of marine or brackish environments could develop. Although marine species dominate over brackish or even freshwater species, it is not possible to address the so-called "marinization" of lagoons due to lack of past data.

Even though we were able to confirm only one hypothesis, the goal of the master's thesis - to give a detailed overview of the biodiversity of microalgae, with a focus on phytoplankton, in the Port of Koper, Škocjanski zatok nature reserve and Stjuža - was achieved. We hope that the results of this work will serve as a basis for further research on brackish and anthropogenically altered habitats and contribute at least a small amount to our knowledge of these dynamic and peculiar environments. Furthermore, the results will be relevant for the preparation of a new assessment of the status of the marine environment according to descriptor D2 - non-indigenous species, as set out in the Marine Strategy Framework Directive 2008/56/EC (MSFD). In the near future, the results of the thesis could also be taken into account in the implementation of the Water Framework Directive 2000/60/EC (WFD), as phytoplankton with its biomass and community structure is one of the biological elements for the assessment of the ecological status of heavily modified (Port of Koper) and transitional water bodies (e.g. lagoons). Currently, only phytoplankton biomass is used for the such assessments, but in the future the results of this study could be used for the development of the metodology based on phytoplankton community structure.

6 DALJŠI POVZETEK V SLOVENSKEM JEZIKU

Magistrska naloga je bila izdelana na Morski biološki postaji (Nacionalni inštitut za biologijo) v okviru projekta »Spremljanje vrstne pestrosti in abundance tujerodnih vrst v slovenskem morju«, čigar naročnik je Ministrstvo za kmetijstvo, gozdarstvo in prehrano in tudi ARRS raziskovalnega programa »Raziskave obalnega morja«.

Namen naloge je bilo ugotoviti, kakšna je biodiverziteta mikroalg, zlasti fitoplanktona v brakičnih in antropogeno preoblikovanih habitatih – v Luki Koper, Stjuži in Škocjanskem zatoku, ter rezultate primerjati z referenčno postajo v obalnem morju (LTER 000F). Antropogeno preoblikovani habitati, kot so pristanišča, lagune, mokrišča in območja marikulture so pogosto recipientsko okolje za tujerodne organizme. Tujerodna vrsta je vrsta, podvrsta ali takson nižje kategorije, ki je vnesena izven območja naravne razširjenosti v neko novo območje, s pomočjo človeka, bodisi namerno ali nenamerno. To vključuje kateri koli razvojni stadij organizma, ki je sposoben preživetja in razmnoževanja (Pyšek in sod. 2009). Vrste, katerih izvor je nejasen in jih ne moremo opisati kot tujerodne ali avtohtone, imenujemo kriptogene vrste (Carlton 1996). Podkategorijo tujerodnih vrst, ki so se izven območja svoje naravne razširjenosti (zgodovinske ali trenutne) tudi uveljavile in imajo negativne vplive na biološko raznovrstnost, ekosistemske funkcije, socio-ekonomske vrednote in zdravje ljudi, imenujemo tujerodne invazivne vrste. Te vrste se po navadi hitro razmnožujejo in lahko svoj areal zelo hitro širijo (Pyšek in sod. 2009).

Mikroalge so bile vzorčene enkrat mesečno mesečno med aprilom 2018 in majem 2021 na vzorčnih mestih v Luki Koper, Stjuži in Škocjanskem zatoku. Za vzorčenje smo uporabili fitoplanktonsko mrežico (KC Denmark) z očesi velikosti 20 µm, vzorce pa shranili v temne steklenice volumna 0,5 l. Na terenih smo povzorčili tudi okoljske parametre (temperatura, slanost) z WTW Multi 3620 IDS sondo. Vzorci so bili ob prihodu iz terena fiksirani s 4% formaldehidom (predhodno nevtraliziranim s heksaminom) in shranjeni do pregleda.

Za namen pregledovanja vzorcev je bil uporabljen invertni epifluorescentni mikroskop Axio Observer Z1 (ZEISS) z integriranim digitalnim fotoaparatom AxioCam Mrc5 (ZEISS). Z namenom čim bolj natančne analize vzorca je bilo pregledano celotno dno komorice in sicer pri 200x povečavi. Nekatere manjše in/ali težje določljive vrste smo pogledali tudi pri 400x in 1000x povečavi. Nekaj dinoflagelatov, ki so bili posebno zahtevni za identifikacijo, smo obarvali z barvilom Calcofluor white M2R (Fritz in Triemer 1985), kar nam je omogočilo ogled ploščic celične stene ali teke, katerih razporeditev je pomemben taksonomski znak. Za identifikacijo določenih taksonov smo uporabili vrstični elektronski mikroskop (SEM) TESCAN MIRA. Pred pregledom pod elektronskim mikroskopom smo vzorce dehidrirali z alkoholno vrsto in jih posušili na zraku s pomočjo heksametildisilazana (HMDS) in jih naparili s 5nm mešanice Au-Pd s pomočjo Q150R S/E/ES Plus sputter (Quorum) naparjevalnika. Vzorce, v katerih so nas zanimale predvsem diatomeje smo pripravili po protokolu Trobajo in Mann (2019), pri čemer smo jih fiksirali s 30% EtOH, ter jih pred sušenjem očistili s 65-70% HNO3. Vsi vzorci, pregledani s pomočjo SEM-a so bili opazovani pod visokim vakuumu, pri energiji 2 keV, tokom 100 pA in s SE in In-Beam SE detektorji. Opažene organizme smo določili do najnižjega možnega taksona, pri čemer smo si pomagali z najrazličnejšimi fitoplanktonskimi določevalnimi ključi in ostalo strokovno literaturo, ter spletnimi viri.

Diverziteto mikroalg z različnih območij smo analizirali s pomočjo programa PRIMER-7, iskali pa smo podobnosti med leti, sezonami in lokacijami. Za vpogled v sezonsko dinamiko smo vzorce razdelili v štiri sezone: pomlad (april – junij), poletje (julij – september), jesen (oktober – december) in zima (januar – marec). Podobnosti med vzorci smo izračunali s pomočjo Sørensenovega indeksa (Sørensen, 1948), podobnosti in razlike pa predstavili s pomočjo nemetričnega multidimenzionalnega skaliranja (nMDS).

Skupno smo identificirali 284 različnih taksonov, od tega jih največ pripada razredoma Dinophyceae (126) in Bacillariophyceae (125). Precej manj jih pripada skupinam Coccolithophyceae (15), Dictyochophyceae (4), Ebriida (1), Euglenophyceae (2), Chlorophyta (3), Ochrophyta (2), Cyanophyceae (6). Največ taksonov je bilo določenih v Luki Koper (249). V Škocjanskem zatoku smo določili 151 taksonov, v Stjuži pa 101. 24 vrst, ki smo jih določili lahko povzroča škodljiva cvetenja alg (HAB), ena vrsta (Pseudo-nitzschia multistriata) je bila identificirana kot tujerodna (NIS), dva taksona (Azadinium caudatum var. margalefii in Merismopedia sp.) pa kot kriptogena. 34 od 284 taksonov je bilo določenih prvič, od tega jih je nekaj značilnih predvsem za sladkovodna in brakična okolja. Med 34 prvič določenimi taksoni je bilo 12 planktonskih in 12 bentoških organizmov, ter 10 takšnih, ki se lahko pojavljajo tako v pelaških, kot bentoških habitatih.

S pomočjo nMDS smo rezultate iz Luke Koper (Fig. x) primerjali z rezultati iz referenčne postaje LTER 000F (Fig. X) in ugotovili, da se v Luki Koper rezultati tekom sezon spreminjajo bolj, kot na referenčni postaji, kjer so si vrste ves čas (ne glede na sezono) 40% podobne. Tako v Luki, kot tudi na LTER 000F sta si jesen in zima precej podobni, nekoliko manj pa pomlad in poletje. SIMPER analiza je prav tako pokazala, da je povprečna podobnost v Luki Koper nižja kot na LTER 000F. Najmanjša podobnost med vrstami v Luki Koper je spomladi (33.92), najvišja pa pozimi (47.53). Na LTER 000F so si vrste najmanj podobne pozimi (57.89), najbolj pa poleti (68.71).

Da smo lahko primerjali biodiverziteto izbranih brakičnih okolij med sabo in z referenčno postajo, smo uporabili rezultate od poletja (avgust) in jeseni (november) 2020, saj smo takrat vzorčili na vseh omenjenih območjih. Nemetrični MDS model je pokazal, da se biodiverziteta na postaji referenčni postaji LTER 000F najbolj razlikuje od drugih treh območij, sta si poletni in jesenski vzorec 60% podobna. Pri ostalih vzorcih sicer zaznavamo, da vzorci iz istih območij gravitirajo skupaj, vendar pa je možno opaziti nekaj sezonske dinamike. Izjema je poletni vzorec iz Škocjanskega zatoka, ki se od drugih močno razlikuje, kar bi lahko pripisali temu, da smo v njem identificirali precej manj vrst, kot v drugih vzorcih.

Na začetku priprave magistrske naloge smo si zadali štiri hipoteze:

1. Brakični oz. antropogeno preoblikovani habitati (Škocjanski zatok, Stjuža, Luka Koper) so si med seboj podobni po sestavi mikroalg.
2. Biodiverziteta mikroalg v brakičnih habitatih se razlikuje od tiste v obalnem morju.
3. V brakičnih habitatih najdemo več tujerodnih vrst kot v obalnem morju.
4. V Škocjanskem zatoku in Stjuži je opaziti vrste, ki nakazujejo na marinizacijo lagun.

Potrdili smo samo prvo hipotezo. Biodiverziteta mikroalg v brakičnih, antropogeno preoblikovanih habitatih je bila glede na vrstno sestavo vzorcev in statistične analize podobna. Druge hipoteze kljub jasnim razlikam med referenčno postajo LTER 000F in ostalimi območji vzorčenja v nMDS modelu, nismo mogli potrditi. Način vzorčenja, ter raven analize vzorcev nista bila enaka, zaradi česar je tovrstna primerjava vprašljiva. Tudi tretje hipoteze nismo mogli potrditi, saj smo tekom projekta našli zgolj eno tujerodno vrsto, ki smo jo nazadnje opazili leta 2019. Tovrstno (neredno) opažanje ne potrjuje pričakovanega trenda. Za potrditev četrte hipoteze bi potrebovali natančni opis biodiverzitete mikroalg v Škocjanskem zatoku in Stjuži iz preteklosti, da bi lahko primerjali trend. Ker tovrstnih rezultatov nimamo, torej kljub temu, da je večina najdenih taksonov sicer res morska, hipoteze ne moremo potrditi.

Glavni cilj naloge, pripraviti podroben pregled biodiverzitete mikroalg s poudarkom na fitoplanktonu v Luki Koper, Stjuži in Škocjanskem zatoku in ugotoviti, kako se prisotnost in abundanca taksonov spreminja glede na lokacijo in čas vzorčenja, je bil dosežen. Rezultati bodo pomembni tudi za pripravo nove presoje stanja morskega okolja po deskriptorju D2 - tujerodne vrste, kot to določa Okvirna direktivi o morski strategiji 2008/56/ES (ODMS). Rezultati bodo koristni tudi pri uresničevanju Okvirne direktive o vodah 2000/60/ES (ODV) v prihodnosti, saj je eden izmed bioloških elementov za oceno ekološkega stanja v močno preoblikovanih vodnih telesih (Luka Koper) in vodnih telesih tranzicijskih voda (lagunah) tudi fitoplankton oziroma njegova biomasa in vrstna sestava.

Trenutno se za oceno stanja uporablja le biomasa fitoplanktona, saj metodologija za oceno stanja po vrstni sestavi še ni razvita, v prihodnosti pa bodo podatki pridobljeni v tej nalogi koristni pri njenem razvoju.

7 LITERATURA IN VIRI

Adamič M. O. 2002. Geomorfološke značilnosti Tržaškega zaliva in obrobja. Dela 18: 143–155.

Batelli C., Gregorič N. 2020. First report of an aegagropilous form of Rytiphlaea tinctoria from the lagoon of Strunjan (Gulf of Trieste, Northern Adriatic). Annales, Series historia naturalis 30: 61–68.

Brush Mark J., Mozetič P., Francé J., Aubry Fabrizio B., Djakovac T., Faganeli J., Harris Lora A., Niesen M. 2021. Phytoplankton dynamics in a changing environment. Malone Thomas C., Malej A., Faganeli J. (ur). Coastal ecosystems in transition: A comparative analysis of the Northern Adriatic and Chesapeake Bay, Geophysical Monograph 256: 49-74.

Cabrini M., Fornasaro D., Cossarini G., Lipizer M., Virgilio D. 2012. Phytoplankton temporal changes in a coastal northern Adriatic site during the last 25 years. Estuarine, Coastal and Shelf Science 115: 113–124. http://dx.doi.org/10.1016/j.ecss.2012.07.007.

Cabrini M., Cerino F., de Olazabal A., Di Poi E., Fabbro C., Fornasaro D., Goruppi A., Flander Putrle V., Francé J., Gollasch S., Hure M., Lipej L., Lučić D., Magaletti E., Mozetič P., Tinta T., Tornambè A., Turk V., Uhan J., David M. 2019. Potential transfer of aquatic organisms via ballast water with a particular focus on harmful and non-indigenous species: A survey from Adriatic ports. Marine Pollution Bulletin 147: 16-35.

Cahoon L. 1999. The role of benthic microalgae in neritic ecosystems. Oceanography and marine biology 37: 47–86.

Carlton J. T. 1996. Biological invasions and cryptogenic species. Ecology 77: 1653–1655.

Cerino F., Fornasaro D., Kralj M., Giani M., Cabrini M. 2019. Phytoplankton temporal dynamics in the coastal waters of the north-eastern Adriatic Sea (Mediterranean Sea) from 2010 to 2017. Nature conservation 34: 343-372.

Cibic T., Cerino F., Karuza A., Fornasaro D. 2018. Structural and functional response of phytoplankton to reduced river inputs and anomalous physical-chemical conditions in the Gulf of Trieste (northern Adriatic Sea). Science of The Total Environment 636: 838–853.

Clarke K. R., Gorley R. N. 2015. PRIMER v7: User Manual/Tutorial. Devon, United Kingdom, PRIMER-E Ltd.

Corriero G., Pierri C., Accoroni S. et al. 2016. Ecosystem vulnerability to alien and invasive species: a case study on marine habitats along the Italian coast. Aquat. Conserv. 26, 392–409. http://dx.doi.org/10.1002/aqc.2550.

Cullen J. J. 1985. Diel vertical migration by dinoflagellates: roles of carbohydrate metabolism and behavioral flexibility. V: Raukin M. A., Checkley D., Cullen J. J., Kitting C., Thomas P. (ur). Migration: mechanisms and adaptive significance: 135–152.

Čermelj B., Mozetič P., Bajt O., Turk V., Vrišer B., Kovač N., Lipej L., Vukovič A. 2000. Raziskave in monitoring Škocjanskega zatoka - pregled stanja kakovosti vode in sedimenta v vodni laguni znotraj rezervata in kakovosti vode Badaševice. Piran, Nacionalni inštitut za biologijo - Morska biološka postaja Piran: 71.

Dimiza M. D., Triantaphyllou M. V. 2014. New evidence for the ecology of Helicosphaera carteri in polluted coastal environments (Elefsis Bay, Saronikos Gulf, Greece). Journal of Nannoplankton Research 34: 37–43.

Dodge J. D. 1982. Marine Dinoflagellates of the British Isles. London, Her Majesty's Stationary Office.

European Communities. 2000. Directive 2000/60/EC of the European parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy. Official Journal of the European Communities 43 (L327): 75.

Faust M. A., Gulledge R. A. 2002. Identifying Harmful Marine Dinoflagellates. Washington, DC, Department of Systematic Biology – Botany.

Flander Putrle V., Mozetič P., Francé J., Ozod-Seradj D., Turk V., Tinta T., Lipej L., Orlando Bonaca M., Mavrič B., Uhan J., Klun K., Grego M. in Šiško M. 2015. Port Baseline Survey in Port of Koper, Slovenia (BALMAS, Act. 5.1). Final Report. Piran, National Institute of Biology (NIB) – Marine Biology Station.

Fonda U., Franco S., Ghirardelli P., Malej E. A. 1992. Outline of oceanography and the plankton of the Adriatic Sea. In: Colombo G., Ferrari I ., Ceccherelli V. U., Rossi R. (eds). Marine eutrophication and population dynamics. Olsen & Olsen: 347–365.

Francé J., Mozetič P. 2006. Ecological characterization of toxic phytoplankton species (Dinophysis spp., Dinophyceae) in Slovenian mariculture areas (Gulf of Trieste, Adriatic Sea) and the implications for monitoring. Marine Pollution Bulletin 52: 1504–1516.

Francé J., Obal R., Mozetič P. 2013. Možne so tri vrste zastrupitev: o toksičnosti školjk in prepovedih njihove prodaje v povezavi s pojavljanjem toksičnega fitoplanktona v slovenskem morju. Vestnik Veterinarske zbornice Slovenije 8 (2): 13–17.

Francé J. 2020. Škodljiva cvetenja alg - na sledi sprememb v slovenskem morju. Alternator 17: 1-5.

Fritz L., Triemer R. E. 1985. A rapid simple technique utilizing Calcofluor White M2R for the visualization of dinoflagellate thecal plates. Journal of Phycology 21: 662–664.

Gómez F. 2003. Checklist of Mediterranean free-living dinoflagellates. Botanica Marina 46: 215–242.

Graham L., Wilcox L. W., Graham J. 2009. Algae (2nd edition). San Francisco, Pearson Educations.

Grilli F., Accoroni S., Acri F., Aubry F. B., Bergami C., Cabrini M., Campanelli A., Giani M., Guicciardi S., Marini M., Neri F., Penna A., Penna P., Pugnetti A., Ravaioli M., Riminucci F., Ricci F., Totti C., Viaroli P., Cozzi S. 2020. Seasonal and Interannual Trends of Oceanographic Parameters over 40 Years in the Northern Adriatic Sea in Relation to Nutrient Loadings Using the EMODnet Chemistry Data Portal. Water 12 (8): 1–22.

Gu H., Luo Z., Zeng N., Lan B., Lan D. 2013. First record of Pentapharsodinium (Peridiniales, Dinophyceae) in the China Sea, with description of Pentapharsodinium dalei var. aciculiferum. Phycological Research 61: 256–267.

Guiry M. D., Guiry G. M. 2022. AlgaeBase. World-wide electronic publication, National University of Ireland, Galway. https://www.algaebase.org (datum dostopa 15. 5. 2021).

Helcom. 2021. Guidelines for monitoring of phytoplankton species composition, abundance and biomass. Helsinki, Baltic Marine Environment Protection Commission.

Hemaiswarya S., Raja R., Carvalho I. S. 2013. Microalgae taxonomy and breeding. Biofuel Crops. Production, Physiology and Genetics: 44–53.

Honsell, G., Poletti, R., Pompei, M., Sidari, L., Milandri, A., Casadei, C., Viviani, R. 1996. Alexandrium minutum Halim and PSP contamination in the Northern Adriatic Sea (Mediterranea Sea). In: Yasumoto, T., Oshima, Y., Fukuyo, Y. (Eds.), Harmful and Toxic Algal Blooms. Intergovernmental Oceanographic Commission of UNESCO, pp. 77–80.

Hoppenrath M., Elbrächter M., Drebes G. 2009. Marine Phytoplankton. Stuttgart, Schweizerbart Science Publishers.

Kraberg A., Baumann M., Dürselen C. D. 2010. Coastal Phytoplankton-Photo Guide for Northern European Seas. Munchen, Alfred Wegener Institute for Polar and Marine Research.

Krajinski Park Strunjan. https://parkstrunjan.si (datum dostopa: 4. 5. 2022).

Kraus R., Ninčević-Gladan Ž., Auriemma R., Bastianini M., Bolognini L., Cabrini M., Cara M., Čalić M., Campanelli A., Cvitković I., Despalatović M., Dragičević B., Drakulović D., Dulčić J., Flander-Putrle V., Grati F., Grego M., Grilli F., Jaklin A., Janeković I., Kolitari J., Lipej L., Magaletti E., Marini M., Matić-Skoko S., Mavrič B., Mikuš J., Mozetič P., Orlando-Bonaca M., Petović S., Precali R., Supić N., Trabucco B., Travizi A., Žuljević A. 2019. Strategy of port baseline surveys (PBS) in the Adriatic Sea. Marine Pollution Bulletin 147: 47–58.

Larink O., Westheide W. 2011. Coastal Plankton-Photo Guide for European Seas. V: Wiltshire K. H. in Boersma M. (ur.). Munchen, Alfred Wegener Institute for Polar and Marine Research.

Lassus P., Chomérat N., Hess P., Nézan E. 2016. Toxic and Harmful Microalgae of the World Ocean: Micro-algues toxiques et nuisibles de l'océan mondial. Denmark, International Society for the Study of Harmful Algae in United Nations Educational, Scientific and Cultural Organisation.

Lipej B., Lipej L., Kerma S. 2020. Škocjanski zatok Nature Reserve: a case study of a protected urban wetland area and tourist attraction. V: Koderman M., Opačić V.T. (ur). Challenges of tourism development in protected areas of Croatia and Slovenia. Koper in Zagreb, Založba Univerze na Primorskem in Hrvatsko geografsko društvo.

Logar J. 2012. Conference paper.

Lopes V. R., Vasconcelos V. M. 2010. Planktonic and benthic cyanobacteria of European brackish waters: a perspective on estuaries and brackish seas. European Journal of Phycology 46 (3): 292–304.

Lundholm N., Churro C., Frag S., Hoppenrath M., Iwataki M., Larsen J., Mertens K., Moestrup Ø., Zingone A. (Eds). 2009. onwards. IOC-UNESCO Taxonomic Reference List of Harmful Micro Algae. Accessed at https://www.marinespecies.org/hab on 2022-08-18. doi:10.14284/362 (datum dostopa: 17. 5. 2021).

Malačič V. 1991. Estimation of the vertical eddy diffusion coefficient of heat in the Gulf of Trieste (Northern Adriatic). Oceanologica Acta 14: 23–32.

Malačič V., Celio M., Čermelj B., Bussani A., Comici C. 2006. Interannual evolution of seasonal thermohaline properties in the Gulf of Trieste (northern Adriatic) 1991–2003. Journal of Geophysical Research 111: 1–16.

Malej A., Mozetič P., Malačič V., Terzić S., Ahel M. 1995. Phytoplankton responses to freshwater inputs in a small semi-enclosed gulf (Gulf of Trieste, Adriatic Sea). Marine Ecology Progress Series 120: 111–121.

Malone T. C., Malej A., Faganeli J. 2021. Introduction: Coastal Ecosystem Services at Risk. V: MALONE, T. C., MALEJ, A., FAGANELI, Jadran (ed.). Coastal ecosystems in transition: a comparative analysis of the northern Adriatic and Chesapeake Bay. 1st ed. American Geophysical Union: Wiley, 2021. Str. 1-6.

Margalef R. 1978. Life-forms of phytoplankton as survival alternatives in an unstable environment. Oceanologoca Acta 1: 493–509.

Mavrič B., Orlando-Bonaca M., Fortič A., Francé J., Mozetič P., Slavinec P., Pitacco V., Trkov D., Vascotto I., Zamuda L., Lipej L. 2021. Spremljanje vrstne pestrosti in abundance tujerodnih vrst v slovenskem morju. Piran, Nacionalni Inštitut za Biologijo - Morska Biološka Postaja.

McDermott G., Raine R. 2006. The Dinoflagellate Genus Ceratium in Irish Shelf Seas. Galway, The Martin Ryan Institute.

McLusky D. S., Elliott M. 2007. Transitional waters: A new approach, semantics or just muddying the waters?. Estuarine, coastal and shelf science 71: 359-363.

Miller C. B. 2004. Biological oceanography. Oxford, Blackwell Publishing.

Mozetič B., Lipej B., Šalaja N. 2010. Zeleno srce Kopra – vodnik po Naravnem rezervatu Škocjanski zatok. Ljubljana, Društvo za opazovanje in proučevanje ptic Slovenije.

Mozetič P., Fonda-Umani S., Cataletto B., Malej A. 1998. Seasonal and inter-annual plankton variability in the Gulf of Trieste (northern Adriatic). ICES Journal of Marine Science 55: 711–722.

Mozetič P., Francé J., Lucic P., Lipej L. 2015. Additional record of the non-indigenous copepod Pseudodiaptomus marinus (Sato, 1913) in the Adriatic Sea. Acta adriatica 56 (2): 275–282.

Pećarević M., Mikuš J., Bratoš Cetinić A., Dulčić J., Čalić M. 2013. Introduced marine species in Croatian waters (Eastern Adriatic Sea). Mediterranean Marine Science 14 (1): 224–237.

Pociecha A., Solarz W., Najberek K., Wilk-Wozniak E. 2016. Native, alien, cosmopolitan, or cryptogenic? A framework for clarifying the origin status of rotifers. Aquatic Biology 24: 141–149.

Pompei M., Pigozzi S., Cangini M. 2003a. Marine Dinoflagellates. Material of UNEP training course, Cesenatico, 9-13 June 2003. Italy, Centro Ricerche Marine - Laboratorio Fitoplankton.

Pompei M., Pigozzi S., Cangini M. 2003b. Marine Diatoms. Material of UNEP training course, Cesenatico, 9-13 June 2003. Italy, Centro Ricerche Marine - Laboratorio Fitoplankton.

Povezanost pristanišča. https://luka-kp.si/slo/povezanost-pristanisca (datum dostopa: 17. 5. 2021).

Pyšek P., Hulme P. E., Nentwig W. 2009. Glossary of the main technical terms used in the handbook. Daisie (ur). Handbook of Alien Species in Europe. Invasing Nature - Springer Series in Invasion Ecology 3: 375–379.

Sorensen Index Calculator. https://calculator.academy/sorensen-index-calculator/ (datum dostopa: 6.6.2022)

Rak G., Zec D., Kostelac M. M., Joksimović D., Gollasch S., David M. 2019. The implementation of the ballast water management convention in the Adriatic Sea through States' cooperation: The contribution of environmental law and institutions. Marine Pollution Bulletin 147: 245–253.

Renaud S., Klaas C. 2001. Seasonal variations in the morphology of the coccolithophore Calcidiscus leptoporus off Bermuda (N. Atlantic). Journal of Plankton Research 23 (8): 779–795.

Reynolds C. S. 2006. The Ecology of Phytoplankton. New York, Cambridge University Press.

Rich V. I., Maier R. M. 2015. Aquatic Environments. V: Pepper I.L., Gerba C.P., Gentry T.J. (ur). Environmental Microbiology (Third Edition). Academic Press: 111–138.

Rodríguez-Gómez C. F., Aké-Castillo J. A., Vázquez G. 2019. Short-term Responses of the Bloom-Forming Dinoflagellate Peridinium quadridentatum in Tropical Coastal Waters: Environmental Variables and Phytoplankton Community. Journal of Coastal Research 92: 22–32.

Round F.E. 1971. Benthic marine diatoms. Oceanography and marine biology: an annual review 9: 83–139.

Salmaso N., Naselli-Flores L., Padisák J. 2015. Functional classifications and their application in phytoplankton ecology. Freshwater Biology 60: 603–619.

Sedmak B., Fanuko N. 1991. Occurrence of Dinophysis spp. and toxic shellfish in the Northern Adriatic. Journal of Applied Phycology 3: 289–294.

Snoeijs-Leijonmalm P., Schubert H., Radziejewska T. 2017. Biological Oceanography of the Baltic Sea. Dordrecht, Springer.

Šajna N., Kaligarič M. 2005. Vegetation of the Stjuža coastal lagoon in Strunjan landscape park (Slovenia): A draft history, mapping and nature-conservancy evaluation. Annales 15: 79–90.

Šalaja N., Mozetič B. 2005. Škocjanski zatok – oaza na pragu Kopra. Svet ptic 10 (4): 188–21.

Tomas C. R. 1996. Identifying Marine Diatoms and Dinoflagellates. San Diego CA, Academic Press.

Totti C., Civitarese G., Acri F., Barletta D., Candelari G., Paschini E., Solazzi A. 2000. Seasonal variability of phytoplankton populations in the middle Adriatic sub-basin. Journal of Plankton Research 22 (9): 1735–1756.

Trobajo R., Mann D. G. 2019. A rapid cleaning method for diatoms. Diatom Research 34 (2): 115–124.

Turk Dermastia T., Cerino F., Stanković D., Francé J., Ramšak A., Tušek-Žnidarič M., Beran A., Natali V., Cabrini M., Mozetič P. 2020. Ecological time series and integrative taxonomy unveil seasonality and diversity of the toxic diatom Pseudo-nitzschia H. Peragallo in the northern Adriatic Sea. Harmful Algae 93 (9): 1–20.

Twrdy E., Hämäläinen E. 2017. A green port – Case study of Port of Koper. Conference paper.

Učakar T. 2009. Geografija naravnega rezervata Škocjanski zatok. Diplomsko delo, Univerza v Ljubljani.

Vale M. A., Ferreira A., Pires J. C. M., Goncalves A. L. 2020. CO2 capture using microalgae. V: Rahimpour M.R., Farsi M., Makarem M.A. (ed). Advances in Carbon Capture. Woodhead Publishing 17: 381–405.

Vascotto I., Mozetič P., Francé J. 2021. Phytoplankton Time-Series in a LTER Site of the Adriatic Sea: Methodological Approach to Decipher Community Structure and Indicative Taxa. Water 13: 2045. https://doi.org/10.3390/w13152045.

Viličić D. 2002. Fitoplankton Jadranskoga Mora – biologija i taksonomija. Zagreb, Školska Knjiga.

Viličić D., Djakovac T., Burić Z., Bosak S. 2009. Composition and annual cycle of phytoplankton assemblages in the northeastern Adriatic Sea. Botanica Marina 52: 291–305.

WFD CIS. 2003. Transitional and Coastal Waters, Typology, Reference Conditions and Classification Systems. Published by the Directorate General Environment of the European Commission, Brussels. Guidance Document No. 5.

Wojtal A. Z. 2003. Diatoms of the genus Gomphonema Ehr. (Bacillariophyceae) from a karstic stream in the Krakowsko-Częstochowska Upland. Acta Societatis Botanicorum Poloniae 72 (3): 213–220.

WoRMS Editorial Board. 2022. World Register of Marine Species. https://www.marinespecies.org (datum dostopa: 15.5.2021).

APPENDIX

APPENDIX A *Microalgal taxa found in brackish environments.*

	Port of Koper	Škocjanski zatok	Stjuža
DIATOMS / Bacillariophyceae			
Achnanthes brevipes	+	+	
Achnanthes cf. *longipes*	+	+	
Achnanthes sp.	+	+	
Actinocyclus senarius	+		
Actinocyclus sp.	+	+	
Amphiprora spp.		+	
Amphiprora/Entomoneis	+	+	
Amphora spp.	+	+	
Asterionellopsis glacialis	+	+	
Asteromphalus cf. *flabellatus*	+	+	
Asteromphalus cf. *hyalinus*	+		
Asteromphalus cf. *parvulus*		+	
Asteromphalus spp.	+	+	
Bacillaria paxillifera	+	+	
Bacteriastrum furcatum	+	+	
Bacteriastrum hyalinum		+	
Bacteriastrum jadranum	+		
Bacteriastrum spp.	+	+	
Biddulphia alternans	+		
Cerataulina pelagica	+	+	
cf. *Craticula cuspidata*		+	
cf. *Cymbella*	+	+	
cf. *Diatoma vulgaris*	+		+
cf. *Lauderia confervacea*	+		
cf. *Entomoneis alata*	+		
cf. *Pinnularia*	+	+	
cf. *Placoneis pseudanglica*		+	
Chaetoceros affinis	+	+	+
Chaetoceros anastomosans	+		
Chaetoceros brevis	+	+	
Chaetoceros cf. *borealis*		+	

Chaetoceros cf. *constrictus*	+	+	
Chaetoceros cf. *diversus*	+		
Chaetoceros cf. *subtilis*	+		
Chaetoceros curvisetus	+		
Chaetoceros dadayi	+		+
Chaetoceros danicus	+	+	
Chaetoceros decipiens/lorenzianus	+	+	+
Chaetoceros dydimus	+	+	+
Chaetoceros lauderi	+		
Chaetoceros rostratus	+		
Chaetoceros sp.1	+	+	+
Chaetoceros spp.	+	+	+
Chaetoceros vixvisibilis	+		
Cocconeis cf. *sawensis*		+	
Cocconeis scutellum	+		+
Cocconeis spp.	+	+	+
Corethron sp.		+	
Coscinodiscus spp.	+	+	+
*Cyclotell*a spp.	+		
Cylindrotheca closterium	+	+	+
C*ymbella* spp.		+	+
Dactyliosolen fragilissimus	+		+
Detonula pumila	+		
Diploneis crabro	+	+	+
Diploneis spp.	+		+
Ditylum brightwellii	+		
Entomoneis decussata	+	+	
Eucampia cornuta	+	+	+
Eucampia zodiacus	+	+	+
Eupyxidicula turris		+	
Fragilaria spp.	+		
Fragilariopsis spp.	+		
Gomphonema cf. *acuminatum*	+		
Guinardia flaccida	+	+	+
Guinardia striata	+	+	+
Gyrosigma fasciola	+	+	
Gyrosigma sp.	+	+	+
Hemiaulas hauckii	+	+	+

Hemiaulus sinensis	+	+	+
Lauderia annulata	+		
Leptocylindrus convexus	+	+	
Leptocylindrus danicus	+	+	+
Leptocylindrus mediterraneus	+		+
Licmophora spp.	+	+	+
Lioloma cf. *pacificum*	+	+	+
Lithodesmium undulatum	+	+	
Lyrella sp.	+		+
Melosira moniliformis	+	+	
Melosira nummoloides	+		
Melosira sp.	+		
Meuniera membranacea	+		
Navicula cf. *subrostellata*		+	
Navicula spp.	+	+	+
Neocalyptrella robusta	+	+	
Nitzschia cf. *incerta/lorenziana*	+	+	+
Nitzschia cf. *kurzeana*		+	
Nitzschia cf. *sigmoidea*		+	
Nitzschia longissima	+	+	+
Nitzschia spp. *(small type)*	+	+	
Odontella aurita	+		
Paralia sulcata	+	+	+
Petrodictyon gemma	+	+	
Pinnularia spp.	+		
Pleurosigma angulatum	+	+	+
Pleurosigma cf. *balticum*	+		
Pleurosigma spp.	+	+	+
Podocystis adriatica		+	
Podosira stelligera	+	+	+
Proboscia alata	+	+	+
Proboscia indica		+	+
Psammodictyon panduriforme		+	
Pseudo-nitzschia multistriata	+		
Pseudo-nitzschia delicatissima group	+	+	+
Pseudo-nitzschia seriata group	+	+	+
Pseudo-nitzschia spp.	+		+

Pseudosolenia calcar-avis	+	+	
Rhizosolenia cf. *imbricata*	+	+	
Rhizosolenia cf. *styliformis*	+		
Rhizosolenia spp.	+		+
Skeletonema costatum s.l.	+	+	
Striatella unipunctata	+	+	+
Surirella sp.	+		+
Thalassionema frauenfeldii	+		
Thalassionema nitzschioides	+		
Thalassionema spp.	+	+	+
Thalassiosira rotula	+		
Thalassiosira spp.	+	+	+
Toxarium undulatum			+
Triceratium favus			+
Trieres mobiliensis	+	+	
Tryblionella sp.			
diatomeae non ident.	+	+	+
DINOFLAGELLATES / Dinophyceae			
Akashiwo sanguinea	+	+	+
Alexandrium insuetum	+	+	
Alexandrium cf. *minutum*	+	+	+
Alexandrium cf. *tamarense*	+		
Alexandrium pseudogonyaulax	+	+	
Alexandrium spp.	+	+	+
Amphisolenia sp.		+	
Archaeperidinium minutum	+	+	+
Azadinium caudatum var. *margalefii*	+	+	
Ceratocorys horrida	+		
cf. Pentapharsodinium	+		
Cochlodinium spp.	+	+	
Coolia monotis		+	
Corythodinium recurvum	+		
Corythodinium tesselatum			+
Dinophysis acuminata	+		
Dinophysis acuta			+
Dinophysis caudata	+	+	
Dinophysis cf. *similis*			

Dinophysis fortii	+	+	+
Dinophysis hastata	+		
Dinophysis nasuta	+		
Dinophysis norvegica	+		
Dinophysis ovum	+		
Dinophysis parva	+		
Dinophysis sacculus	+	+	+
Dinophysis spp.	+		
Dinophysis tripos	+		+
Diplopsalis group	+	+	+
Dissodinium pseudolunula	+		+
Gonyaulax digitale	+		
Gonyaulax cf. *diegensis*		+	
Gonyaulax fragilis	+	+	
Gonyaulax polygramma	+		+
Gonyaulax scrippsae	+		+
Gonyaulax spinifera	+		
Gonyaulax spp.	+		
Gonyaulax verior	+	+	+
Gymnodinium cf. *fuscum*	+		
Gymnodinium spp.	+		+
Gyrodinium spp.	+		+
Heterocapsa cf. *triquetra*	+		
Heterocapsa group	+	+	+
Lingulodinium polyedra	+		
Mesoporos perforatus	+		
Noctiluca scintillans	+	+	
Ornithocercus magnificus			+
Oxyphysis oxytoxoides	+		
Oxytoxum sceptrum	+		+
Oxytoxum scolopax	+		
Oxytoxum spp.	+		
Oxytoxum tesselatum	+		
Pentapharsodinium cf. *dalei*	+		
Peridinium quadridentatum	+	+	
Phalacroma mitra	+	+	
Phalacroma rotundatum	+	+	+
Podolampas bipes	+		

Podolampas cf. *elegans*	+		
Podolampas palmipes	+	+	+
Pronoctiluca sp.	+		
Prorocentrum aporum	+	+	+
Prorocentrum balticum	+		
Prorocentrum cf. *formosum*	+	+	+
Prorocentrum compressum	+	+	
Prorocentrum cordatum	+		
Prorocentrum dactylus	+	+	+
Prorocentrum gracile	+	+	
Prorocentrum lima	+	+	+
Prorocentrum micans	+	+	+
Prorocentrum scutellum	+		+
Prorocentrum spp.	+	+	
Prorocentrum triestinum		+	
Protoceratium reticulatum	+		
Protoperidinium bipes	+		
Protoperidinium brevipes	+		+
Protoperidinium cerasus	+	+	+
Protoperidinium cf. *claudicans*	+		
Protoperidinium conicoides	+	+	
Protoperidinium cf. *curvipes*	+		
Protoperidinium cf. *latidorsale*	+		
Protoperidinium cf. *oblongum*	+	+	+
Protoperidinium cf. *pallidum*	+		
Protoperidinium cf. *pellucidum*	+		
Protoperidinium cf. *subinerme*	+		
Protoperidinium cf. *thorianum*	+		
Protoperidinium conicoides	+	+	
Protoperidinium conicum	+	+	
Protoperidinium crassipes	+		
Protoperidinium curtipes	+		
Protoperidinium depressum	+	+	+
Protoperidinium diabolus	+	+	+
Protoperidinium divergens	+		+
Protoperidinium minutum	+		
Protoperidinium mite	+		
Protoperidinium ovum	+		
Protoperidinium pellucidum	+	+	

Protoperidinium pentagonum	+		
Protoperidinium pyriforme	+	+	+
Protoperidinium spp.	+		
Protoperidinium steinii	+	+	+
Protoperidinium tuba	+	+	+
Pseliodinium vaubanii	+		+
Pyrophacus horologicum	+		
Pyrophacus sp.	+		
Pyrophacus steinii	+		
Scaphodinium mirabile	+	+	
Scrippsiella spp.	+	+	+
Scrippsiella trochoidea	+		
Triadinium polyedricum	+		
Tripos azoricus	+	+	
Tripos candelabrum	+		+
Tripos extensus	+	+	
Tripos furca	+	+	+
Tripos fusus	+	+	+
Tripos hexacanthus	+		
Tripos horridus	+	+	+
Tripos lineatus	+	+	+
Tripos longirostrus	+		
Tripos macroceros	+		
Tripos massiliense	+		
Tripos muelleri	+	+	+
Tripos pentagonus	+		
Tripos setaceum	+		
Tripos teres	+		
Tripos trichoceros	+	+	+
dinoflagellatae non ident.	+	+	+
COCCOLITHOPHORES / Coccolithophyceae			
Acanthoica quattrospina	+	+	
Calcidiscus leptoporus	+		
Calciopappus sp.		+	
Calciosolenia brasiliensis	+		
Calciosolenia murrayi	+		+
Calciosolenia sp.	+		
Calyptrosphaera oblonga	+		

Coronosphaera mediterranea	+		
Emiliania huxleyi	+	+	+
Helicosphaera cf. *carteri*	+		
Ophiaster hydroideus	+		
Rhabdolithes claviger	+	+	+
Syracosphaera pulchra	+	+	+
Syracosphaera spp.	+		
Coccolithophycea/ order Primnesiales			
Chrysochromulina spp.	+	+	
SILICOFLAGELLATES / Dictyochophyceae			
Dictyocha crux	+		
Dictyocha fibula	+	+	+
Octactis octonaria	+	+	
Octactis speculum	+		
Ebriida			
Hermesinum adriaticum	+	+	+
Euglenophyceae			
Eutreptiella sp.	+	+	
euglenoficeje non. ident. (sp. 1)	+		
Chlorophyta			
Halosphaera viridis	+		
Pediastrum sp.		+	
Scenedesmus sp.	+	+	
Ochrophyta			
Dinobryon spp.	+		
Meringosphaera mediterranea	+	+	
CIANOBACTERIA / Cyanophyceae			
cf. *Anabaena* sp.	+		
Lyngbya sp.		+	
Nostoc sp.		+	
Merismopedia sp.		+	+
Oscillatoria sp.	+	+	
Synechococcus sp.		+	