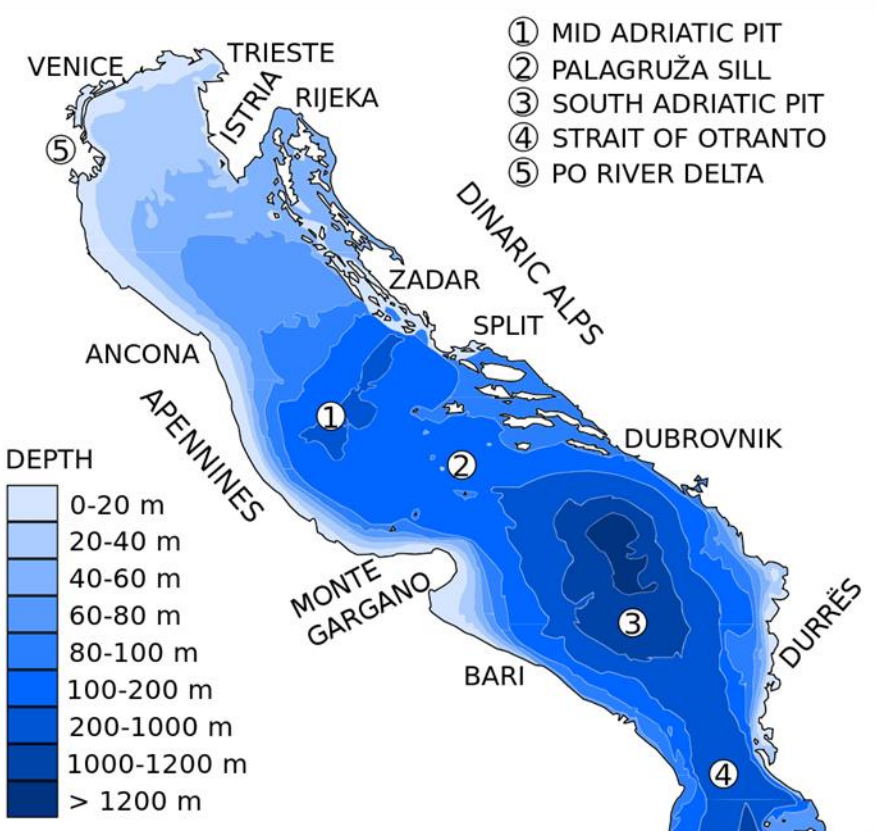
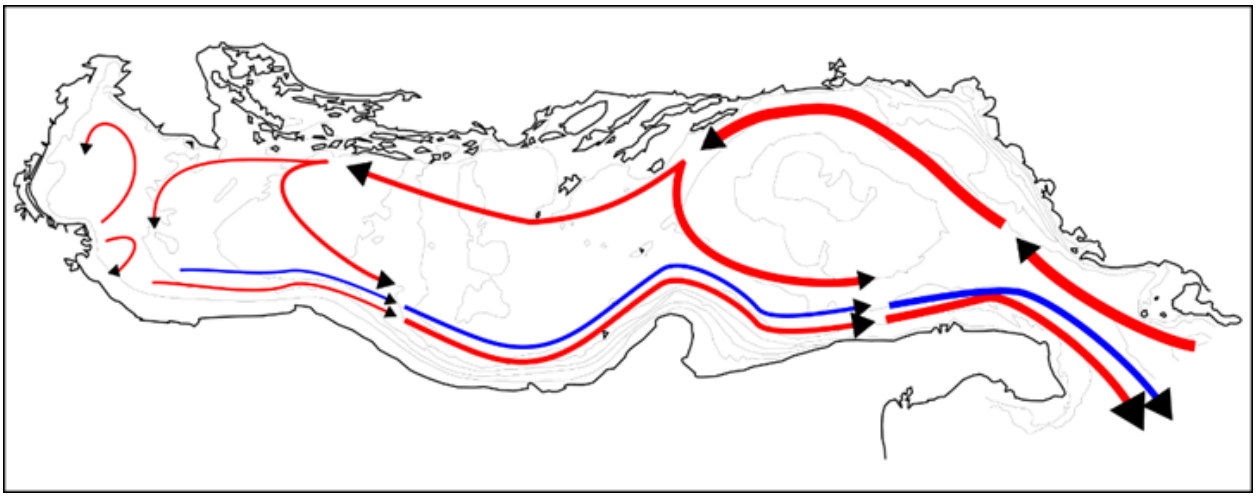
DAY 1

INTRODUCTION

The Adriatic sea is located between the Italian and the Balkan peninsulas and is the northernmost arm of the Mediterranean Sea. It is divided into three basins: northern, central and southern basin, where the northern one is the shallowest (maximum depth less than 50 m) and the southern one the deepest (maximum depth 1.233 m) (figure below).



The general flow direction of surface currents in the Adriatic is counterclockwise: the water flows along the Croatian coast from south to north and flows out on the Italian side, with several transverse flows (figure below). Consequently, the Northern Adriatic is influenced by highly saline, oligotrophic water coming from the southern part by Eastern Adriatic Current. Meanwhile, the West Adriatic Current transfers nutrients from north to south Adriatic along the western coast of the Adriatic Sea. This results in a south to north gradient in nutrient concentrations in the Adriatic.



* red – surface currents, blue – deep currents

However, the Northern Adriatic also exhibits west to east gradients of physicochemical and biological parameters as a result of Po River's freshwater discharge. Discharges of the Po River and different circulation patterns were identified as major drivers of nutrient input and distribution (Cozzi and Giani, 2011; Degobbis et al., 2000), and thus phytoplankton community composition in the whole area (Viličić et al., 2009). Due to the ecosystem in the Northern Adriatic being highly variable, it shows high species diversity.

Due to the high amount of nutrients, primary production in the Northern Adriatic is among the highest in the Mediterranean while the rest of the Adriatic shows low productivity. Strong northern wind bora cools the sea which results in water of higher density and triggers water circulation in the Adriatic. Cold water then reaches the rest of the Mediterranean due to wind and deep-sea currents, simultaneously bringing nutrients to the area and consequently acting as a food source for the whole Mediterranean. The Northern Adriatic is thus of key importance for the whole Mediterranean ecosystem.

Generally, the annual pattern of phytoplankton biomass in temperate systems is thought to have two major maxima, in spring and autumn (Legendre, 1990). For the northern Adriatic, though, four major peaks were reported by Bernardi Aubry et al. (2012) (February, May, July and September). These blooms are dominated by diatoms (Marić et al., 2012), with the exception of May when nanoflagellates co-dominate (Bernardi Aubry et al., 2012; Mozetič et al., 2012). Phytoplankton biomass reaches its maximum amounts earlier in the Northern Adriatic (winter-spring) compared to the Southern part (spring). Blooms on the south are as well dominated by diatoms.

MATERIALS AND METHODS

Sampling

To investigate seasonal dynamics and stability of the Mediterranean, sea water samples were collected during monthly cruises in the Adriatic Sea from January 2010 until December 2015 (refers to physical data; chemical data were collected by July 2015, and phyto data by January 2014). At each station, an SBE 25 Sealogger CTD probe was used to record conductivity, depth, temperature, density, salinity and dissolved oxygen in the water column. Water samples for nutrient and phytoplankton abundance analyses were collected using 5 L Niskin bottles. Samples for phytoplankton abundance were filtered after collection using a 300 µm filter to remove zooplankton and fixed with neutralized formaldehyde (2% final concentration). Zooplankton samples were collected using a 200 µm mesh plankton net. Vertical net hauls were performed from 2 m above seafloor up to the surface. The samples were fixed with neutralized formaldehyde (2% final concentration).

Sample analysis

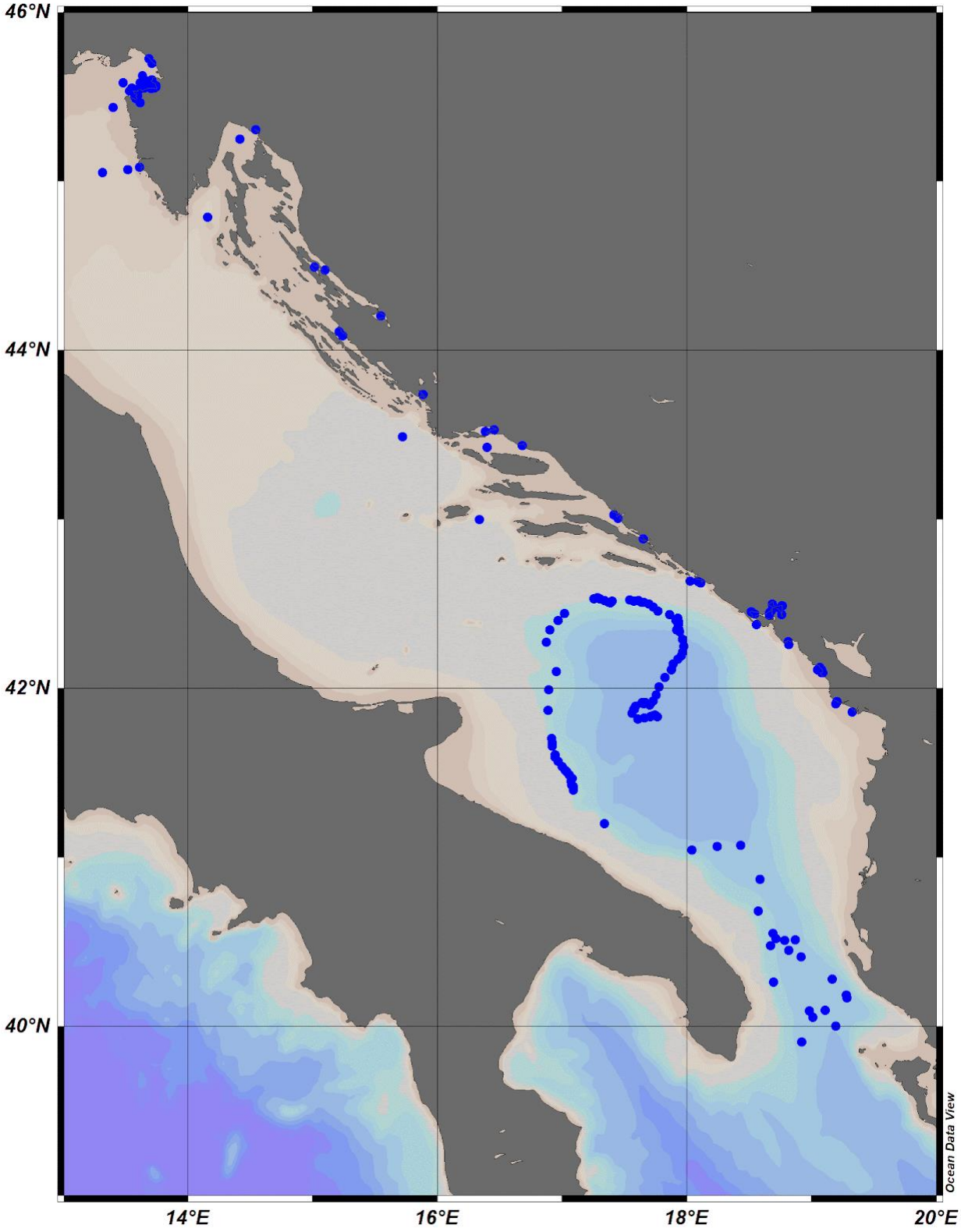
Sample analyses were performed in the laboratories of Center for Marine Research, Ruđer Bošković Institute, in Rovinj, Croatia.

Nutrients: nitrate (NO_3^-), nitrite (NO_2^-), orthophosphate (PO_4^{3-}) and orthosilicate (SiO_4^{4-}) were measured by spectrophotometric methods (Parsons et al., 1984). Ammonium (NH_4^+) was analyzed by a modified technique of the indophenol method (Ivančić and Degobbis, 1984). Measurements were performed on a Shimadzu UV-Mini 1240 spectrophotometer with 10 mm cells.

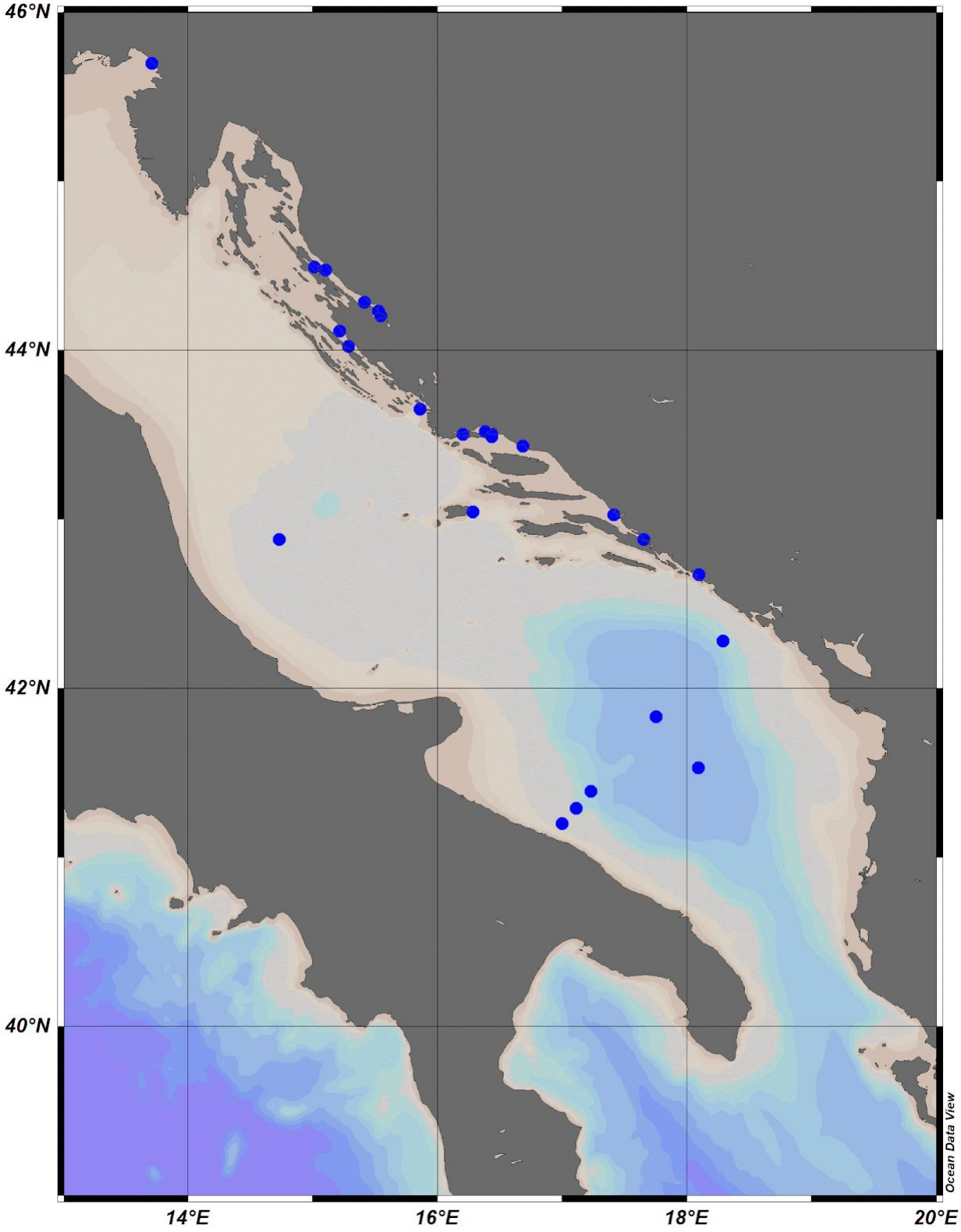
A 500 mL subsample for the determination of chlorophyll a was filtered onto Whatman GF/C filters and immediately frozen at −20 °C until analysis (within a week). Total chlorophyll a concentrations were determined on a Turner TD-700 fluorometer (Parsons et al., 1984) after three hours of extraction in 90% acetone (in the dark, with grinding).

200 mL of fixed phytoplankton samples were sedimented for 40 h (Hasle, 1978). A subsample of 50 mL was taken for counting phytoplankton cells using Axiovert 200 light invert microscope (Zeiss GmbH, Oberkochen, Germany) following the Utermöhl method. The same procedure was followed for zooplankton samples.

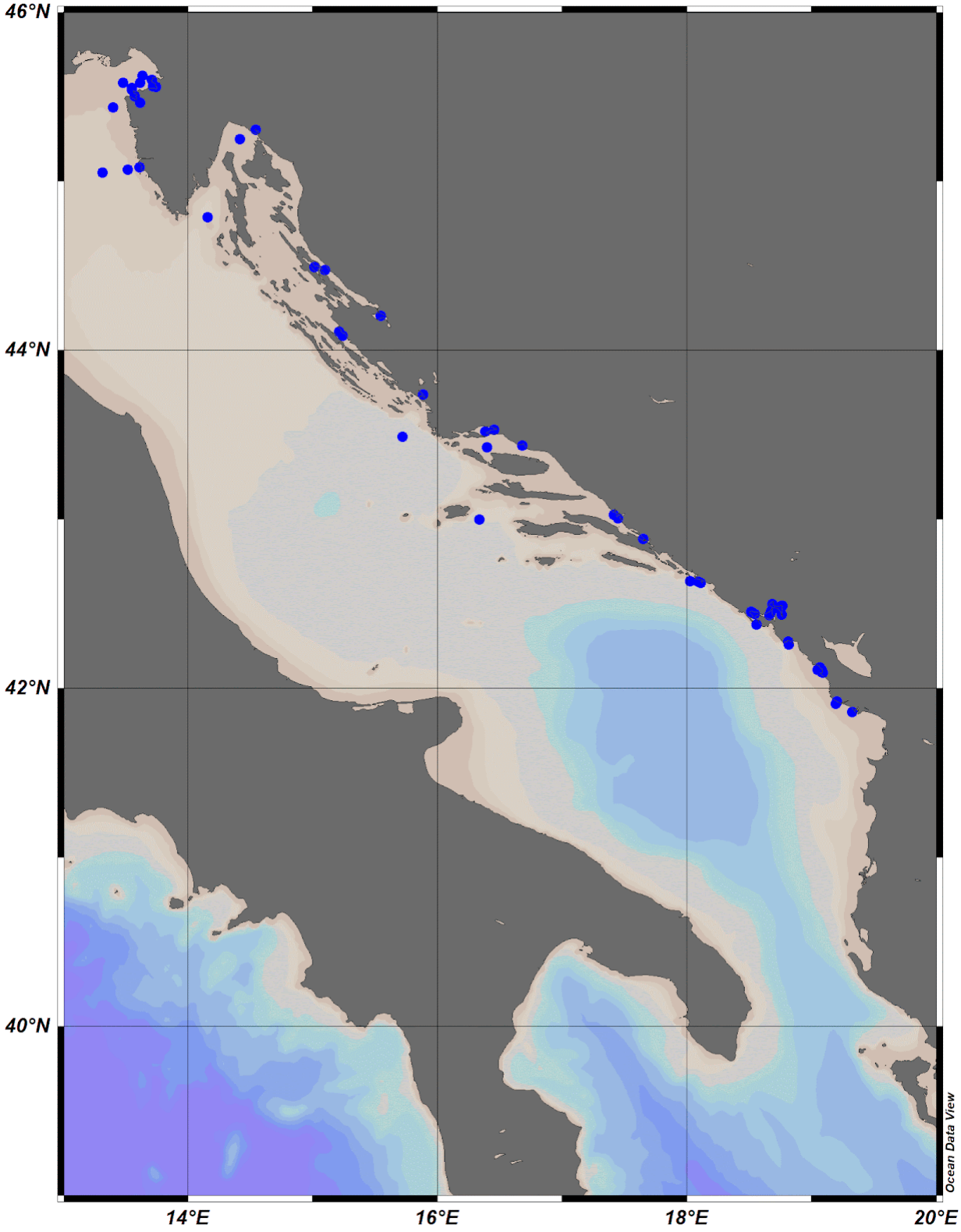
Physical data sampling stations



Phytoplankton data sampling stations



Chemical data sampling stations



DATA ANALYSIS

For the first day your task is to analyze oceanographic datasets. You are provided with three datasets obtained from publicly available data. Samples were taken across the Adriatic sea (shown in the maps above) from 2010 to 2015, and are stored in `physics.csv`, `chemistry.csv` and `biology.csv`.

Your tasks include exploratory analysis, data cleaning and pre-processing. Even though this seems daunting, it is a major part of working in science.

Task 1

a Load the `physics.csv` file into your favourite software for data analysis (R, python, Excel...).

b Plot all possible time dependent variables:

- Temperature
- Salinity
- pH
- Conductivity
- Dissolved Oxygen
- Chlorophyll A

c From examining the plots, detect outliers and explain the possible causes.

d Exclude the outlining data points and repeat the plots with cleaned data.

Task 2

After data clean up, your group's task is to separate data by seasons. Show the same dependent variables from Task 1, but depending on seasons, and then answer the questions:

a Which data shows significant differences between seasons? Reinforce your answer with quantitative measures.

b How are the observed differences connected to the seasonality?

Task 3

In this task your group aims to explore the data on living organisms from the Adriatic sea. To do so load in the `biology.csv` data set and make two plots:

1. Bar plot of total number of observations by kingdom
2. Bar plot of total abundance by kingdom

Examine the resulting plots and answer the following questions:

a How many taxonomic groups do you identify?

b Are all the bars visible in both plots, and if not, explain why? What method would you use to resolve this issue?

c Which kingdom is the most dominant? What species does it include and why are they prevalent?

Task 4

Your group should assign a season to each data point in the biology dataset. Show graphically the seasonal abundance of organisms considering kingdoms (similar to the physical dataset).

a Is it possible to determine the differences between groups? Do the necessary data transformation.

b What are the organisms that are not assigned to any kingdom? To which kingdom do they belong? Add literature reference for your research.

c Based on your research, assign the unknown organisms and build new plots.

Task 5

All that remains is to analyze the `chemistry.csv` dataset. Load the `chemistry.csv` file and answer the questions:

a Plot all possible time dependent variables:

- Nitrite
- Nitrate
- Ammonium
- Silicate
- Phosphate

b From examining the plots, detect outliers and explain the possible causes.

c Exclude the outlining data points and repeat the plots with cleaned data.

d There is an important piece of metadata missing from the provided three datasets. What is it?

DAY 2

PART I

Task 1

Water quality is important both in nature and in the human environment. The analysis of some factors of water quality is described below. The first of these parameters is dissolved oxygen. One of the most commonly used methods for its determination is the Winkler method, which is based on oxygen tying into the tetravalent manganese compound.

In the first step, 5 drops of manganese(II)sulphate solution are added to 50 mL of the water sample. Next, 5 drops of potassium iodide and sodium hydroxide solution are added to form create a brown precipitate (in this step, oxygen tying into the manganese-containing compound forming $MnO(OH)_2$). The precipitate disappears with the addition of a few drops of sulfuric acid and the solution becomes transparent. This solution is then transferred to a 10 mL vessel where 2 drops of the starch solution are added. 760 µL of sodium thiosulphate solution is used for titration of the prepared solution.

a How much sodium thiosulphate (amount) is needed to titrate the solution containing 1 mmol of dissolved oxygen?

The concentration of thiosulphate solution was determined by titrating an iodine solution with a known concentration. This solution was prepared as follows. 267.5 mg of potassium iodate was dissolved in 1 L of distilled water and an aliquot of 25 mL was transferred to an Erlenmeyer flask. Potassium iodide (excess) and the sulfuric acid solution were then added to the same flask. 15 mL of the solution with unknown thiosulphate concentration is used for titration of the prepared solution.

b Calculate the oxygen mass concentration.

Task 2

One of the indicators of water quality is the dissolved nitrite content. There are several methods for nitrite determination, and most of those described so far are based on the Griess reaction. The Griess reaction refers to the condensation of nitrite with aniline. The resulting adduct then reacts with another aromatic amine (usually naphthylamine).

In our laboratory, 4-aminobenzenesulfonic acid and *N*-(1-naphthyl)ethylenediamine were used to determine nitrite concentration (in a suitable acidic medium).

a Draw the structure of the azo dye formed in this process and draw the condensation mechanism of nitrite and

4-aminobenzenesulfonic acid.

The absorbance maximum of the dye produced by this reaction is observed at 525 nm. To determine the molar absorption coefficient, a series of solutions of known concentration were prepared. The solutions were prepared by dissolving a given amount of sodium nitrite (Table 1, first column) in distilled water (100 mL). The prepared stock solutions were diluted 30 times after which 1 mL of this solution was pipetted into the cuvette. A solution of 4-aminobenzenesulfonic acid (0.5 mL), a solution of hydrochloric acid (0.5 mL), and a solution of the mentioned amine (0.5 mL) were then added to the cuvette. All reagents were added in excess and the optical path length was 10 mm. The measured absorbances for the given solutions are shown in the second column.

b Determine the molar absorption coefficient at a given wavelength (525 nm).

Table 1. Sodium nitrite masses used for the preparation of the standard solutions and corresponding absorbance values at 525 nm with an optical path length: l = 10 mm (T = 293 K).

$m(NaNO_2)$ / mg	A(525 nm)
0.5	0.0254
1.5	0.0761
2.5	0.1271
3.5	0.1703
4.5	0.2278
5.5	0.2789
6.5	0.3298
7.5	0.3858
8.5	0.4305
9.5	0.4790
10.5	0.5440
11.5	0.5833
12.5	0.6350
13.5	0.6857
14.5	0.7550

After determining the molar absorption coefficient, an analysis of nitrite in a real sample was performed. 1 mL of seawater was pipetted into the cuvette, then 4-aminobenzenesulfonic acid solution (0.5 mL), hydrochloric acid solution (0.5 mL), and *N*-(1-naphthyl)ethylenediamine solution (0.5 mL) were added to the cuvette. With an optical path length of l = 1 cm, the absorbance at 525 nm was 0.234.

c Determine the molar concentration and mass concentration of the nitrite anion in a mentioned seawater sample.

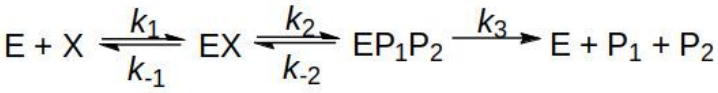
There are three water samples in front of you (labels **A**, **B** and **C**). Small vials with labels **1** and **2** contain acidified solutions of naphth-1-ylamine (solution **1**) and 4-aminobenzenesulfonic acid (solution **2**).

Add half of a dropper with solution **1** and half of a dropper with solution **2** to each of the three samples obtained.

d Indicate which of the samples are negative and which are positive for nitrites.

Task 3

One of the parameters that determine the quality of seawater is the amount of phosphates present. Besides the inorganic form, phosphate can also be part of various organophosphorus compounds. The scheme shown below describes the process of enzymatic hydrolysis (enzyme **E**) of an organophosphate (molecule **X**), producing phosphate **P1** and the product **P2**:



a Use a steady-state approximation and derive the expression for the rate of phosphate (**P1**) formation.

b Derive the expression describing the dependence of the concentration of molecule **X** on time. Use the following approximations: $k_1 \approx k_2 \approx k_3$, $k_1 > k_{-1}$, and $k_2 > k_{-2}$.

c An experiment was performed in the laboratory with the isolated enzyme **E** ($c = 1 \cdot 10^{-6}$ mol dm^{-3}). Knowing that (with the assumptions from subtask b) the constants are: $k_1 = 5 \cdot 10^5$ dm^3 mol^{-1} s^{-1}, $k_2 = 4.5 \cdot 10^5$ s^{-1}, and $k_3 = 4 \cdot 10^5$ s^{-1}, determine the time at which the substrate concentration is 10% of the initial substrate concentration. The initial substrate concentration is half of the enzyme concentration.

Task 4

The organic substances present in water are determined by non-specific indicators, including the measurement of oxygen consumption. The organic substances are oxidized at the boiling point with a standard solution of potassium dichromate and sulphuric acid.

The chlorides, which can be partially oxidized to chlorine by potassium dichromate solution, and reducing substances such as hydrogen sulphide, sulphides, hydrogen peroxide, etc. interfere with the determination. On the one hand, the negative influence of chlorides can be eliminated by removing them in the form of practically insoluble silver chloride by using silver sulphate. On the other hand, hydrogen sulphide and sulphides can be oxidized in a parallel water sample (without heating), and the amount of potassium dichromate used for this purpose is subtracted from the total amount of potassium dichromate consumed in the sample oxidized at the boiling point.

The excess potassium dichromate is titrated with ammonium iron(II)sulphate hexahydrate using ferroin as an indicator.

a Why is the back titration performed?

b Write a redox reaction for titrating an excess of potassium dichromate with ammonium iron(II)sulphate hexahydrate and give the oxidation states of the atoms and the number of electrons exchanged.

c What colour change do you expect to see during the titration? Explain your answer in detail.

d Calculate the mass of oxygen in a sample of seawater and express it in mg O_2/ L if the procedure is as follows:

About 0.2 g silver sulphate and 6 mL potassium dichromate, c = 0.0417 mol L^{-1}, were added to a 10 mL seawater sample. Then 14 mL of concentrated sulphuric acid with silver sulphate was added and the system was boiled for 2 h. The system was then cooled. After cooling, 6 drops of ferroin indicator were added and titrated with ammonium iron(II)sulphate hexahydrate solution, c = 0.05 mol L^{-1}. The equivalence point was reached with the addition of 10 mL of titrant.

PART II

Water quality is very important for achieving good environmental status of marine waters. The main nutrient sources in the sea are the load brought in by water from the land across the river, and the direct discharge of wastewater on the shores. 78% of total nitrogen and 95% of total phosphorus come from the load introduced by water. The two most important sources of water-borne nutrients are diffuse sources, in which agricultural activities account for the largest share (45% of total nitrogen and 45% of total phosphorus) and point sources, in which municipal wastewater accounts for the largest share (12% of total nitrogen and 20% of total phosphorus).

Task 1

a Increase in the concentrations of various components of N and P in the sea can lead to which process? Explain briefly this process and how it affects marine biodiversity and food web. Support the answer with literature.

b Climate changes are the cause of two additional problems. How do you think short and wet winter will affect on the nutrient load in the sea, and how will affect the temperature increase? Support the answer with literature.

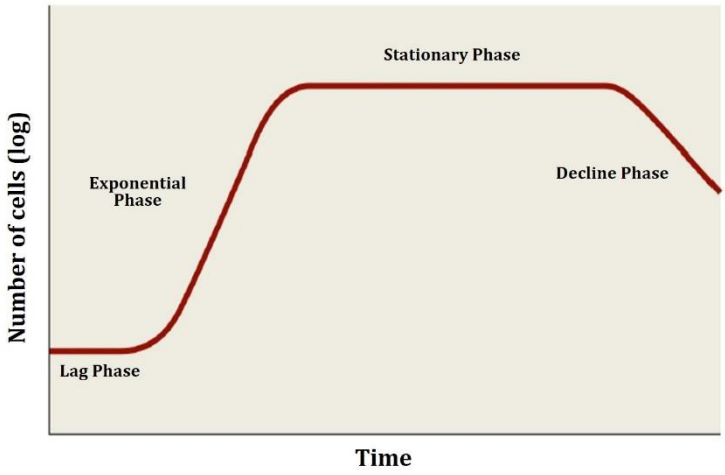
In addition to the negative effect of N, P and their components, these macronutrients can have a positive effect on the development of life in aquatic ecosystems. Diatoms are one of the most numerous groups of microalgae, responsible for as much as a quarter of all photosynthetic production in the world (Sumper and Bruner, 2006). They belong to the class Bacillariophyta which is divided into two orders: Centrales and Pennales (Viličić, 2002). They can be found in any aquatic or moist medium, from marine habitats such as oceans, seas and lagoons to rivers, lakes and even the smallest puddles. They are dominant in most coastal seas and are one of the main components of phytoplankton. They usually stay close to the surface due to light sources and nutrients. The already mentioned macronutrients N, P, but also Si are important for their growth, as well as micronutrients (mostly dissolved iron) and vitamins (thiamine, cyanocobalamin and in some cases biotin) (Lebeau and Robert, 2003b). Diatoms require water rich in nutrients, especially silicates, as evidenced by the fact that they use over 6.7 billion tons of silicon per year from the ocean in which they are located (Treguer et al., 1995). Under nutrient limitation conditions, cells accumulate lipids, primarily triacylglycerols. Under adverse conditions, they can form permanent cysts (spores), called hypnospores. They create a hard silicified shell with the aim of sinking into colder water that is richer in nutrients, waiting for favourable conditions again (French and Hargraves, 2004).

Task 2

Investigate whether diatoms react to changes in Si, N and P concentrations in the sea? What can happen to diatoms by limiting a particular macronutrient? Why? Support the answer with literature.

a At higher concentrations of which macronutrient do diatoms grow best? Why? Support the answer with literature.

b There are several stages of growth in algal culture (figure below).



What can happen to the exponential phase at higher silicate concentrations?

Why does the dying phase occur?

c What is biosilicification?

d What can happen in the event of a sudden decrease in the number of diatoms in the sea? Support the answer with literature.

DAY 3

CASE STUDY

Today you are provided with marine measurements (`2019_data_Day3.csv`) from the year 2019 which is infamously known to be the year when an incident caused by human error happened in the northern Adriatic Sea. The data were gathered by experts from the Center for Marine Research Rovinj under the supervision and coordination of the Ministry of Environmental Protection and Energy to gain an insight into the implications of this local environmental disaster on marine life. Your task today is to analyse the provided data and discover how this ecological event is reflected in the chemical and physical parameters.

Your observations of significant deviations from normal values, together with literature sources on the possible reasons for the mentioned dissimilarities, should be presented in the form of an essay. One of the aims of your report is to raise awareness of the potentially negative anthropological impacts on marine life, but also to provide some ideas and suggestions on how well-designed human activities to meet economic needs can still create the conditions for sustainable development - a development that meets today's needs while not jeopardizing the needs of future generations.