

European Union Science Olympiad



Jahresbericht 2016/17

Mag. Peter Holub

Regionales Netzwerk für Naturwissenschaften und Mathematik Kärnten

Vom



gefördert



European Union Science Olympiad

Die EUSO ist ein naturwissenschaftlicher Teamwettbewerb der Europäischen Union für Biologie, Chemie und Physik. Österreich war 2017 zum schon zehnten Mal mit zwei Teams bei der EUSO, die heuer in Kopenhagen stattfand, vertreten.

Das Credo der EUSO

- begabten SchülerInnen die Möglichkeit geben, ihre Talente zu entfalten
- Das Interesse an Wissenschaft und des forschenden Lernens zu wecken bzw. zu fördern
- Durch die Eindrücke und Erfahrungen der EUSO auf eine mögliche Teilnahme an weiteren Internationalen Olympiaden vorzubereiten

Ziel des Wettbewerbs

- Öffentliche Interesse auf die naturwissenschaftliche Ausbildung lenken
- Ermittlung der besten SchülerInnen der Europäischen Union im naturwissenschaftlichen Bereich
- Wertschätzung der Wissenschaft in der Allgemeinheit
- Intensivierung der Zusammenarbeit zwischen europäischen Bildungssystemen
- Individuelle Ideen und Konzepte innerhalb der gesamten Europäischen Union zu verbreiten
- Vorbereitung europäischer SchülerInnen auf die Internationalen Facholympiaden

Mehr dazu unter: www.euso.eu und www.euso.at

Inhalt

1. Vor den Vorhang	4
2. Vorbereitungswoche Graz	5
3. Trainingstage an der BIKO mach MINT in Klagenfurt	6
4. EUSO 2017 in Dänemark	6
5. Team AUSTRIA 2017	7
6. Einmal Silber, einmal Bronze für Österreich!	7
7. Medallenspiegel	8
8. Aufgabenstellungen 2017	9
9. Unterstützung durch	53

1. Vor den Vorhang

Ich möchte mich im Namen aller beteiligten SchülerInnen zu Beginn bei zwei KollegInnen bedanken, die in den letzten Jahren viel zum sehr guten Abschneiden unserer Teams bei den internationalen Wettbewerben beigetragen haben, deren berufliche Laufbahn sie ab dem Herbst 2017 jedoch leider zu anderen Aktivitäten führt.

Mag. Sabine Seidl, die mich oft vertreten hat und die auch bei der Organisation der EUSO 2015 in Klagenfurt maßgeblich beteiligt war, steht im Herbst als Mentorin für Chemie nicht mehr zur Verfügung. Mag. Christine Ottowitz bleibt den begabten SchülerInnen zwar im Rahmen von „Biologie im Team“ erhalten, beendet aber ebenfalls ihre Tätigkeit als Mentorin für Chemie. Ich wünsche beiden viel Erfolg in ihrer nächsten Lebensphase und bedanke mich auch persönlich von ganzem Herzen.

Als Mentor für Chemie konnte mit Mag. Karl Brachtl ein äußerst erfahrener Chemiker gewonnen werden. Als Mentorin für Biologie ist ab dem Herbst 2017 Dr. Christina Morgenstern, die bereits in Estland als Mentorin dabei war und die ebenfalls eine führende Rolle bei der Organisation der EUSO 2015 in Klagenfurt gespielt hat, in unser Team gekommen.

Team AUSTRIA bei der EUSO 2017 in Kopenhagen



Mag. Dieter Winkler, Othmar Gritzky, Jakob Pretenthaler, Markus Rupp, Jesse Ziegler, Christina Lassnig, Zarije Ademi, Mag. Sabine Seidl, Mag. Peter Holub, Mag. Christine Ottowitz, Dr. Christina Morgenstern, Josefine Neerup-Lundh(Guide), von links nach rechts

2. Vorbereitungswoche Graz

36 SchülerInnen aus sechs Bundesländern, erstmalig mehr Mädchen als Buben, wurden, organisiert vom Fachdidaktikzentrum Physik, vom 27.02.-3.03. 2017 an der Karl-Franzens-Universität Graz auf den Teamwettbewerb in Dänemark vorbereitet.

	Familienname	Vorname	Fach	Schule	BL
1	Ademi	Zarije	Physik	Adalbert Stifter Gymnasium	OÖ
2	Antensteiner	Sebastian	Physik	Bischöfliches Gymnasium Graz	St
3	Berghold	Michael	Physik	Gymnasium Zell am See	S
4	Fuchs	Lukas	Physik	Gymnasium Zell am See	S
5	Gritzky	Othmar	Chemie	Gymnasium Dachsberg	OÖ
6	Haas	Fabian	Physik	Ingeborg Bachmann Gymnasium Klagenfurt	K
7	Herwagen	Rouven	Biologie	BRG 18 Wien	W
8	Hohl	Elias	Physik	Bischöfliches Gymnasium Graz	St
9	Holzapfel	Markus	Chemie	Bertha-von-Suttner-Gymnasium	W
10	Hübel	Lorenz	Physik	Sir Karl Popper Schule	W
11	Kalla	Victoria	Biologie	BG/BRG Mössingerstraße Klagenfurt	K
12	Klaus	Elisabeth	Chemie	Europagymnasium Klagenfurt	K
13	Klaus	Sophie	Chemie	Europagymnasium Klagenfurt	K
14	Kögler	Florian	Chemie	Sir Karl Popper Schule	W
15	Lassnig	Christina	Biologie	Peraugymnasium Villach	K
16	Lerchster	Julia	Biologie	BG/BRG Mössingerstraße Klagenfurt	K
17	Obermaier	Sophia	Physik	Bischöfliches Gymnasium Graz	St
18	Perner	Wanda Melina	Chemie	Sir Karl Popper Schule	W
19	Plakolm	Oliver Leon	Biologie	BRG 18 Wien	W
20	Prattes	Lorenz	Physik	Bischöfliches Gymnasium Graz	St
21	Prettenthaler	Jakob	Physik	Bischöfliches Gymnasium Graz	St
22	Rampler	Heike	Biologie	Stiftsgymnasium Admont	St
23	Reinprecht	Johanna	Chemie	BRG Leibnitz	St
24	Reisner	Teresa	Chemie	Modellschule Graz	St
25	Rupp	Markus	Biologie	Peraugymnasium Villach	K
26	Schantl	Michael	Physik	Bischöfliches Gymnasium Graz	St
27	Steiner	Amelie Daniela	Biologie	BRG Leibnitz	St
28	Steinwender	Elena	Chemie	BRG Feldkirchen	K
29	Stepman	Helene	Chemie	Gymnasium Schillerstraße	Vo
30	Stölzl	Theo	Chemie	BG/BRG Carneri	St
31	Vojtech	Victoria	Chemie	Bertha-von-Suttner-Gymnasium	W
32	Wendel	Stephanie	Biologie	Gymnasium Schillerstraße	Vo
33	Winkler	Lucia	Chemie	Bischöfliches Gymnasium Graz	St
34	Wlattnig	Hannah	Biologie	Akademisches Gymnasium Graz	St
35	Ziegler	Jesse	Chemie	Bertha-von-Suttner-Gymnasium	W
36	Zwirner	Chiara	Biologie	Sir Karl Popper Schule	W

3. Trainingstage an der BIKO mach MINT in Klagenfurt

Sechs Jugendliche schafften es in die Qualifikation und somit zum Intensivtraining, das heuer wieder in Kooperation mit dem deutschen EUSO-Nationalteam erstmals am Lakeside Park in Klagenfurt stattfand (2. - 6. April 2017).

Diese Trainingstage mit den deutschen EUSO KandidatInnen waren für die zwei österreichischen Nationalteams erneut eine tolle Herausforderung. Zudem konnten an der BIKO (Bildungskooperation) mach MINT mit modernster Ausrüstung an molekularbiologischen Aufgabenstellungen, entwickelt von Dr. Christina Morgenstern, gearbeitet werden.

Insgesamt beteiligte TrainerInnen in Graz und Klagenfurt

TrainerIn	Stamminstitution	Fach
Mag. Dieter Winkler	Bischöfliches Gymnasium Graz	Physik
Matthias Diez	Karl Franzens Universität Graz	Physik
Mag. Karl Brachtl	Regionales Netzwerk Kärnten	Chemie
Mag. Sabine Seidl	Nawizentrum der Pädagogischen Hochschule Kärnten	Chemie
Mag. Christine Ottowitz	BG/BRG Villach St. Martin	Biologie
Dr. Christina Morgenstern	Nawizentrum der Pädagogischen Hochschule Kärnten	Biologie
Mag. Sigrid Holub	Regionales Netzwerk Kärnten	Biologie
Mag. Peter Holub	Regionales Netzwerk Kärnten	Biologie
Stefan Lobnig	BIKO mach MINT	Physik

4. EUSO 2017 in Dänemark

Die Organisation in Kopenhagen war ausgezeichnet. TrainerInnen und Teams waren im selben Hotel, jedoch beinahe völlig getrennt untergebracht. Das Freizeitprogramm war ansprechend, die Aufgabenstellungen fordernd originell und gut durchdacht. Den VeranstalterInnen gebührt ein großes Lob.

5. Team AUSTRIA 2017

Delegationsleitung: Mag. Peter Holub
Mentorin Biologie: Mag. Christine Ottowitz
Mentorin Chemie: Mag. Sabine Seidl
Mentor Physik: Mag. Dieter Winkler
Observer: Dr. Christina Morgenstern
Team A: Jakob Prettenthaler, Markus Rupp, Jesse Ziegler
Team B: Zarije Ademi, Othmar Gritzky, Christina Lassnig

6. Einmal Silber, einmal Bronze für Österreich!

Die jungen Österreichischen Teams schlugen sich sehr gut. Team A errang eine Silbermedaille, das B-Team Bronze.



Team A Jakob Prettenthaler, Markus Rupp, Jesse Ziegler, vlnr.



Team B Zarije Ademi, Christina Lassnig, Othmar Gritzky, vlnr.

7. Medaillenspiegel

GOLD MEDAILLEN (IN UMGEKEHRTER REIGENFOLGE)

Czech Republic A	5	Gold	Jindřich Jelínek	Jiří Janoušek	Richard Veselý
Germany A	4	Gold	Bruno Ederer	Jakob Schramm	Roman David Ventzke
Croatia B	3	Gold	Luka Bulić Bračulj	Paula Vídas	Petar Škrobo
Estonia A	2	Gold	Karl Paul Parmakson	Kirke Joamets	Kaarel Kivisalu
Hungary A	1	Gold	Ákos Harangozó	Balázs Németh	Bulcsú Fajsi

SILBER MEDAILLEN (IN UMGEKEHRTER REIGENFOLGE)

Denmark 1 A	26	Silver	Astrid Kildelund Rosenkilde	Esben Bjørn Salmonsén	Søren Johannes Heiberg
Slovakia B	25	Silver	Jakub Barat	Michal Lelak	Peter Rukovansky
Italy B	23	Silver	Felix Chippendale	Francesco Debortoli	Jakob Nögler
Sweden B	23	Silver	Andy Fang	Björn Diemer	Linus Persson
Croatia A	22	Silver	Domagoj Perković	Klara Tomašković	Mislav Barić
Austria A	21	Silver	Jakob Pretenthaler	Jesse Ziegler	Markus Rupp
The Netherlands B	20	Silver	Daan Hoogers	Sean Camps	Tido Houtepen
Ireland B	19	Silver	Brian Durkan	Kevin Jansson	Stephen Trenier
Estonia B	18	Silver	Andreas Must	Hanna-Riia Allas	Konstantin Dukatš
Slovakia A	17	Silver	Dominik Kopcak	Emil Lelak	Michal Chovanec
Ireland A	16	Silver	Andrew Nash	Antonia Huang	Theo Bolger
Germany B	15	Silver	Franziska Salome Schwark	Lea Paula Wagner	Sophia Maria Louisa Häußler
Lithuania A	14	Silver	Gediminas Lelešius	Milda Navickaitė	Ričardas Navickas
Luxembourg B	13	Silver	Chris Petit	Daniel Stors	Michel Mertens
Latvia A	12	Silver	Anna Marija Sumrova	Kristers Kokars	Laura Bukevica
The Netherlands A	11	Silver	Lotte Kremer	Marouscha Puister	Stan Koenis
Lithuania B	10	Silver	Ignas Šakuro	Justas Terentjevas	Mindaugas Dženkaitis
Italy A	9	Silver	Alberto Cadorin	Gaia De Paciani	Luca Lazzari
Slovenia B	8	Silver	Aleš Globočnik	Klemen Bogataj	Maša Predin
Hungary B	7	Silver	Csaba Kozma	István Csépanyi	Ivett Mihálicz
Portugal A	6	Silver	Diogo Rodrigues	João Miguel Sousa	Marco António Ribeiro

BRONZE MEDAILLEN (IN ZUFÄLLIGER REIGENFOLGE)

Czech Republic B	Bronze	Adam Mendl	Kateřina Bezányiová	Michal Straka
Romania B	Bronze	Andrei Bordenianu	Miruna Teodora Patru	Razvan Petru Efrim
Denmark 2 B	Bronze	Albert Sandahl Kjølby	Christian Adam Deding Nielsen	Katharina Walsted Lykou
Belgium A	Bronze	Brent Nissens	Thijs Paelman	Toon De Prins
Latvia B	Bronze	Anastasija Šjapina	Anitra Zile	Vilhelms Cinis
Bulgaria A	Bronze	Ivan-Aleksandar Mavrov	Yana Dimitrova	Zdravko Ivanov
Finland A	Bronze	Anton Ruhr	Oona Salonen	Pietari Virkkunen
Greece A	Bronze	Aikaterini Gkrintzia	Panagiotis Makris	Velissarios Christodoulou
Sweden A	Bronze	Henrik Vester	Lucas Johnson	Mustafa Al-Assadi
Denmark 1 B	Bronze	Anna Vingborg	Nanna Frost Falborg	Sif Bjerre Lindby
France A	Bronze	Ahmad Iksi	Noé Perrin	Tilia Pierson
Austria B	Bronze	Christina Lassnig	Othmar Gritzky	Zarije Ademi
Belgium B	Bronze	Adrien Arbalestier	Thomas Rosseel	Thomas Vray
Cyprus B	Bronze	Angelos Assos	Ioannis Kestoras	Savvas Kamenos
France B	Bronze	Mathilde Gosse	Nicolas Brosseau-Habert	Bastien Wagner
Denmark 2 A	Bronze	Jakob Rossander Kristensen	Rasmus Vester Munkner	Rolf Bisgaard Poulsen
Cyprus A	Bronze	Despina Gerolemidou	Matthaios Chouzouris	Xenios Papageorgiou
Bulgaria B	Bronze	Evgeni Statelov	Silvi Koycheva	Slaveya Kostadinova
Slovenia A	Bronze	Ana Meta Dolinar	Jon Judež	Tijan Prijon
Romania A	Bronze	Daria Ioana Radu	Miruna Ioana Belciu	Monica Balanescu
Finland B	Bronze	Oskar Heikkilä	Otto Torkkeli	Reetta Kälvälä
Greece B	Bronze	Kerasoula Christakaki	Nikolaos Kalligas	Panagiotis Karakostidis
Luxembourg A	Bronze	Helena Westermann	Jean-Marc Furlano	Raffaël Marth
Portugal B	Bronze	Alexandra Ribeiro Verdasca	Ana Rita Magalhães Gerales	Filipe Miguel Favita Monteiro

8. Aufgabenstellungen 2017

Task 1

ICE

9th of May 2017



Introduction

Welcome to the Ice Lab. This is going to be your place of work during the next few hours. You are going to investigate the climate and conditions of life in the past.

The increasing amount of greenhouse gases in the atmosphere is changing the climate of the Earth in a decisive way. Experts are engaged in lively discussions about the expected increase in the global mean temperature and whether it will give rise to a violent melting of the polar ice sheets. Among these is the inland ice of Greenland. Such a melting could give rise to increases in the level of the oceans of many meters.

If we are to be able to predict the future climate, there is a pressing need to improve the knowledge and understanding of the climate of the past. One of the tools for this is the investigation of ice cores, samples of the ice cap, drilled from the surface down to the solid rock beneath. In Greenland, the thickness of the ice cap is in some places more than 3000 m. Ice cores represent a source of comprehensive knowledge of the climate in the past. Measurements of the properties of the ice and its content of impurities and air bubbles allow one to investigate the atmosphere, seas and ice caps of the past with a high amount of details.

You are going to investigate ice core samples from the test station NEEM in Greenland, and you will be analysing DNA-fragments in samples from the Dye-3 position. Also, you will determine recent living organisms from so-called cryoconite holes in the arctic ice. You will characterize the climate in earlier times from physical, chemical and biological examinations.

In this way, you can contribute decisively to the understanding of the climate in the present and in past interglacial periods, and thus improve on the understanding of the dynamics of the climate system and on the possibility to predict the reaction of the ice caps to future climate changes.



Figure 1.0. Greenland, located close the north pole. The localizations of the drilling spot NEEM and the Dye-3 position are shown.

Experiment 1 -

32 marks

Introduction

Polar ice cores can be used to provide valuable information on past climate. Willi Dansgaard, a pioneer in polar ice core research, proposed in 1952 that the ratio of the heavy to the light isotopes of oxygen in the water molecules of polar precipitation shows a strong correlation with the temperature of the atmosphere at the time of the snowing event. As a result, study of ice from the deeper layers of the Greenland and the Antarctic ice caps can yield information on past climate spanning tens to hundreds of thousands of years back in time.

Here you will have a unique opportunity to perform measurements and calculations using real ice core samples from the Greenland ice cap. First, you will perform measurements of ice density and later you will reconstruct past Greenland temperatures, using state of the art laser spectroscopy measurements.

Materials

- 1 cylindrical ice core sample, 2 cm thick
- 1 beaker, 1000 mL
- 1 bottle of cold, deionized water
- 1 precision laboratory scale (± 0.1 g)
- 1 potato fork
- 1 thermometer
- 1 plastic calliper
- Millimetre paper
- 2 bottles with samples of water from ice core – will be handed out
- 2 plastic pipettes
- 2 vials

1.1 The density of ice

The top layer of Greenland's ice consists of firn. With the term firn (in German it literally means snow of the last year) we refer to the material that is going through a densification process from snow to solid ice. The first part of this assignment is the experimental determination of the density of a real ice core sample from the Greenland ice cap.

Please pay attention to the following:

The following experiment requires that you work with a piece of ice in an environment with temperature above zero °C. As a result, you are advised to prepare your equipment, read carefully the assignment steps in advance and make a plan as a team before you fetch the ice core sample from the freezer. Extensive delays during this experiment may result in unnecessary melting of the ice core sample and thus poor measurement results.

Question 1.1

Work through the following steps.

Question 1.1.1

- a. Fetch the beaker and cold deionized water from the fridge and fill the beaker with 0.5 L water. Measure the temperature of the water T_w .

➤ Write down the result in the answer sheet, box 1.1.1.

- b. Using the plot provided in **Figure 1.1**, find the density of the water ρ_w for the temperature you measured.

➤ Write down the result in the answer sheet, box 1.1.1.

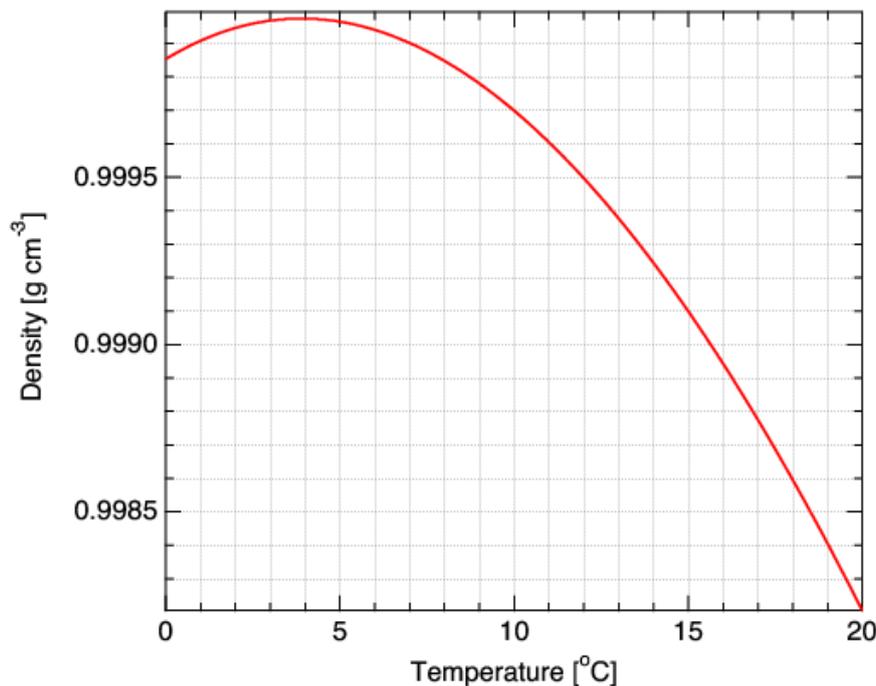


Figure 1.1. Density of water as a function of temperature.

Question 1.1.2

- c. Place the beaker on the scale and measure the mass of the beaker and the water m_{w+g} .

➤ Write down the result in the answer sheet, box 1.1.2.

- d. Fetch the ice core sample from the freezer.
e. Using the plastic calliper measure the diameter D_{ice} and the thickness H_{ice} of your sample.

➤ Write down the result in the answer sheet, box 1.1.2.

f. Place the ice sample in the water. Measure the new mass $m_{g+w+ice}$.

➤ Write down the result in the answer sheet, box 1.1.2.

g. Apply force with the fork to submerge the ice. Record the new reading $m_{g+w+ice+force}$.

➤ Write down the result in the answer sheet, box 1.1.2.

Question 1.1.3

h. Calculate the volume of your sample V_{ice} .

➤ Write down the result in the answer sheet, box 1.1.3.

i. Calculate the mass of the ice core sample m_{ice} .

➤ Write down the result in the answer sheet, box 1.1.3.

j. Use the results of steps h and i to calculate the density of the ice ρ_{ice} .

➤ Write down the result in the answer sheet, box 1.1.3.

k. Use the results from steps g and i to give a second estimate of the ice density ρ'_{ice} . Show the intermediate steps in your calculation.

➤ Write down the result in the answer sheet, box 1.1.3.

Question 1.1.4

In this question, uncertainties of some of the measurements will be estimated using min-max method.

l. Calculate the difference $\rho'_{ice} - \rho_{ice}$.

➤ Write down the result in the answer sheet, box 1.1.4.

m. You have probably observed that the reading you get for $m_{g+w+ice+force}$ when the ice is submerged is somewhat unstable. Give an estimate of the uncertainty $\Delta m_{g+w+ice+force}$.

➤ Write down the result in the answer sheet, box 1.1.4.

n. Based on your answer in step m, give an estimate of the uncertainty $\Delta \rho'_{ice}$.

➤ Write down the estimate in the answer sheet, box 1.1.4.

o. Assuming the quality of the cutting of the sample and the calliper measurement result in an uncertainty of 0.2 mm for both D_{ice} and H_{ice} , calculate the uncertainty of the estimate of V_{ice} and ρ_{ice} (ΔV_{ice} , $\Delta \rho_{ice}$).

➤ Write down the result in the answer sheet, box 1.1.4.

1.2 Densification of snow to ice and pressure in the ice

The pressure needed to force the firm densification process comes from the weight of the material itself. As

a result, the density increases with increasing depth as shown in **Figure 1.2**. The pressure at a depth z is given by the equation:

$$p(z) = \frac{g \cdot m_z}{A}$$

where g is the gravitational acceleration (9.81 ms^{-2}) and m_z is the mass of a column of overburden ice at depth z and with cross sectional area A .

Question 1.2.1

Based on the pressure equation and **Figure 1.2**, answer if the following sentences are true or false.

Statement	True	False
$p(z)$ depends on only the depth z .		
At sufficiently large depths, the pressure $p(z)$ is roughly a linear function of depth.		
$p(z)$ depends on the depth z and the density $\rho(z)$.		
$p(z)$ is independent of depth z .		
$p(z)$ is independent of the cross-sectional area of the column A .		

➤ Tick your answers in the answer sheet, box 1.2.1.

Question 1.2.2

Estimate the pressure and density of the ice at the following depths using **Figure 1.2**.

Depth z [m]	Density ρ [kgm^{-3}]	Pressure p [kPa]
0		
80		
160		
1000		

➤ Give your answers in the answer sheet, box 1.2.2.

Our small drilling machine can retrieve ice cores with a diameter of 74 mm and a length of 1 m from depths up to 350 m.

Question 1.2.3

Calculate the mass of a drilled ice core from these depths.

➤ Give your answers in the answer sheet, box 1.2.3.

Depth z [m]	Mass m [kg]
80	
160	

The big drilling machine can retrieve ice cores with a diameter of 98 mm and a length of 4 m at depths reaching the bottom of the ice cap.

Question 1.2.4

Calculate the mass of an ice core drilled with the big drilling machine at these depths.

➤ Give your answers in the answer sheet, box 1.2.4.

Depth z [m]	Mass m [kg]
1000	
2000	

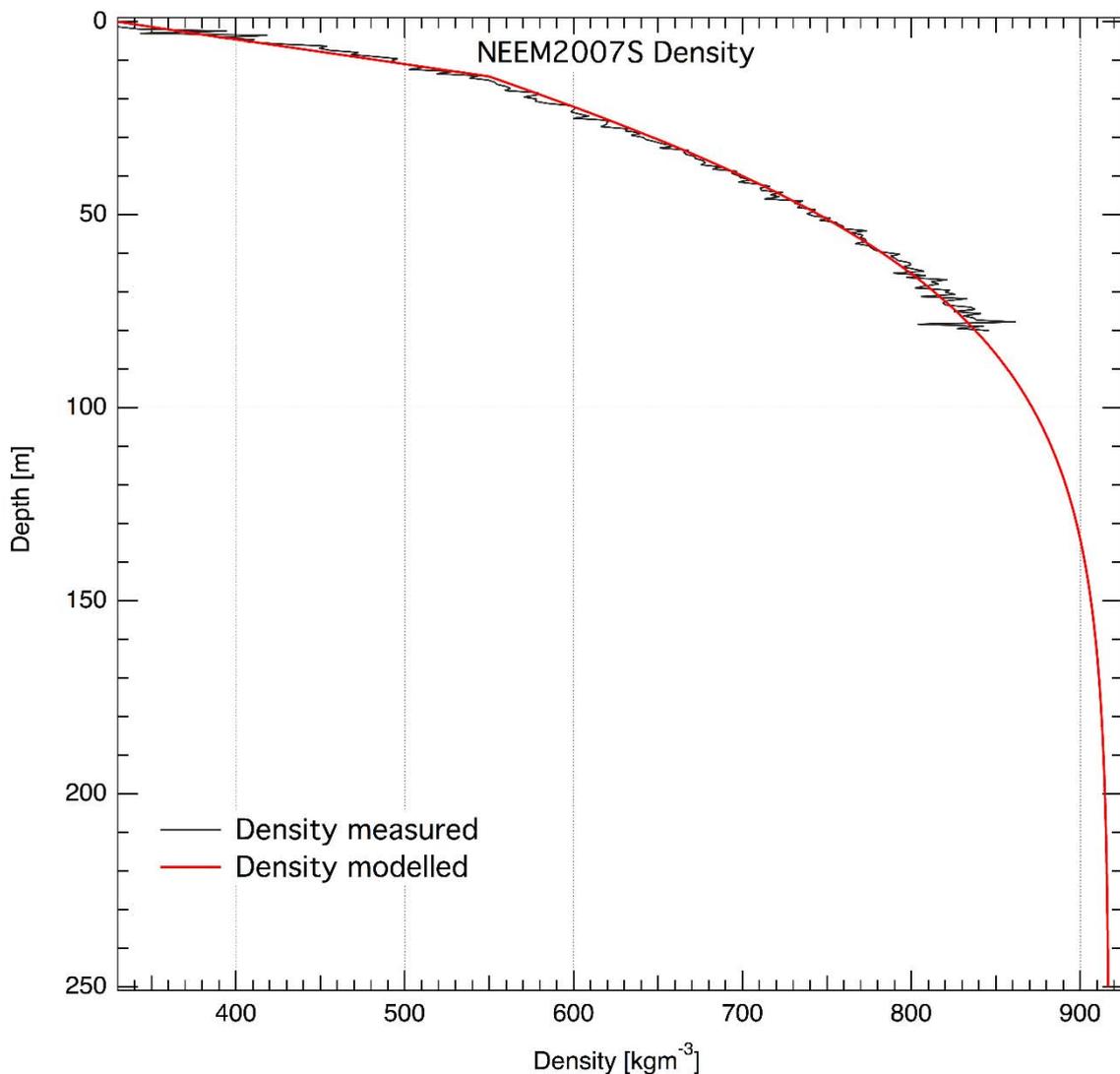


Figure 1.2. Density of inland ice from Greenland as a function of depth (notice the orientation of the axes).

1.3 Dating of ice and isotopes

For the next part of this experiment you will be working with ice core samples from the deeper part of the NEEM ice core. The NEEM ice core was drilled in North-East Greenland between 2007 and 2012 and contained ice from the previous warm interglacial time.

You will be given two bottles with real ice core samples from two different depths of the ice sheet. You will be asked to estimate the age of both samples, calculate the temperature over the ice sheet and in the end, you will be asked to prepare sample vials in order to perform isotopic analysis on a laser spectrometer.

Ice flow

We normally think of ice as a solid material. In reality, ice is a material that under stress can behave like a fluid and no other place in the world presents a better example of this behaviour than the ice caps of Greenland and Antarctica. Each year a new layer of snow falls on the ice cap. After an annual layer of snow has undergone a process of densification, the load of the overburden ice results in a continuous stretching and thinning of the ice layers. As shown in [Figure 1.3](#), an ice layer with an initial thickness of 20 cm (typical for the case of NEEM for present conditions) undergoes a thinning process that has the effect that at a depth of 1000 m the same layer has a thickness of about 10 cm (50% of the initial value).

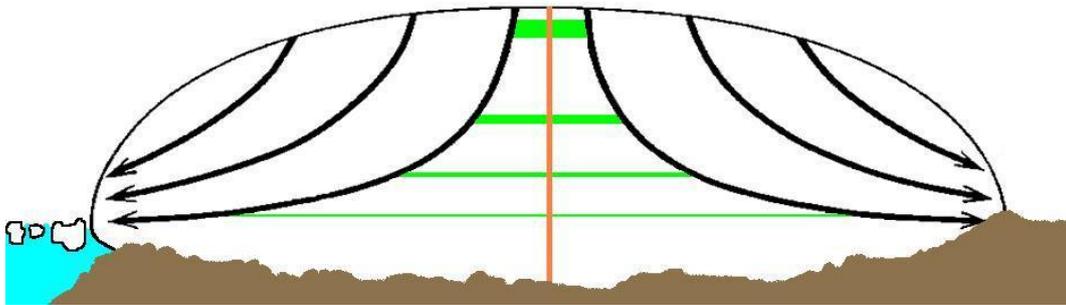


Figure 1.3. Flow of ice

By using very precise and high-resolution measurements of water isotopes and chemical impurities, we are able to measure the thickness of the annual layers in the ice core. In the following table, you can see what a year's layer thickness (**let us call it λ from now on and express it in meters per year**) looks like, based on real measurements of ice from the NEEM ice core.

Question 1.3.1

Based on the data present in **Table 1**, make a graph of λ as a function of depth z on the millimetre paper you are given. Label the graph "Graph 1.3.1" and attach it to the answer sheet.

➤ *Attach "Graph 1.3.1" to the answer sheet.*

Table 1

Depth z [m]	Annual Layer Thickness λ [m/yr]
0	0.25
500	0.20
1200	0.10
1400	0.04
1500	0.0125

Age of the ice

The quantity λ is extremely useful as it can be used in order to calculate the age of a layer of ice. If λ is known as a function of depth and we assume a thin layer with thickness Δz , then the number of years included in this layer Δt will be given by the equation:

$$\Delta t = \frac{1}{\lambda} \cdot \Delta z$$

For a thicker layer between for example the depths z_1 and z_2 the number of years $t_2 - t_1$ will be given by the area under the $1/\lambda$ curve as shown in **Figure 1.4**.

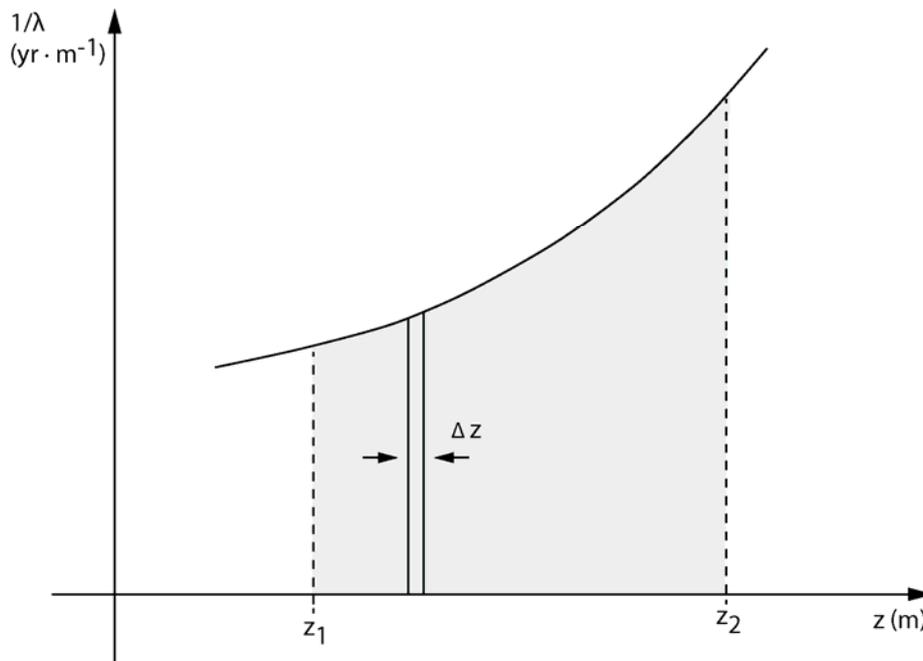


Figure 1.4

Question 1.3.2

Use **Table 1** to calculate the quantity $1/\lambda$ at the depths of 0, 500, 1200, 1400 and 1500 m. Plot the quantity $1/\lambda$ as a function of z . Connect the points with straight lines in order to ease later calculations. Label the graph “Graph 1.3.2” and attach it to the answer sheet.

➤ *Attach “Graph 1.3.2” to the answer sheet.*

Question 1.3.3

The graph you created in 1.3.2 can help you calculate the age of the ice at the given depths. Calculate the age of the ice for $z = 0, 500, 1200, 1400$ and 1500 m (by the term age we mean the total number of years from the surface and until the depth of interest). Create a new graph (“Graph 1.3.3”), where you present the age t as a function of depth z .

➤ *Attach “Graph 1.3.3” to the answer sheet.*

Water isotopic ratios in polar ice

Chemical elements with the same number of protons but a different number of neutrons are called isotopes. The water molecule H_2O can be found in nature in different variants containing different isotopes. The most common water molecule contains ^1H and ^{16}O (where superscripts 1 and 16 denote the number of nucleons), while the two second most common variants are $^1\text{H}^{16}\text{O}^2\text{H}$ and $^1\text{H}_2^{18}\text{O}$.

These molecular variants are called isotopologues. Isotopologues behave identically with respect to their chemical properties, but due to their mass difference, they differ with respect to physical properties, like evaporation and diffusion. It has been shown that the small variations in the isotopic composition of the water molecules in the snow that falls in Greenland are related to the temperature changes over the ice cap. In other words, if we are able to measure the water isotopic composition of the ice core from top to bottom we will be able to get an idea about the temperature history of Greenland from present times to tens of thousands of years in the past.

In isotope geochemistry, the isotopic composition of water is expressed with respect to an international standard water that is called Vienna Mean Ocean Water (VSMOW) and the symbol that is internationally used is the symbol δ ($\delta^{18}\text{O}$ reflects differences in the number of oxygen atoms and $\delta^2\text{H}$ reflects differences in the number of hydrogen atoms). As a rule, the more negative the value of $\delta^{18}\text{O}$ is in the Greenland ice, the colder was the climate at the time when this piece of ice was deposited as snow on top of the Greenland ice sheet. Due to the fact that the quantity δ is a relative quantity, δ is unit-less and typically expressed in

per mille.

You will be given two plastic bottles containing ice core sample in liquid form. The samples are taken from two different depths that are written on each bottle. Together with the bottles, you will be given a diagram presenting the water isotopic profile from the NEEM ice core (**Figure 1.5**) as well as the depth-age relationship for the top 1700 m of the NEEM ice core (**Figure 1.6**).

Question 1.3.4

Estimate the age of both samples and mark them clearly as vertical lines on **Figure 1.5** and **Figure 1.6**. Then, using the isotopic composition plot you were given, estimate approximately the isotopic content $\delta^{18}\text{O}$ you expect for each of the samples.

Which of the two samples do you think originates from a time when climate was significantly colder than now?

➤ Give your answer in the answer sheet, box 1.3.4.

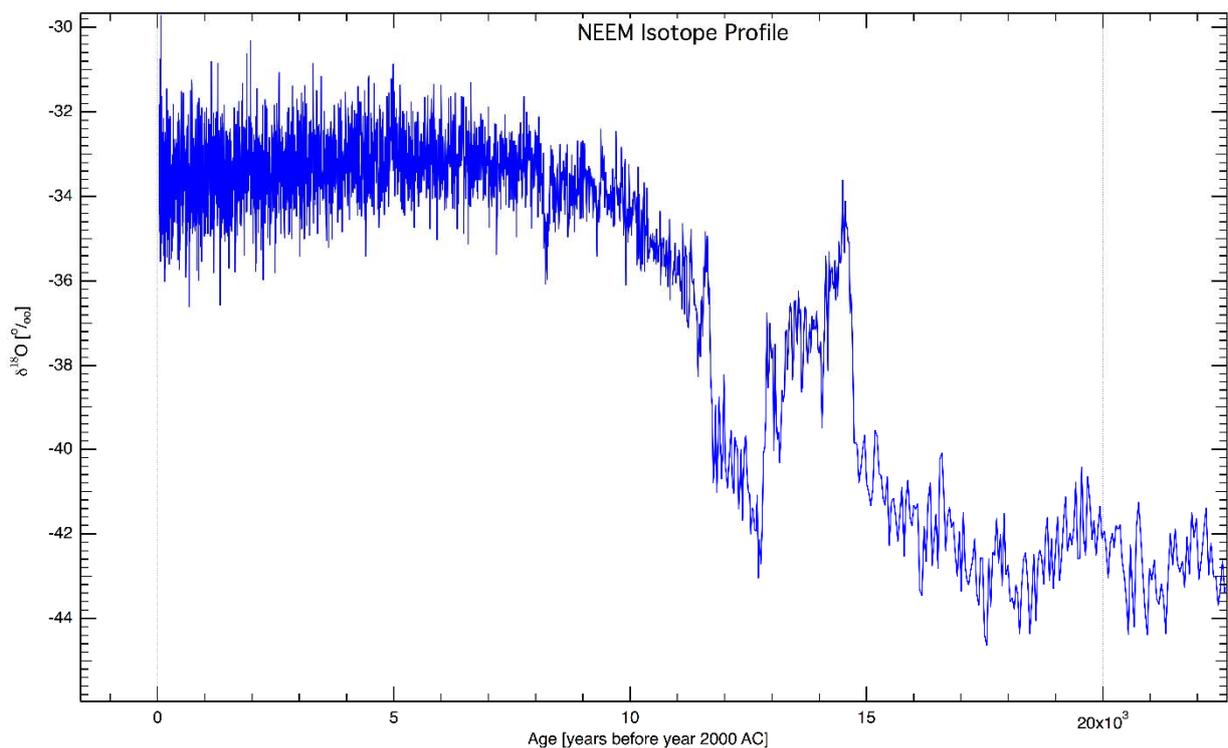


Figure 1.5. $\delta^{18}\text{O}$ as a function of age (in years before year 2000) for the NEEM drilling site.

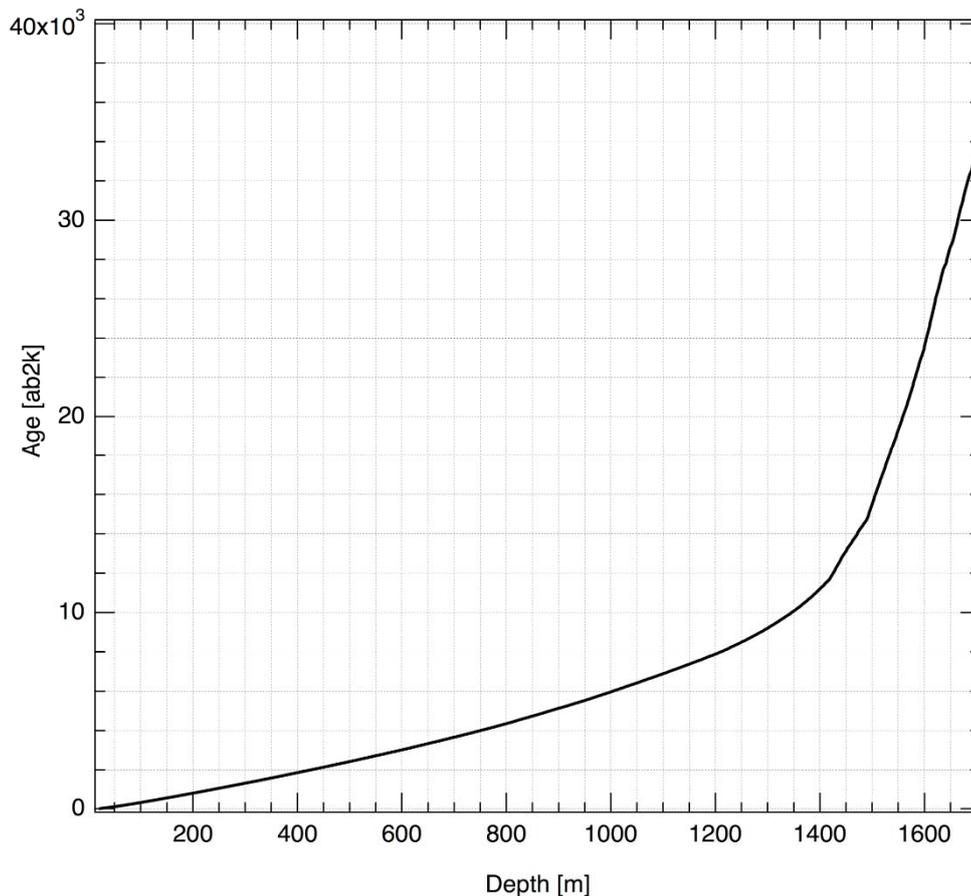


Figure 1.6. The depth-age relationship for top 1700 m of the NEEM ice core.

Question 1.3.5

Two different versions of a temperature – $\delta^{18}\text{O}$ relationship are given by the equations below (T in $^{\circ}\text{C}$ and $\delta^{18}\text{O}$ in per mille). The first one is linear and the second quadratic. Use both these equations to calculate the temperature above the ice cap at the time of deposition for the two samples you were given. What is the difference in temperature between the two samples?

$$T = 1.5 \cdot \delta^{18}\text{O} + 20.45$$

$$T = -0.1 \cdot (\delta^{18}\text{O})^2 - 4.46 \cdot \delta^{18}\text{O} - 72.43$$

➤ Give your answer in the answer sheet, box 1.3.5.

Sample preparation

You will now proceed to the transfer of ice core sample to glass measurement vials. For this, you are given two pipettes, which you can use for this task. You need approximately 1.5 mL of water to be transferred from the sample bottle to the sample vial. Sample preparation is an important step in the process of measuring isotopic ratios and if not done properly it can lead to measurement errors.

Keep in mind the following points when working:

- Evaporation of the sample can alter its isotopic composition.
- Mixing of different waters with the sample will cause isotopic contamination.

Question 1.3.6

Proceed with the sample transfer. Label the sample vials carefully. Your samples will be analysed with a Cavity Ring Down Laser Spectrometer overnight in order to assess the quality of your sample preparation work.

➤ Call a laboratory assistant to fetch and verify your sample.

Question 1.3.7

Answer with True/False the four statements given below.

Statement	True	False
The time that a sample is exposed to lab air can affect the quality of the measurement.		
Only one pipette should be used for the transfer of both samples		
Keeping the samples as cold as possible helps minimizing isotopic fractionation.		
The exact quantity of water transferred to the sample vials is critical for the quality of the isotopic analysis.		

➤ Give your answers in the answer sheet, box 1.3.7.

Experiment 2 32 marks

Copper and zinc content in an ice core

The volcano Laki in Iceland erupted on June 8, 1783 and the eruption lasted until February 1784. The amount of gases and ash emitted into the atmosphere affected the climate in Europe. Chemical analyses of ice cores, obtained from drillings in Greenland give valuable information of such incidents. The ice has been formed from pressurized snow of annual snowfalls. Each annual layer can give information about the temperature and composition of the atmosphere, including a possible content of volcanic ash in the year of the snowfall. Investigations have shown that volcanic ash contains metals.

In this experiment, an annual layer from an ice core is analysed for its content of the metals copper and zinc. By comparison with the results from other drillings, it can then be shown if the content of copper and zinc can be related to the eruption of Laki.

Materials and equipment

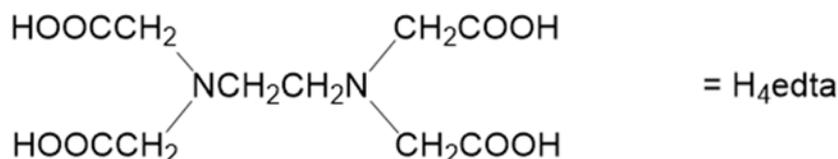
- Funnel
- Burette, 25 mL
- Erlenmeyer flask, 100 mL
- 3× Beaker, 250 mL
- 3× Graduated cylinder, 10 mL
- Pipettes, 5, 10 (3×), 15, 20 (2×), 25 (2×) mL
- Peleus ball
- 6× Volumetric flask, 100 mL
- Volumetric flask, 50 mL
- 10× Plastic cuvette, 10 mm
- Test tube, 20 mL
- 10× Plastic pipettes, 1 mL
- 50 mL 0.20 M CH₃COOH/1.7 M NaCH₃CO₂ buffer solution
- 10 mL 0.50 % Xylenol Orange indicator solution
- 50 mL 0.20 M Na₂S₂O₃ solution
- 250 mL 1.3 M NH₄Cl/7.0 M NH₃, pH = 10 buffer solution
- 100 mL 0.0360 M Cu(ClO₄)₂ solution
- 250 mL 0.0170 M EDTA solution
- 150 mL (Cu/Zn) analysis solution
- Vernier SpectroVis Plus Spectrophotometer
- Computer
- Plastic wash bottle
- Waste container labelled "X"
- Millimetre graph paper

For sample preparation, 180.6 g of ice core was melted and the liquid was quantitatively transferred to a 2000 mL volumetric flask. The flask was then filled up to the mark with water. The solution provided, labelled (Cu/Zn), is 10⁹ times more concentrated than the initial one.

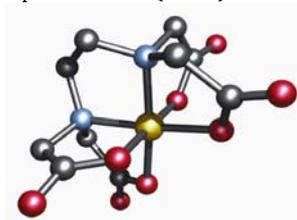
In this experiment, the amount concentration of Cu²⁺ and Zn²⁺ in the analysis solution (Cu/Zn) has to be determined. The concentration of Zn²⁺ is determined by an EDTA-titration and the concentration of Cu²⁺ by spectrophotometry.

The content of Cu^{2+} and Zn^{2+} in the ice core can then finally be calculated.

A. EDTA (EthylenediamineTetraAcetate) titration



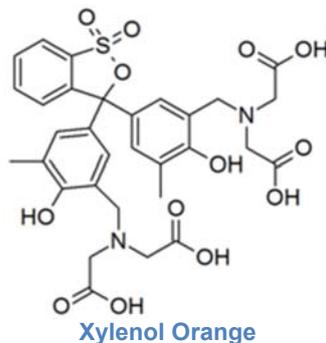
H_4edta , commonly abbreviated EDTA, forms very stable complexes with metal ions M^{q+} through the release of H^+ ions:



$\text{M}(\text{edta})^{(4-q)-}$

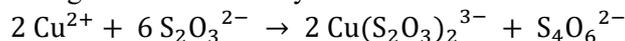
This is why many metal ions in a not too acidic solution can be titrated with EDTA in a so-called complexometric titration.

The equivalence point of the titration is detected by using a metal ion indicator, in this case Xylenol Orange, which is bound to the metal ion with one colour (red) before the equivalence point and after the equivalence point exists as the free indicator with another colour (yellow) at the given pH. As titrator, a solution of $\text{Na}_2\text{H}_2\text{edta} \cdot 2\text{H}_2\text{O}$ (hereafter referred to as EDTA-solution) is used.

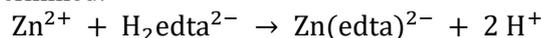


Xylenol Orange

Cu^{2+} is “masked” in the following EDTA-titration by reaction with excess of thiosulphate $\text{S}_2\text{O}_3^{2-}$:



The resulting complex $\text{Cu}(\text{S}_2\text{O}_3)_2^{3-}$ does not react with $\text{H}_2\text{edta}^{2-}$. However, Zn^{2+} does not form a complex with thiosulphate, and as a result, it *does* react with $\text{H}_2\text{edta}^{2-}$. In the following EDTA-titration, only the content of Zn^{2+} is determined:



The burette provided has been filled with deionized water. The burette is first emptied, then rinsed and filled up with the 0.0170 M EDTA-solution. With a pipette, 10.00 mL of the analysis solution (**Cu/Zn**) is transferred to the 100 mL Erlenmeyer flask. Then, 5 mL of ethanoic acid/ethanoate buffer solution (0.20 M CH_3COOH / 1.7 M NaCH_3CO_2) and 5 mL of 0.20 M $\text{Na}_2\text{S}_2\text{O}_3$ solution are added. To the colourless solution, 6 drops of 0.5 % Xylenol Orange solution are added and the solution is then titrated with the EDTA solution until the colour of the solution changes from red to lemon yellow.

Question 2.1

- The volume of titrant V_1 is written on the answer sheet, box 2.1.

The titrated mixture is poured into the waste container labelled X. The titration is repeated twice more and the values of V_1 are written on the answer sheet, box 2.1.

- Calculate the average value $V_{1,av}$ and write the result on the answer sheet, box 2.1.

Question 2.2

In the analysis solution (Cu/Zn), calculate $[Zn^{2+}]$ from the value of $V_{1,av}$.

- Show your calculation and write the result on the answer sheet, box 2.2.

Question 2.3

Why does $Cu(S_2O_3)_2^{3-}$ not react with H_2edta^{2-} ?

- Mark the correct answers on the answer sheet, box 2.3.

Question 2.4

Ethanoic acid, CH_3COOH , is a weak acid with the acid dissociation constant $K_a = 1.78 \cdot 10^{-5} M$, where K_a can be expressed as:

$$K_a = \frac{[H_3O^+] \cdot [CH_3CO_2^-]}{[CH_3COOH]}$$

- Isolate $[H_3O^+]$ in the equation and write the expression on the answer sheet, box 2.4.

Calculate $[H_3O^+]$ in the ethanoic acid/ethanoate buffer solution.

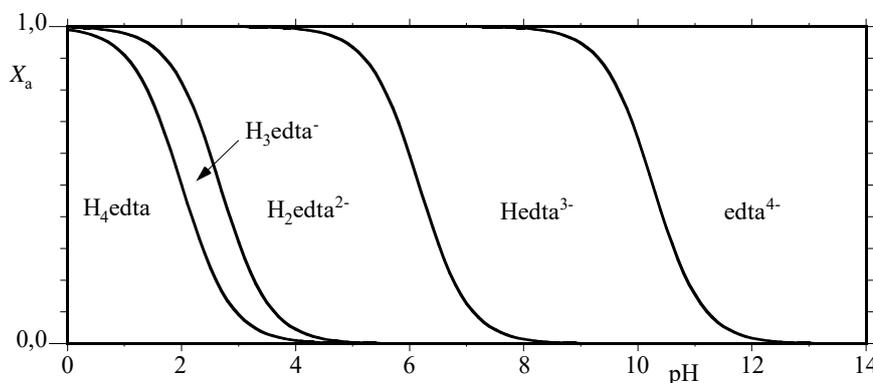
- Show the result on the answer sheet, box 2.4.

Calculate pH in the buffer solution.

- Write the expression and the result on the answer sheet, box 2.4.

For a solution containing a corresponding acid-base pair the mole fraction of acid, X_a , gives the fraction of the corresponding acid-base pair present in the acidic form. The mole fraction of acid, X_a , depends on pH in the solution. This correlation between pH and X_a for a corresponding acid-base pair can be expressed graphically in a so-called Bjerrum plot.

H_4edta is a tetravalent acid with the pK_a values $pK_{a1} = 1.99$, $pK_{a2} = 2.67$, $pK_{a3} = 6.16$, $pK_{a4} = 10.22$. From these values, the Bjerrum plot for the EDTA-system can be constructed:



Bjerrum plot for the EDTA-system.

Question 2.5

Using the Bjerrum plot, **estimate** whether an aqueous solution of $\text{Na}_2\text{H}_2\text{edta} \cdot 2\text{H}_2\text{O}$ is acidic, basic or neutral. Justify the answer by a marking on the pH axis in the Bjerrum plot.

➤ Write the answer on the answer sheet, box 2.5.

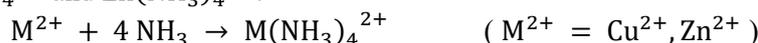
B. Spectrophotometry

Coloured compounds absorb light in the visible range ($\lambda = 400 - 700 \text{ nm}$). In a cuvette containing a solution of a compound S, the absorbance (A) depends on the path length (l), the concentration of S ($[S]$), and the wavelength dependent molar absorption coefficient (ε) of S in the following way:

$$A = \varepsilon \cdot [S] \cdot l$$

This equation is called the Beer-Lambert Law.

The metal ions Cu^{2+} and Zn^{2+} react quantitatively with ammonia in aqueous solution forming the compounds $\text{Cu}(\text{NH}_3)_4^{2+}$ and $\text{Zn}(\text{NH}_3)_4^{2+}$:



At a given wavelength, the absorbance of such a solution is equal to the sum of contributions from all compounds in the solution:

$$A = A(\text{Cu}(\text{NH}_3)_4^{2+}) + A(\text{Zn}(\text{NH}_3)_4^{2+})$$

This can be rewritten as:

$$A = \varepsilon(\text{Cu}(\text{NH}_3)_4^{2+}) \cdot [\text{Cu}(\text{NH}_3)_4^{2+}] \cdot l + \varepsilon(\text{Zn}(\text{NH}_3)_4^{2+}) \cdot [\text{Zn}(\text{NH}_3)_4^{2+}] \cdot l$$

In the entire visible range $\varepsilon(\text{Zn}(\text{NH}_3)_4^{2+}) = 0 \text{ M}^{-1}\text{cm}^{-1}$ and the absorbance of an aqueous solution containing Cu^{2+} , Zn^{2+} and excess of NH_3 is therefore:

$$A = \varepsilon(\text{Cu}(\text{NH}_3)_4^{2+}) \cdot [\text{Cu}(\text{NH}_3)_4^{2+}] \cdot l$$

The absorbance is determined using a Vernier SpectroVis Plus Spectrophotometer connected to a computer and cuvettes with a path length of $l = 1.00 \text{ cm}$ are used. In the following an ammonium/ammonia-buffer solution (1.3 M NH_4Cl / 7 M NH_3 , pH = 10) is used as a source of ammonia and a 0.0360 M solution of copper(II) perchlorate, $\text{Cu}(\text{ClO}_4)_2$, as a source of Cu^{2+} .

Six solutions (1-6) with known concentrations of $\text{Cu}(\text{NH}_3)_4^{2+}$ are prepared by transferring aliquots of 0.0360 M $\text{Cu}(\text{ClO}_4)_2$ to six separate 100 mL volumetric flasks using pipette volumes 0, 5.00, 10.00, 15.00, 20.00, and 25.00 mL, respectively. To each flask is then added 20.00 mL ammonium/ammonia buffer solution (1.3 M NH_4Cl / 7 M NH_3 , pH = 10). The flasks are filled to the mark with water and shaken thoroughly.

Question 2.6

For each of the solutions 2-6 calculate the concentration of $\text{Cu}(\text{NH}_3)_4^{2+}$.

➤ Write the answers on the answer sheet, box 2.6.

Using solution 1 as a blank, measure the absorbance at $\lambda = 618 \text{ nm}$ (A_{618}) of solutions 2-6. (Appendix C gives instructions for use of the spectrophotometer.)

➤ Write the results on the answer sheet, box 2.6.

Using a pipette, 25.00 mL of the analysis solution (**Cu/Zn**) is transferred to a 50 mL volumetric flask. Then 10.00 mL ammonium/ammonia-buffer solution (1.3 M NH_4Cl / 7 M NH_3 , pH = 10) is added using a pipette. The flask is filled to the mark with water and shaken thoroughly. This solution is labelled 7.

Question 2.7

Measure the absorbance at $\lambda = 618 \text{ nm}$ (A_{618}) of solution 7.

➤ Write the result on the answer sheet, box 2.7.

Plot the values of A_{618} vs. $[\text{Cu}(\text{NH}_3)_4^{2+}]$ for solutions 2-6 on the supplied graph paper (label the graph "Graph 2.7"). Draw a line that best fits the points and calculate the slope and y-intercept.

➤ Add Graph 2.7 to the answer sheet.

Question 2.8

Find the slope and y-intercept.

- Show your readings on the graph paper,
and show your calculations and results on the answer sheet, box 2.8.

Question 2.9

Calculate the molar absorption coefficient, ϵ , for $\text{Cu}(\text{NH}_3)_4^{2+}$ at $\lambda = 618 \text{ nm}$.

- Write the calculation and result on the answer sheet, box 2.9.

Question 2.10

Using the A_{618} -value of solution 7 calculate $[\text{Cu}^{2+}]$ in the analysis solution (**Cu/Zn**).

- Write the calculation and result on the answer sheet, box 2.10.

Using a graduated cylinder, transfer 10 mL of the analysis solution (**Cu/Zn**) to a test tube. Add the ammonium/ammonia buffer solution (1.3 M NH_4Cl / 7 M NH_3 , pH = 10) dropwise. After the addition of a few drops of the buffer solution a precipitate is formed. Add more buffer solution dropwise with stirring until the precipitate is completely redissolved.

Question 2.11

Suggest, with a chemical formula, what the identity of the precipitate could be.

- Write the answer on the answer sheet, box 2.11.

- Write a balanced reaction scheme for the formation of the precipitate on the answer sheet, box 2.11.

C. The ice core

Question 2.12

Calculate the content of Cu^{2+} and Zn^{2+} in $\mu\text{g/g}$ in the ice core (1 $\mu\text{g} = 10^{-12} \text{ g}$). Molar masses: Cu: 63.54 g/mol; Zn: 65.38 g/mol.

- Write the calculations and results on the answer sheet, box 2.12.

In another drilling from Greenland, eight ice cores from depths of 67.155 – 67.785 m containing annual layers from the time period 1782–1785 have been analysed for the content of copper and zinc with the following results:

Depth (m)	Sample number	Cu ($\mu\text{g/g}$)	Zn ($\mu\text{g/g}$)
67.155–67.23	1	0.46	35
67.23 –67.305	2	1.6	26
67.315–67.40	3	2.9	42
67.74 –67.485	4	6.4	35
67.485–67.555	5	2.0	11
67.555–67.625	6	20.5	490
67.645–67.745	7	3.2	21
67.745–67.785	8	1.2	24

It was concluded that the relatively high content of copper and zinc in sample number 6 could be related to the eruption of Laki. By comparison with your results, estimate if the content of copper and zinc in your ice core can be related to the eruption of Laki.

- Mark the correct answer on the answer sheet, box 2.13.

Experiment 3 20 marks

Metazoan life in extreme environments

Many different types of extreme and harsh habitats exist around the globe. In such habitats, living organisms are challenged by extremes in physicochemical factors, such as temperature, water availability, salinity, pH, and oxygen tension. In order to cope with these hostile environments, organisms require special adaptations and only the most resistant survive. Organisms that live in the Arctic are, obviously, adapted to very low temperatures. Cryoconite holes (created when dust melts through the snow or ice) offer a niche for selected algae, bacteria and metazoans. In this exercise, you are provided with a sample that mimics the material found in a cryoconite hole.



Figure 3.1. Cryoconite hole.

Materials

- Petri dish with sediment sample
- Stereo microscope
- Dissecting needle
- Identification Key ([Appendix A1](#))
- Image Key ([Appendix A2](#))

Find and identify metazoan lifeforms

Question 3.1

Find and identify active and moving microscopic metazoan lifeforms (up to 1 mm long) in the sediment sample using the stereo microscope and the “Identification Key” ([Appendix A1](#)).

➤ *Fill in the answers in the answer sheet.*

Note that:

1. Images ([Appendix A2](#)) are provided of animals that are present in the sample *AND* of animals that are *NOT* microscopic and/or *NOT* present in the sediment sample. Numbers marked with an asterisk (*) in the "Identification Key" ([Appendix A1](#)) refer to a specific image in [Appendix A2](#).
2. You should consult the "Terminology" given below before filling in the answers.
3. Filling in the answer sheet correctly - i.e. with *exactly* the microscopic metazoans that are actually present in the sample will result in full points.

Terminology

Metazoan	A multicellular animal with cells differentiated into tissues.
Radial symmetry	The animal has a central axis along which it can be divided into a number of mirror images. The animal has no left and right side.
Appendages	External protuberances from the animal's body, e.g. legs or antennae.
Cilium (plural cilia)	Slender, hair like process extending from the surface of a cell. Cilia may be motile or non-motile.
Scalids	Spinose appendages that function in locomotion, chemo- and mechano- reception.
Articulated	Consisting of sections united by joints.

Experiment 4 26 Marks

Ancient Greenlandic habitats

Deep below the ice cap all the way down at bedrock, remnants of life that existed before the ice covered the landscape can be found. Samples have been taken 2-3000 meter down in the ice (See **Figure 4.1**) from the ice core drilling site Dye-3. By looking at the basal ice, which contains soil particles, it is possible to find soil material that has been scraped up by the movement of the ice. Hence, the basal ice may contain a lot of ancient genetic material, which can give us an indication of the past climate and plant diversity at the time before ice covered Greenland. Ancient soil DNA has been extracted from this material.

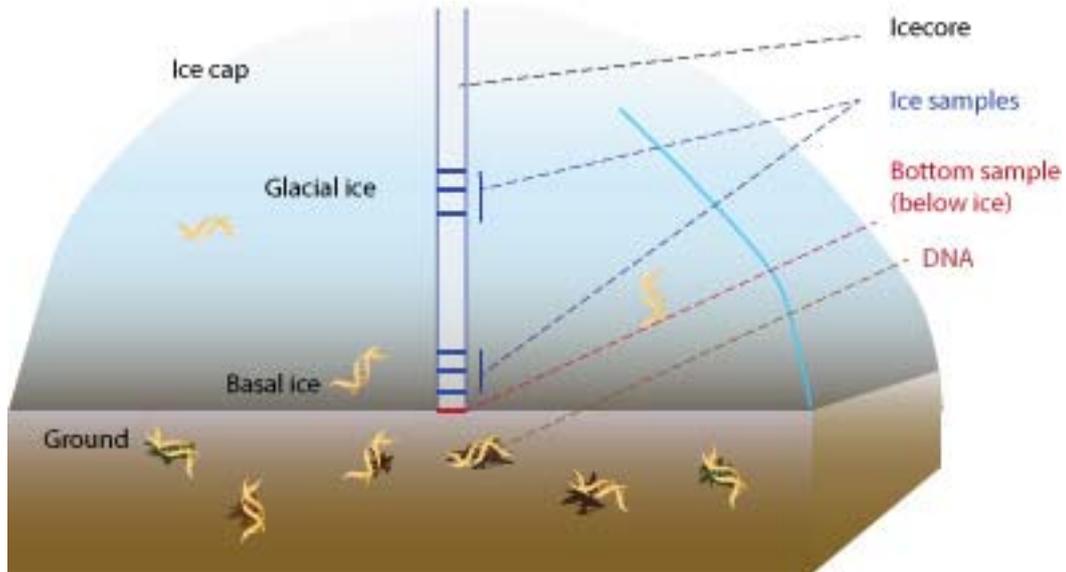


Figure 4.1. Illustrating the ice cap.

In this experiment, you will analyse DNA from such samples and determine what kind of habitat existed in Greenland before the ice was formed. You will look for the presence of indicator plants, which thrive in certain climate niches and have specific temperature requirements for survival and growth. The different growth conditions required for the specific plant families can tell us something about the temperature ranges of their habitat, length of summer and winter period and if for example forest existed. From this kind of information, you will be able to explain what kind of habitat you would expect to see in Greenland before the great ice cap was formed.

A PCR (Polymerase Chain Reaction) has been performed to amplify the DNA we would like to analyse. Your job will be to analyse the ancient environmental DNA by gel-electrophoresis and you will compare these results to DNA libraries of modern day plants.

To see the presence of a specific plant family, primers¹ have been designed to target a specific region in the genome for each indicator family of interest. If an indicator family is present in your sample, you will see a band on your gel.

Materials

- 1 gel cassette
- 1 FlashGel® Dock with cables and power supply
- 1 Power source
- 1 tube of buffer, labelled "Buffer 4"
- 1 micropipette 2-20 μL
- Micropipette tips

¹ Primers are short single stranded DNA fragments (20-30 nucleotides long) that are designed to bind to a complementary DNA strand. During a PCR, the DNA polymerase will start copying the complementary DNA strand from the positions of the primer. Hence, the complementary DNA strand will be amplified.

- PCR-tubes containing DNA samples with primers for indicator **families** (for Experiment 4A)
- PCR-tubes containing DNA samples with primers for indicator **genera** (for Experiment 4B)
- Lint free wipes
- Disposal bag for waste
- Appendix B

Taxonomic tree

You will be looking for specific plant families and genera in your samples. These families and genera are used as climate indicators, since these can only grow if the climate fits their needs. When analysing your results, you will need [Appendix B](#), [Figure 4.2](#) and [Figure 4.3](#).

Kingdom

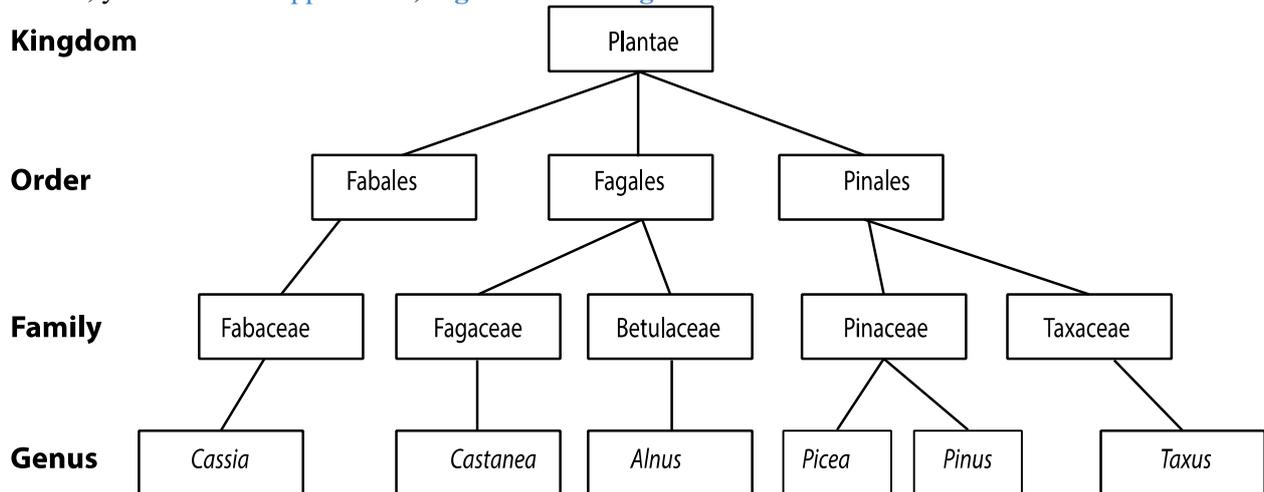


Figure 4.2. Taxonomic tree with indicator families and indicator genera.
Note that this taxonomic tree is not complete.

Electrophoresis

You will be using the electrophoresis technique as part of your investigation on the ancient DNA fragments in the ice.

Question 4.1

➤ Write the letters for the correct words in the answer sheet, box 4.1.

Words

A Amino acid	G Base	M Wells	S Deoxyribose-molecules
B DNA-ladder	H DNA-fragments	N Fatty acids	T Phosphate-groups
C Glucose molecules	I Ditches	O Shortest	U Carbohydrates
D Charge	J Length	P Longest	V Loading dye
E Molecules	K Negative pole	Q Net negative charge	W Positive pole
F Net positive charge	L Proteins	R Size	X Macromolecules

Text

An electrophoresis is conducted in an electrophoresis cassette. Samples are loaded into the { Word 1 } of a gel, which is covered with buffer.

When placed in an electric field, macromolecules such as { Word 2 } and { Word 3 } can be

separated. The separation depends on the different { Word 4 } and { Word 5 } of the molecules.

Nucleic acids migrate towards the { Word 6 } as they have a { Word 7 } due to the { Word 8 }. Proteins, however, could migrate towards either the positive or the negative pole, since their overall charge depends on their { Word 9 } composition.

Experiment 4A. Analyse the DNA samples using gel-electrophoresis

Ancient DNA bound to soil particles preserved in the basal ice under the ice cap is a glimpse into ancient natural history. Since the material has been frozen and kept oxygen free, nucleotide sequences have been preserved, however, in a highly degraded state. We can use the remaining short degraded pieces of DNA code as a taxonomic library of the previous genera from this geographical area of Greenland. See the list below of the different families we are looking for, in the samples obtained by PCR-amplification using family-specific primers:

- A1. Taxaceae
- A2. Pinaceae
- A3. Betulaceae
- A4. Fabaceae
- A5. Fagaceae

The different DNA samples indicate different indicator **families** that we are looking for.

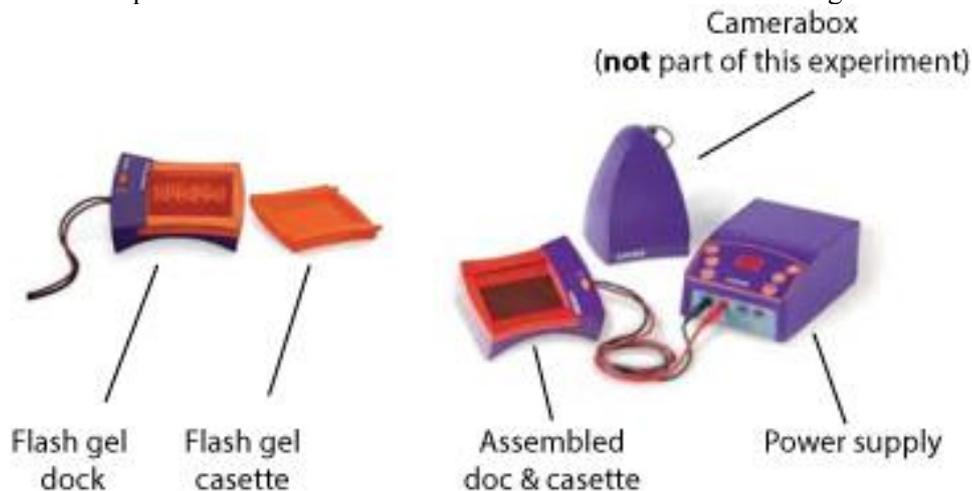


Figure 4.3 The Lonza Flash gel system

Instructions

1. Tear open pouch and remove cassette.
2. Remove seals from cassette.
3. Make sure sample wells become flooded with "Buffer 4" and remove buffer from cassette, but do not remove buffer from the wells. If in doubt, please ask the instructor – there will be no penalty for asking!
4. Insert cassette into dock.
5. Load samples from PCR tubes (5 μ L pr. sample) as shown in the [Figure 4.4](#) below. Remember to write down which samples are loaded where.

NB: Use only 5 wells, as another 6 wells will be needed later on.

6. Plug in cables as indicated by colour code to the power source.
7. Set power to: Voltage 195 V DC or as posted at lab table, Power 15 W or as posted, Current: 25 mA, and time to 10 min.
8. Start the run on the power source.

9. Observe the experiment every minute by turning on the light on the light switch. Let the experiment run for maximum 9 minutes.

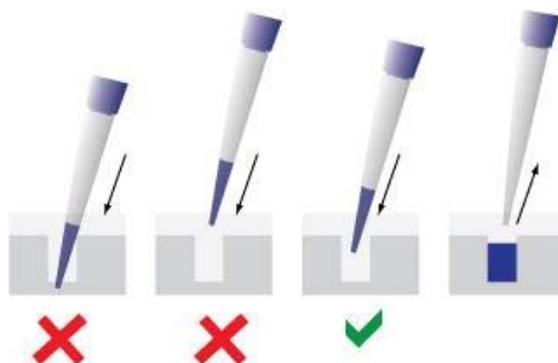


Figure 4.4. Application of a sample on to the gel.

Analysing the results of experiment 4A

Question 4.2

If you obtain a signal on the gel, the family was present. Write the names of the families you have analysed in the gel boxes in the answer sheet and indicate if the family was present or not present in your sample. This is done by writing + (present) or – (not present) on the gel template in the answer sheet under the name of the family.

- Write the names of the families you have analysed on the answer sheet, box 4.2.

Question 4.3

Based on the data from experiment A and the information in [Appendix B](#), decide whether the following statements about Greenlandic flora are true or false.

Statements	True	False
No plants were present at the time		
Only four families existed at the time		
The winters were below $-2\text{ }^{\circ}\text{C}$ and the summers were above $10\text{ }^{\circ}\text{C}$		
Only three families existed at that time		
Nothing can be concluded about the temperature by information based on only the families		
Greenland had a forest at the time		

- Tick the answers in box 4.3 on the answer sheet.

Question 4.4

Which families would be relevant to investigate for further analysis? Taxaceae, Fagaceae, Pinaceae, Fabaceae and/or Betulaceae?

- Highlight your answer by drawing a ring around the names on the answer sheet, box 4.4.

Experiment 4B. Analyse DNA samples using gel-electrophoresis

The DNA samples indicated which indicator **genera** we should be looking for.

See the list below of the different genera we are looking for, in the DNA-samples obtained by PCR-amplification using genera-specific primers:

- B1. Alnus
- B2. Picea

- B3. Pinus
- B4. Taxus
- B5. Cassia
- B6. Castanea

Instructions

1. Make sure sample wells become flooded with buffer and remove buffer 4 from cassette, but do not remove buffer in the wells.
2. Insert cassette into dock.

NB: Be aware to use the wells NOT used in the previous experiment!

3. Load samples from PCR tubes (5 µL pr. sample). Remember to write down which samples are loaded where.
4. Plug in cables as indicated by colour code to the power source.
5. Set power to: Voltage 195 V DC or as posted at lab table, Power 15 W or as posted, Current: 25 mA, and time to 10 min.
6. Start the run on the power source.
7. Observe the experiment every minute by turning on the light on the light switch. Let the experiment run for maximum 9 minutes.

Analysing the results from experiment 4B

As in experiment 4A use the attached taxonomic tree and the table showing the indicator genera.

Question 4.5

Write the names of the genera you have analysed in the boxes and indicate if the **genus** was present or not present in your sample.

➤ *This is done by marking either + (present) or – (not present), on the answer sheet, box 4.5.*

Question 4.6

Based on experiment 4B, Appendix B, and the above answers, what kind of ecosystem was dominant at the location of Dye-3?

Statement	
Rainforest.	
Deciduous temperate forest.	
Mire (a wetland terrain without forest cover, dominated by living, peat-forming plants).	
Meadow (an open area with grassland).	
Boreal forest with a mix of conifers and deciduous trees.	

➤ *Tick the correct answer on the answer sheet, box 4.6.*

Question 4.7

We want to be sure that the DNA from the basal ice samples are really representing the ancient ecosystems and not just contaminations from the air that was transported to Greenland from other areas through time.

Where would you take control samples in the ice core (see [Figure 4.1](#)) to check for airborne exotic DNA?

Statement	True	False
In the centre of the glacial ice core and close to the basal ice where exotic plant DNA might have been incorporated together with air, airborne contaminants and snow.		
In the clean glacial ice much closer to the surface than to the basal ice.		
Only on top of the ice cap since this place is most likely to be contaminated.		
Atmospheric air samples since this is where the contaminants are.		
Atmospheric air samples and top of the ice cap since both contain the contaminants.		

➤ Tick your answers on the answer sheet, box 4.7.

Question 4.8

From the indicator genera, make an analysis on what the climate most likely looked like at the time these organisms were living in Greenland – what are the upper and lower temperature boundaries? Use [Appendix B](#).

Statement	True	False
Summers are more than 10 °C warm.		
Winters are down to -40 °C.		
Winters are not colder than -17 °C.		
Winters does not go below -1 °C.		

➤ Tick the answers on the answer sheet, box 4.8.

Backtracking the protein sequence

The researchers at Dye-3 found a fraction of a protein when studying their findings. Your job is to backtrack it to mRNA and choose a possible specific primer for testing it using PCR (polymerase chain reaction).

The protein sequence is:

Met-Phe-Asp-Gln-Asp-Tyr-Trp

Question 4.9

Using the genetic code ([Figure 4.5](#)), calculate the possible number of mRNA combinations of the protein sequence.

		Second Letter					
		U	C	A	G		
1st letter	U	UUU Phe UUC UUA Leu UUG	UCU Ser UCC UCA UCG	UAU Tyr UAC UAA Stop UAG Stop	UGU Cys UGC UGA Stop UGG Trp	U C A G	
	C	CUU Leu CUC CUA CUG	CCU Pro CCC CCA CCG	CAU His CAC CAA Gln CAG	CGU Arg CGC CGA CGG	U C A G	
	A	AUU Ile AUC AUA AUG Met	ACU Thr ACC ACA ACG	AAU Asn AAC AAA Lys AAG	AGU Ser AGC AGA AGG Arg	U C A G	
	G	GUU Val GUC GUA GUG	GCU Ala GCC GCA GCG	GAU Asp GAC GAA Glu GAG	GGU Gly GGC GGA GGG	U C A G	

Figure 4.5.

➤ Write your answer on the answer sheet, box 4.9.

Question 4.10

Which of these mRNA sequences is one of the possible combinations of the protein sequence?

- 5'-AUG UUU GAU GAG GAC UAU UGG-3'
- 5'-AUG UUC CCA CAG GAC UAC UGG-3'
- 5'-AUG UUC GAU CAG GAC UAC UGG-3'
- 5'-AUG UUU GAU GGA GAU UAU UGG-3'
- 5'-AUG GGA GAU CAG GAU UAU UGG-3'

➤ Write your answer on the answer sheet, box 4.10.

Question 4.11

You are only asked to choose one primer 12 bp long, although this is far too short to be specific, when it comes to real DNA-analysing. Normally you will use both a forward and a reverse primer. Which of these would you use as a specific primer for further analysis?

3'-CTC CTG ATA ACC-5'
3'-GTT CTG ATG ACC-5'
3'-GTC CTG ATG ACC-5'
3'-CTT CTA ATA ACC-5'
3'-GTC CTA TTA ACC-5'

➤ Write your answer on the answer sheet, box 4.11.

Now when we know some of the plants and temperature conditions of Greenland before the great ice cap formed and covered most of the land area, we want to know when this ice build-up began in the area of the Dye-3 core.

The basal ice has been dated by a combination of four dating techniques; see below. Two of the methods are based on physics and two are based on biology, and the basic theory behind them will be described below.

1. The first physics method to date the ice is based on isotope decay of radioactive isotopes in the ice, like ^{10}Be and ^{36}Cl . These two isotopes are present in the atmosphere and get incorporated into the ice cap together with the snow. This method estimates the ratio of decay of the $^{10}\text{Be} / ^{36}\text{Cl}$ isotopes, which is occurring exponentially with time. Therefore, this method will provide an age estimate based on how long the isotopes and the air have been incorporated in the ice. This is theoretically equal to the age of the ice.
2. Another physics method is optical stimulated luminescence (OSL) dating. This method estimates the time since the soil particles last received any daylight, and hence the time since they got incorporated into the ice. By applying a strong laser beam on the soil particles one can estimate the amount of light reflected back from particles of the soil minerals: feldspar or quartz. The amount of light reflected back is proportional to the last time the particles were exposed to light. This is also theoretically giving us an estimate of when the ice formed and thereby its age.
3. The first biological method applied is called amino acid racemization (AAR). This is measuring the decay of amino acids from biological tissue in organisms. The decay rate in certain amino acids is constant in cold temperatures. Therefore, we can theoretically estimate the age of the ice based on the level of decay of these amino acids within the basal ice samples.
4. The second biological method is based on DNA and the molecular clock theory. Since DNA molecules degrade with time and obtain certain erroneous mutations at a certain rate, we can compare the ancient DNA sequences with modern ones in specific gene regions. Thereby we can get an estimate of the age of the ancient DNA sequences. This will indicate when the organism lived and hence when the ice started to build up and incorporated the DNA sequences.

All four dating techniques have been developed fairly recently to be applied on samples of basal ice, since no other well-tested methods have been available for this kind of material. Each of these four methods are therefore prone to provide some level of uncertainties. It was therefore decided to combine the four different methods to get a consensus age from all of them.

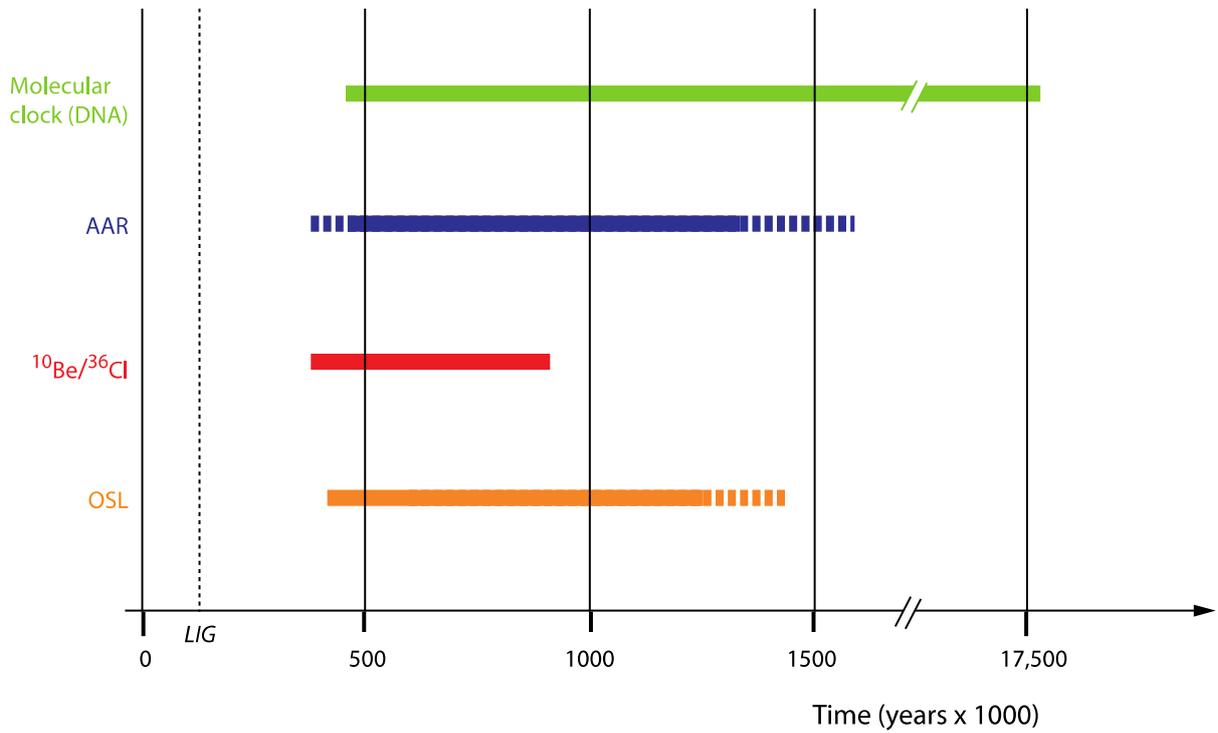


Figure 4.6. Four dating methods used to determine the age of the ancient DNA in the ice cap. Horizontal timeline: Years before present x 1000. *LIG*: *Last interglacial period*, approx. 120,000 ybp. Vertical axis: The four methods. Dashed lines indicate uncertainty of the maximum or minimum age.

Question 4.12

By using **Figure 4.6**, can you determine the last time that forest occurred in the Dye-3 location from a combination of the four methods?

➤ *Tick the answers on the answer sheet, box 4.12.*

END OF TASK 1: ICE

Task 2

OCEAN

11th of May 2017



Introduction

Njord is a fish farmer who grows fish in the Kattegat. He has busy days running a family business, so Njord gets a lot of help from his smart 16-year-old daughter Freja.

Recently Njord decided that instead of buying the small creatures his fish eat, he should produce it locally, starting with fresh microalgae. Photosynthetic algae need water, carbon dioxide, light and nutrients to grow. Nutrients can be costly. It takes a lot of energy to remove nitrogen from the atmosphere and phosphorous has to be mined and will run out someday. An alternative to using these resources is wastewater, which needs to have these things removed before it's discharged in the environment. Anaerobically treated wastewater will have high nitrogen (N) and phosphorus (P) content, but not much organic carbon left, and has been shown to be a good algae growth medium.

One day, Freja went to help her father and she saw he had bought a fancy set of experimental photo bioreactors, filled them with wastewater and a panel of LED's were shining on them.

NOTICE: Note that part B has some time-consuming steps and you may want to read ahead and have one team member start them first.



Kattegat, the sea between Denmark and Sweden.

1. Algae production and wastewater treatment measurements using spectrophotometry.

Njord started pumping wastewater into his reactor at a steady rate to his algae photo bioreactors, left the LEDs on and forgot about them while he went out in the Kattegat to fix some fish cages. When he came back, he noticed that some algae had grown in the reactor and that they were waiting for him in the big tank he set up to collect the effluent. Not knowing how to analyse what had happened, he called Freja. Njord asked:

- A. How much algae am I producing?
- B. How much carotenoids are the algae producing?
- C. How much nutrients are they removing from the wastewater? Will we get credit for that?

Freja dutifully came by and observed the system. She also set up a large plastic container on a big scale and waited an hour to see that 21.5 L of algae suspension had come out of the reactor. (She assumed a density of 1 kg/L.) Then she collected a few samples to take to measure at her high school. She labelled them:

- Sample 1: "Influent" - the wastewater flowing into the photo bioreactor system.
- Sample 2: "Effluent" - the liquid with algae flowing out of the photo bioreactor system.

Now you have to help Freja figure out how to answer Njord's questions using the tools she had, especially spectrophotometry.

A spectrophotometer is a device where light of a chosen wavelength is detected after it passes through a cuvette containing a sample. The amount of light absorbed by the sample is called Absorbance (A).

$$A = -\log_{10} \frac{l}{l_0}$$

l_0 is the incident light intensity and l is the intensity of the transmitted light.

Absorbance is proportional to the concentration of the sample (c) and the distance the light travels (d) and a substance specific constant (ϵ). Most commonly in spectrophotometers the cuvette thickness (d) is 1 cm.

$$A = \epsilon \cdot c \cdot d$$

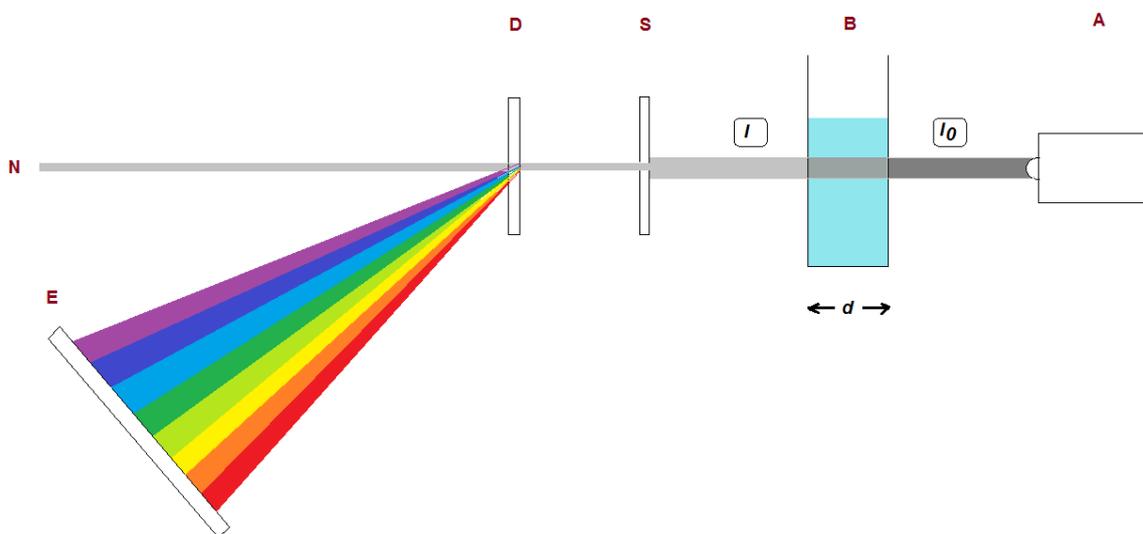


Figure 1.1. Spectrophotometer principle. From right: Lamp (A), Cuvette (B), Slit (S), Diffraction grating (D), Direct beam (N, not used), Detector array (E).

A photo of the actual inside is shown in Appendix B.

There, the diffraction grating is mounted askew to increase resolution and the slit is not visible.

Materials

Shared between teams

- Heat blocks set at 60 °C
- Ice container
- Freezer
- Shovel for ice

At each station

- Laptop with LoggerPro
- Calculator
- Spectrophotometer with SpectroVis optical fibre for light emission
- Black cloth for spectrophotometer
- Centrifuge for 2 mL tubes with instruction
- Vortex mixer
- White light source
- 4 Racks for 50/15/2 mL tubes
- Lens paper for cuvette cleaning
- 2 Timers
- Dispenser with deionized water
- Ice bowl
- Waste bin for solid waste
- Waste container for organic+inorganic waste
- Plastic beaker 250 mL
- Dispenser with paper towels
- Soap
- Safety glasses

For each team

- A box with:
 - Visocolor school kit for Ammonium and Phosphate determination
 - 3 glass vials with lid
 - Plastic spoon
- 50 mL influent wastewater in a glass bottle with a screw lid
- 50 mL effluent water with algae in a glass bottle with a screw lid
- Centrifuge tubes
 - 2 x 50 mL graded tubes (notice where the 50 mL mark is)
 - 6 x 15 mL graded tubes
 - 16 x 2 mL graded tubes
- Cuvettes of thickness of 1.00 cm
 - 16 x 4,5 mL
 - 4 x 1.5 mL
- 20 disposable pipettes of 1.0 mL with 0.25 mL graduation
- 25 mL of 96% ethanol in a pipette bottle
- LED box
- Plastic ruler 20 cm
- 3 Pencils
- List book
- Black waterproof marker pen
- Safety gloves

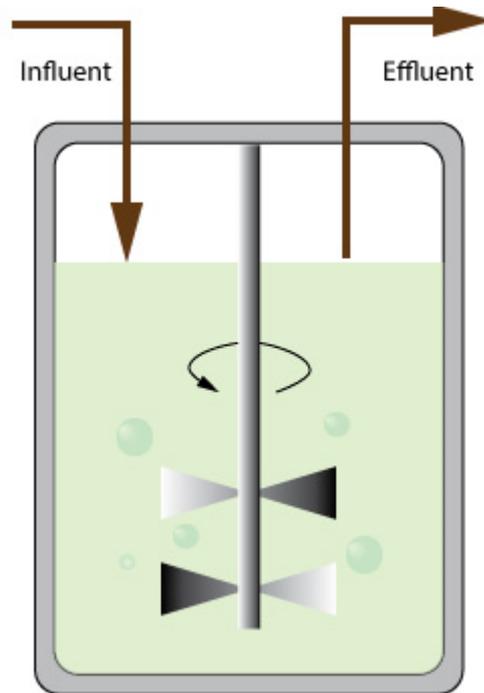


Figure 1.2. Simplified flow diagram.

For these exercises, we can think mostly about the feed (influent) and the effluent.
A detailed diagram of a lab scale algae reactor is shown in the Appendix A1.

A. How much algae am I producing?

When algae technologists ask this question, they want a result in terms of mass of algae dry weight per volume of photobioreactor per unit of time. Often $\text{g} \cdot \text{L}^{-1} \text{ day}^{-1}$ is used. We call this volumetric productivity (VP). In a continuous reactor, volumetric productivity can be easily calculated by multiplying the Dilution rate (D) with the concentration of algae.

$$VP = D_w \cdot D,$$

where D_w is the dry-weight concentration in the units $\text{g dry-weight per L}$, and the dilution rate is in the units day^{-1} .

Dilution rate can be measured like this, Freja found on Wikipedia:

Dilution rate

At steady state, the specific growth rate (μ) of the microorganism is equal to the dilution rate (D). The dilution rate is defined as the flow of medium per time (F) over the volume of culture (V) in the bioreactor

$$D = \frac{\text{Medium flow rate}}{\text{Culture volume}} = \frac{F}{V}$$

Question 1.1

Given Freja collected 21.5 L/hour of effluent and the reactor has a volume of 400 L, what is the dilution rate in units day^{-1} ?

➤ Write your calculations and answer in the answer sheet, box 1.1.

Spectrophotometry is commonly used by biotechnologists as a quick way to measure the dry weight of cell biomass in a system. Since cells both absorb and scatter light, it is not a perfect measure, so you have to be sure to use it in a limited range of cell density. Still it is much faster than filtering, drying and weighing biomass. The first step is to calculate the Dry Weight concentration (D_w) in g/L - from the measured absorbance at 750 nm (A_{750}), with a formula, which Freja has provided for you:

$$D_w = 0.4561 \frac{\text{g}}{\text{L}} \cdot (\text{Absorbance at 750nm})$$

Measure absorbance

Turn on and calibrate the spectrophotometer with deionized water.

Question 1.2

Measure a complete absorbance spectrum for all wavelengths with the effluent algae suspension in the cuvette of 1 cm thickness; see [Figure 1.1](#). Save the spectrum from the File menu – your team will need it in Question 2.3.

Measure the effluent algae absorbance at 750 nm in a 1.00 cm cuvette.

Remember to mix before measurement, as it settles very fast.

➤ Write your measurements in the answer sheet, box 1.2.

Use the formula to calculate the dry weight concentration in g/L.

➤ Write your calculations and answer in the answer sheet, box 1.2.

Question 1.3

How much algae are we making? In other words, what is the volumetric productivity in $\text{g} \cdot \text{L}^{-1} \text{day}^{-1}$?

➤ Write your calculations and answer in the answer sheet, box 1.3.

Question 1.4

How much would the reactor produce if operated at the same rate for one year? (Show your work.)

➤ Write your calculations and answer in the answer sheet, box 1.4.

B. How much carotenoids are the algae producing?

A lot of the reason that fish are considered a healthy food is because they eat organisms that eat algae. Many algae contain carotenoids, auxiliary photo pigments. Carotenoids, especially Astaxanthin, are a small part of the salmon diet, but they account for the nice pink-red colour and a significant portion of the cost. As strong anti-oxidants, these molecules are also considered a healthy part of the human diet. Because both chlorophylls and carotenoids absorb light at around 450-500 nm ([Figure 1.3](#)), it can be tricky to measure carotenoids directly in cell extracts. Therefore, scientists have developed ways of estimating the carotenoid content by comparing the peaks at different wavelengths.

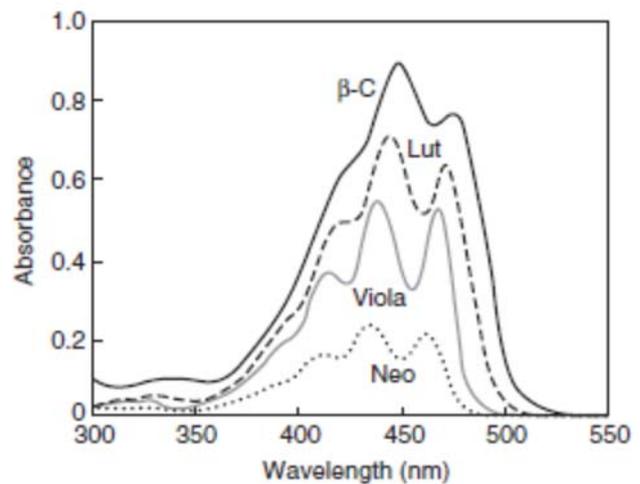
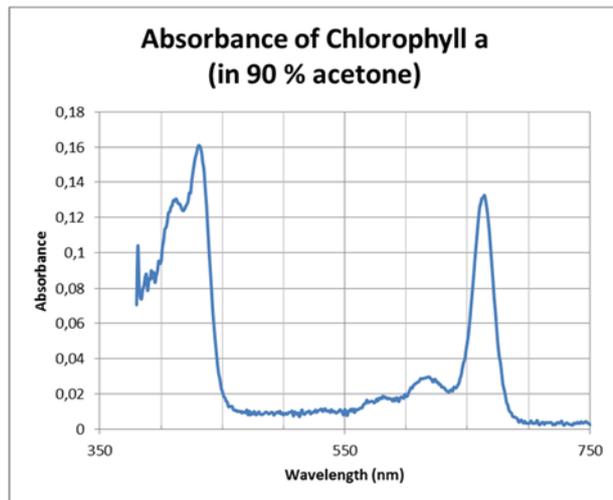


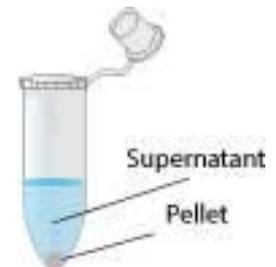
Figure 1.3. Absorbance spectra of chlorophyll a in acetone compared to the carotenoids beta-carotein, lutein, violaxanthin, and neolaxanthin (From Lichtenhaler and Buschmann 2001).

Follow the protocol below to extract and measure the pigments:

Be aware that this experimental step is a bottleneck and time consuming.

Protocol

1. Balance the centrifuge – look at the instructions placed by the centrifuge. Each tube has to have a tube placed opposite with the same volume, so the weight is equally distributed.
2. 2 mL culture is centrifuged at 6000 rpm for 5 minutes.
3. Remove and discard the supernatant. Take care – the pellet can be on the tube side.
4. 2 mL 96% Ethanol is added to the pellet. Shake and mix the tube to begin suspending the pellet.
5. Hold the tube on the Vortex mixer for 5 minutes to fully disrupt the pellet.
6. Incubate at 60 °C for 40 minutes. Then in the ice bath for 15 minutes.
7. Centrifuge again.
8. Transfer the supernatant to the cuvette, being careful not to disturb the pellet. Insert the cuvette in the spectrophotometer in an orientation to let the light travel 1.00 cm through the cuvette. Measure the absorbance spectrum of the supernatant.



Use the equations below to calculate the concentrations of chlorophyll a (c_a), chlorophyll b (c_b) and total carotenoids ($c_{(x+c)}$):

In a solution with Ethanol 96%:

$$c_a = (13.36 \text{ mg/L}) \cdot A_{664} - (5.19 \text{ mg/L}) \cdot A_{649}$$

$$c_b = (27.43 \text{ mg/L}) \cdot A_{649} - (8.12 \text{ mg/L}) \cdot A_{664}$$

$$c_{(x+c)} = ((1000 \text{ mg/L}) \cdot A_{470} - 2.13 c_a - 97.64 c_b) / 209$$

where A_{470} is absorbance measured at 470 nm, A_{649} absorbance measured at 649 nm, and A_{664} absorbance measured at 664 nm.

Question 1.5

What values did you measure for A_{470} , A_{649} , and A_{664} ?

➤ Write your answer in the answer sheet, box 1.5.

Question 1.6

What did you calculate for c_a , c_b , and $c_{(x+c)}$?

➤ Write your calculations and answer in the answer sheet, box 1.6.

Question 1.7

How much chlorophyll (a+b) and carotenoids do the algae contain in mg per gram dry weight?

➤ Write your calculations and answer in the answer sheet, box 1.7.

Question 1.8

What are the production rates of these molecules?

➤ Write your calculations and answer in the answer sheet, box 1.8.

C. How much nutrients are the algae removing from the wastewater?

To answer this, Freja gets two simple colorimetric test kits that the high school had in the closet and follows the instructions. Measure N and P in both the influent and the effluent waters.

Note: The concentrations of N and P in the synthetic influent differ from the concentrations in normal wastewater, in order to make it possible to measure with little practice.

Use the centrifuge to remove the algae from at least 4 x 2 mL of effluent sample. Save the supernatant for testing. The algae pellet is not needed.

Analyse Ammonium

Dilute the influent 50 times before analysis using plastic pipette and graded test tube. The effluent does not need dilution.

1. Fill a glass vial with the sample to the 5 mL mark, using a plastic pipette
2. Add 10 drops of the reagent labelled NH₄ - 1
3. Seal the vial and mix
4. Add 1 level measuring spoonful of the reagent labelled NH₄ - 2 (the spoon is in the kit)
5. Seal the vial and shake until the powder has dissolved
6. Wait 5 minutes
7. Open the vial and add 4 drops of the reagent labelled NH₄ - 3
8. Seal the vial and mix
9. Wait 7 minutes
10. Read the absorbance in the spectrophotometer at 700 nm
11. Use the standard curve in [Figure 1.4](#) to determine the concentration

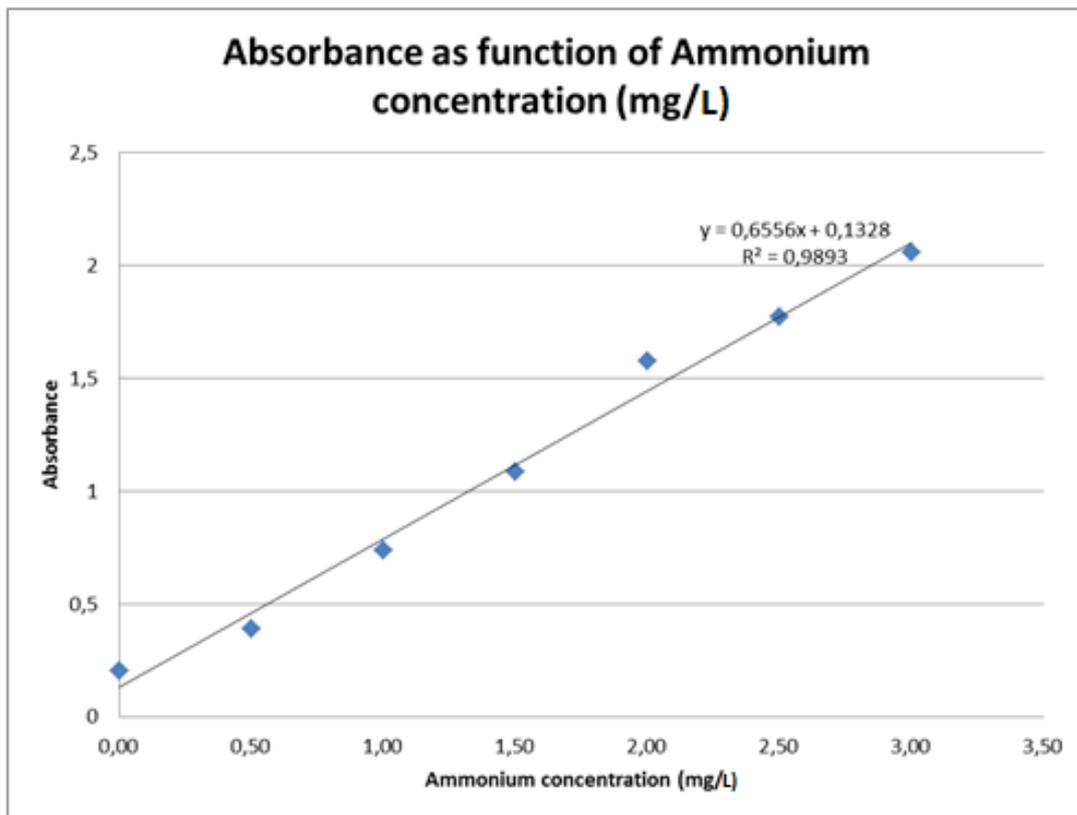


Figure 1.4. Standard curve for absorbance-ammonium concentration correlation.

Question 1.9

What values (see the answer sheet) for NH_4^+ did you determine for influent?

What values (see the answer sheet) for NH_4^+ did you determine for effluent?

What is the NH_4^+ removal percentage?

What is the NH_4^+ removal rate in $\text{mg} \cdot \text{L}^{-1} \cdot \text{d}^{-1}$?

➤ Write your calculations and answer in the answer sheet, box 1.9.

Analyse Phosphate

We expect much higher levels in both influent and effluent of PO_4^{3-} , so please dilute your original influent and effluent samples 10 times before starting the test, using plastic pipette and graded test tube. Make sure to rinse and dry vials that you reuse.

1. Fill a glass vial to the 5 mL mark with the sample, using a plastic pipette
2. Add 6 drops of the reagent labelled $\text{PO}_4 - 1$
3. Seal the vial and mix
4. Add 6 drops of the reagent labelled $\text{PO}_4 - 2$
5. Seal the vial and mix
6. Wait 10 minutes
7. Measure Absorbance at 700 nm
8. Use the standard curve in [Figure 1.5](#) to determine the concentration

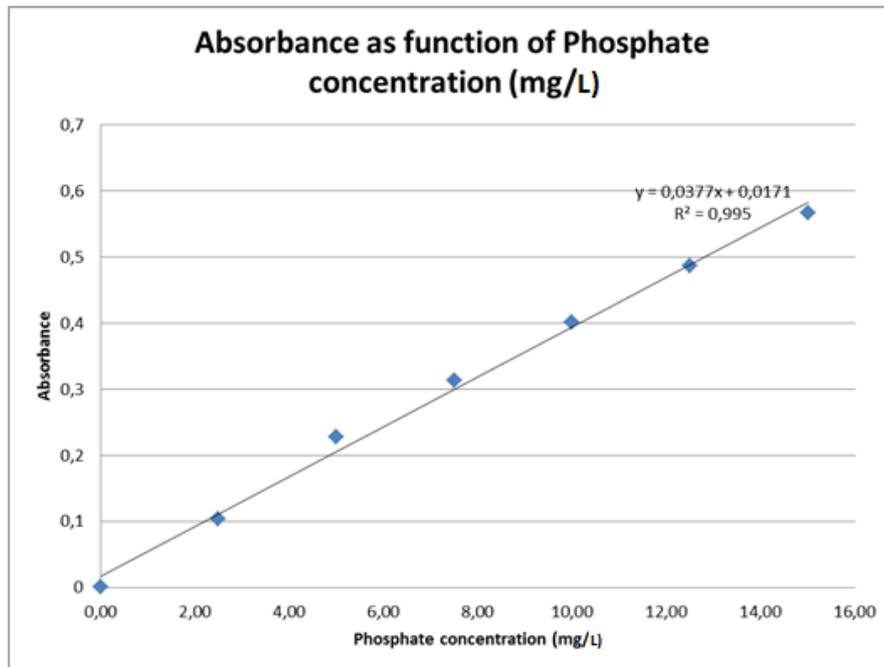


Figure 1.5. Standard curve for absorbance-phosphate concentration correlation.

Question 1.10

What values (see the answer sheet) for PO_4^{3-} did you determine for influent?

What values (see the answer sheet) for PO_4^{3-} did you determine for effluent?

What is the PO_4^{3-} removal percentage?

What is the PO_4^{3-} removal rate in $\text{mg}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$?

➤ Write your calculations and answer in the answer sheet, box 1.10.

Question 1.11

Use nutrient removal rate and volumetric productivity to estimate the N and P content of the algae.

➤ Write your calculations and answer in the answer sheet, box 1.11.

Question 1.12

What is the annual saving in DKK/L reactor, given the Danish tax of 5 DKK per kg N and 110 DKK per kg PO_4^{3-} ?

➤ Write your calculations and answer in the answer sheet, box 1.12.

2. Designing an illumination system for algae production using LEDs.

After analysing the absorbance spectra Freja asked her father, “Why do you use day-light LEDs?” And Njord answered: “Because I suppose the algae need daylight to grow!” “Yes”, Freja said, “That is true, but they only need part of it.”

Compare your absorption measurements in **Question 1.2** on algae in water with the spectrum in **Figure 1.3** of chlorophyll a in acetone.

Question 2.1

Which colours can Freja identify in **Figure 1.3** as relevant for the photosynthesis? State the wavelength intervals for chlorophyll a. (Note that in acetone these are shifted relative to those you would observe in water for your algae.)

➤ Write your answer in the answer sheet, box 2.1.

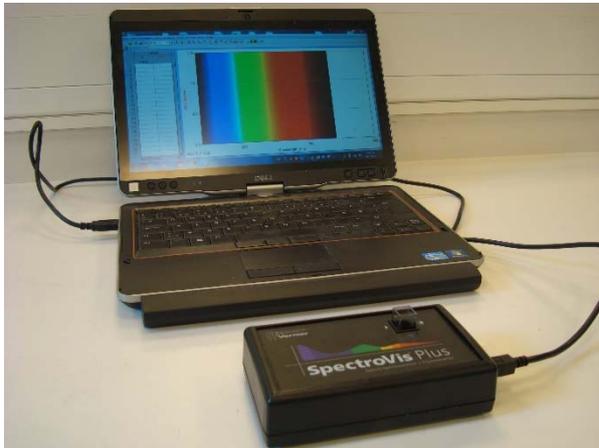
Freja continued:

“Let me illustrate how much you can save on your electricity bill by providing just the necessary light. I will take the red part of the spectrum as an example.”

Question 2.2

Which spectra does Freja have to compare for her example?

➤ Tick your answer or answers in the answer sheet, box 2.2.



Absorption measurement.

Spectrophotometer with cuvette and cable connected to a computer with LoggerPro.



Emission measurement.

Spectrophotometer with optical fibre unit and cable connected to a computer with LoggerPro.

Figure 2.1a



Figure 2.1b. Close-up of fibre unit mounted for emission measurements. Note the matching arrows.

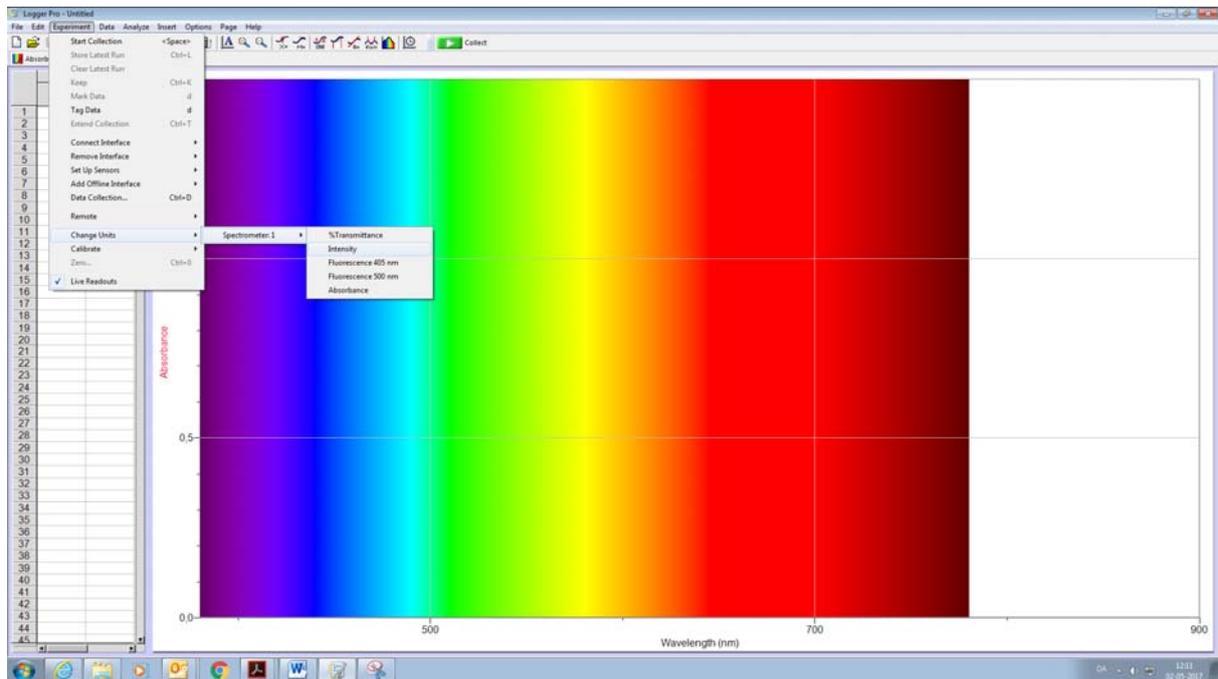


Figure 2.2. “Change units” under “Experiment” for setting of spectrophotometer mode.
 Choose “Absorption” for absorption and “Intensity” for emission spectra.
 Look inside the spectrophotometer in Appendix B.

Measure the absorption peak of the algae in the red part of the spectrum (or retrieve the spectrum saved in Question 1.2)! Keep the graph, but change the setting to “intensity” in Logger Pro (follow the link Experiment -> Change Units ->...). Notice, that the absorption graph now shows a dip, where light is absorbed.

In the following, where you are going to investigate the emitted light spectrum from various light sources, keep this setting.

Question 2.3

Measure the emission spectrum of the red LED in the multicolour LED bar! Show this spectrum together with the spectrum from Question 2.2 in the same graph with intensity on the y-axis².

➤ *Print your graph with team number. Mark it with “Graph 2.3”.*

Question 2.4

How many nanometres should the red LED light be shifted to match the red absorption dip for the algae?

➤ *Write your answer in the answer sheet, box 2.4.*

Add your readings of the necessary numbers to “Graph 2.3”.

“OK”, Freja said. “Let us study this. It does not sound right that the plants can only grow at very particular colours. Let us study the literature...”

Aha, Njord says, I think I have found it. “Plants can collect photons also with higher energy and transport it to the chlorophyll for use there. (You see, light comes in quanta called photons, and are only absorbed in full quanta.) The collection is done by carotenoids. You see the graph below that shows the CO₂ consumption of algae illuminated by light of different frequencies. It shows that the green alga, Chlorella, collects photons between 400 nm and 680 nm with nearly the same efficiency, called the quantum efficiency η_{λ} . More precisely: When the energy is transported to chlorophyll a, it is absorbed there with an efficiency η_{λ} called the quantum efficiency.”

² When you shine light from a strong light source into the spectrophotometer, the graph may get a flat top in certain intervals, due to saturation. You can avoid this either by reducing the sample time (follow the link Experiment -> Set up sensors -> Spectrometer...), or more simply, by moving the light source a bit away from the instrument (taking care to avoid stray light from other sources).

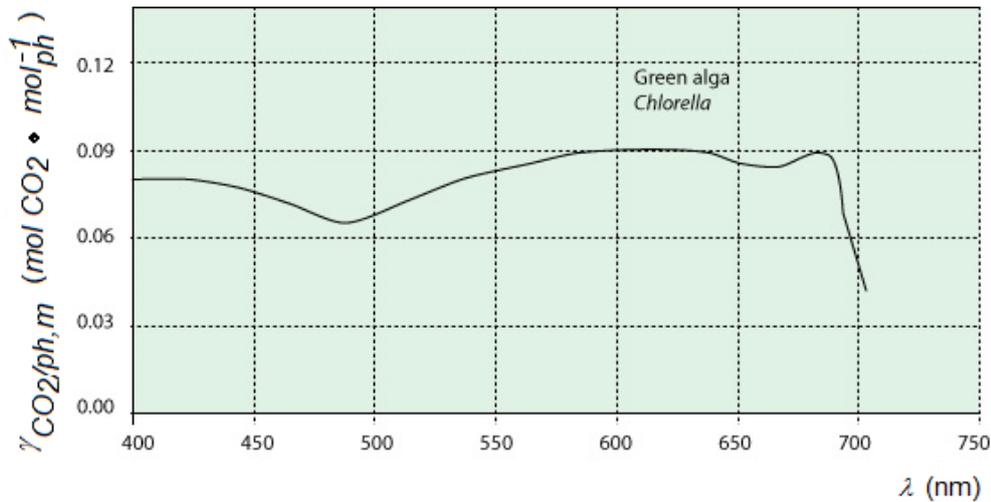


Figure 2.3. The CO₂ consumption in mole/mole photons absorbed as a function of photon wavelength. This consumption can be taken to be proportional to the quantum efficiency η_{λ} .

After Emerson, Robert, and Charlton M. Lewis. "The dependence of the quantum yield of Chlorella photosynthesis on wave length of light." American Journal of Botany (1943): 165-178.

Question 2.5

What process in the plant does the consumption of CO₂ signify?

➤ Tick your answer in the answer sheet, box 2.5.

Question 2.6

Measure the emission spectrum for the green LED in the LED bar! Read off the peak wavelength. For Chlorella, in Figure 2.3, find the ratio of the quantum efficiency at this wavelength relative to the quantum efficiency at the wavelength of the red LED peak. Show how you read off the necessary numbers from the graph.

➤ Print the graph and write your calculations and answer in the answer sheet, box 2.6.

"So, it is not such a big waste after all to use my white light LEDs", said Njord, "They seem to match quite perfectly the wavelength interval which results in photosynthesis, also called "the photosynthetic active regime" (PAR). You see the spectrum for yourself!"

Question 2.7

Measure the spectrum of the white light LED bulb and compare it with the absorption spectrum of the algae.

➤ Print the graph and write your answer in the answer sheet, box 2.7.

"Well, it is more complicated than that. You lose more than the quantum efficiencies indicate!" replied Freja. "You see, light comes in quanta called photons, and are only absorbed in full quanta. The energy of a quantum of light at a certain frequency is proportional to the frequency." The constant h of proportionality is called Planck's constant and its value is $h = 6.626 \cdot 10^{-34}$ J/Hz or $h = 4.136$ meV/THz (note: 1 THz = 10^{12} Hz). The latter unit contains the energy unit electronvolt and is practical for atomic processes. One electronvolt is the energy that an electron gains when it goes through a one volt battery, $1 \text{ eV} = 1.602 \cdot 10^{-19}$ J, and 1 Hz is 1 s^{-1} . The speed of light c is the wavelength times the frequency.

Question 2.8

Using the table in the answer sheet, calculate the energy in eV of a photon at the peak wavelength of the green light LED, at the peak wavelength of the red-light LED and at the red absorption dip of your algae sample.

➤ Write your answer in the answer sheet, box 2.8. State the wavelengths you use for your calculation and state the related frequencies of these wavelengths.

Question 2.9

For one photon reaction step to take place, how much energy (in eV) needs to be absorbed at the red dip in your algae graph from Question 2.3? How many percent of energy is wasted if this is provided by a green, respectively red LED and the photon energy is transported by the carotenoids to the chlorophyll? What are the respective efficiencies in energy consumption?

➤ Write your answer in the answer sheet, box 2.9.

Question 2.10

How many percent light energy is lost if Njord uses green LEDs to “feed” the red absorption in the chlorophyll instead of LEDs optimized to that absorption? Remember to take into account that red, yellow and green LEDs are similar in construction and can be assumed to convert the electric input energy to light energy output with equal efficiency. Take also into account the quantum efficiencies. Finally, note the algae can also obtain energy for use in the chlorophyll through the absorption of green light by the carotenoids.

➤ Write your answer in the answer sheet, box 2.10.

3. Predator-Prey interactions.

NB: You will have to use and study the Appendix C carefully in order to solve this part.

Freja said to her father Njord, “The microalgae are very good to use as feed for copepods. The copepods can afterwards be used as feed for fish. In school, we have taken some videos of how fast different species of Copepods can swim and the size of the escape distance from a fish.”

The videos are placed on the desktop. By using video analysis in “Logger Pro”, find out which of the two copepods species we shall choose as feed for the fish, when the fish has a reaction distance to the copepod prey of 5 mm and attacks at a speed of 200 mm/s over a distance of 20 mm. Look in Appendix C1 for introduction to Copepods and Appendix C2 for Experimental filming-setup.

Freja asked Njord to read the following!

We experimentally investigate ‘predator detection distance’ and ‘escape speed’ of two different copepod species. We introduce one species at the time in the aquarium to observe their predator detection distance and escape speed. This gives us two movies to analyse: movie 1 shows the escape behaviour of the species *Centropages hamatus*, movie 2 shows the escape behaviour of the species *Temora longicornis*. Both movies are filmed at 500 frames per second, and the horizontal width of the movie frames is 39 mm.

Open the movies in Logger Pro. Get readings of time in seconds by setting the “Movie Options” – “frame rate”. Use the “Set Scale” function to convert distances to mm.

Question 3.1

For each of the 2 copepods, by performing the 13 steps below, determine:

- the predator detection distance (in mm) of the two copepod species (i.e. the distance between the tip of pipette and the copepod at the time it starts escaping).
- the jump distance (in mm) of the two copepod species.
- the jump speed (in mm/s) of the two copepod species.

➤ Write your answer in the answer sheet, box 3.1.

1. At what time (in s) does the copepod start the jump?
2. At what time (in s) does the copepod end the jump?
3. What is the distance (in mm) in the z-direction from pipette tip to the copepod when the copepod starts the jump?
4. What is the distance (in mm) in the z-direction from pipette tip to the copepod when the copepod ends the jump?
5. Print the graph of z as a function of time for the jump, and attach it to the answer sheets.
6. What is the distance (in mm) in the x-direction from pipette tip to the copepod when the copepod starts the jump?

7. What is the distance (in mm) in the x-direction from pipette tip to the copepod when the copepod ends the jump?
8. What is the distance (in mm) in the y-direction from pipette tip to the copepod when the copepod starts the jump?
9. What is the distance (in mm) in the y-direction from pipette tip to the copepod when the copepod ends the jump?
10. Print x and y as a function of time in the same graph, and attach it to the answer sheets. Remember to write your country and team before printing.

For the following three steps, you can use the equations below the questions to calculate distances and speeds in three dimensions.

11. What is the distance (in mm) from pipette tip to the copepod when the copepod starts the jump \approx 'Predator detection distance'?
12. What is the jump distance (in mm)?
13. What is the escape speed (in mm/s)?

Equation 1: 'Predator detection distance'

$$d_{predator} = \sqrt{(x_{start} - x_{pipette})^2 + (y_{start} - y_{pipette})^2 + (z_{start} - z_{pipette})^2}$$

Equation 2: 'Jump distance'

$$d_{jump} = \sqrt{(x_{start} - x_{end})^2 + (y_{start} - y_{end})^2 + (z_{start} - z_{end})^2}$$

Equation 3: 'Escape speed'

$$v_{escape} = \frac{d_{jump}}{\Delta t}$$

x_{start} , y_{start} , z_{start} , are respectively the x , y and z position of the copepod at the start of the jump, and x_{end} , y_{end} , z_{end} , are the x , y and z position of the copepod at the end of the jump.

Question 3.2

We know that the fish species in our aquaculture system can detect copepods at a distance of 5 mm. Furthermore, it attacks at a constant speed of 200 mm/s over a distance of 20 mm.

Can the copepods escape from an attacking fish? To answer this, assume one dimensional motion, and use the copepod escape speeds as averages for their movement. Visualize by drawing three lines on a graph for positions (on the secondary axis) of the fish and the two copepods versus time (primary axis) from the time where the copepods start jumping.

➤ Write your answer in the answer sheet, box 3.2.

Question 3.3

Which species is the most appropriate prey species for feeding the fish in our aquaculture system?

➤ Write your answer in the answer sheet, box 3.3.

Question 3.4

To which animal group do the copepods belong?

➤ Write your answer in the answer sheet, box 3.4.

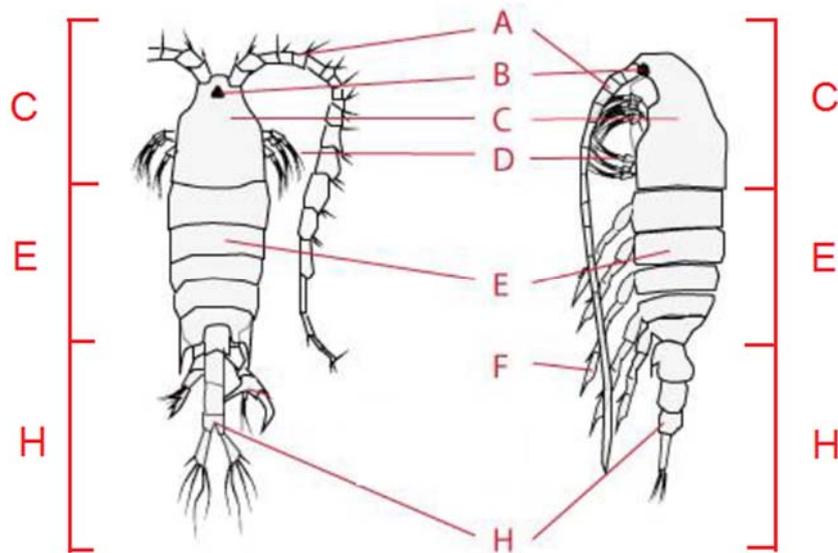


Figure 3.1. Schematic drawing of a copepod, seen from two sides.

Question 3.5

From which sides are the copepods in **Figure 3.1** depicted?

- *Tick the correct answers in the answer sheet, box 3.5.*

Question 3.6

Can you name the parts with the letters?

- *Give the relevant letters (**Figure 3.1**) to the names and write your answer in the answer sheet, box 3.6.*

Question 3.7

Many copepods contain oil. Which advantages can it give the copepods to synthesize oil?

- *Tick the correct answer or answers in the answer sheet, box 3.7.*

END OF TASK 2: OCEAN

9. Unterstützung durch

Land Kärnten

The logo for Land Kärnten features the text "LAND" on the left, a stylized flag icon in the center consisting of a yellow square above a red square, and the text "KÄRNTEN" on the right.

Klagenfurt am Wörthersee

The logo for Klagenfurt am Wörthersee features a stylized graphic of a bird or wing above the text "Klagenfurt" in a large, serif font, with "am Wörthersee" in a smaller, sans-serif font below it.

Industriellenvereinigung Kärnten

The logo for Industriellenvereinigung Kärnten features the letters "iv" in a white font inside a blue square, followed by the text "INDUSTRIELLENVEREINIGUNG KÄRNTEN" in a blue, sans-serif font.

Regionales Netzwerk für
Naturwissenschaften und Mathematik Kärnten

The logo for the Regional Network for Natural Sciences and Mathematics in Carinthia features the letters "RN" in a large, bold, yellow font, followed by "KÄRNTEN" in a grey, sans-serif font. Above "KÄRNTEN" is the word "Jugend" in a yellow, italicized font, and "Regionales Netzwerk" is written in a small, grey font between "RN" and "KÄRNTEN".

IMST- Innovationen machen Schulen Top



Anton Paar

The logo for Anton Paar features a stylized graphic of a sphere with a red and grey orbital path above the text "Anton Paar" in a bold, red, sans-serif font.