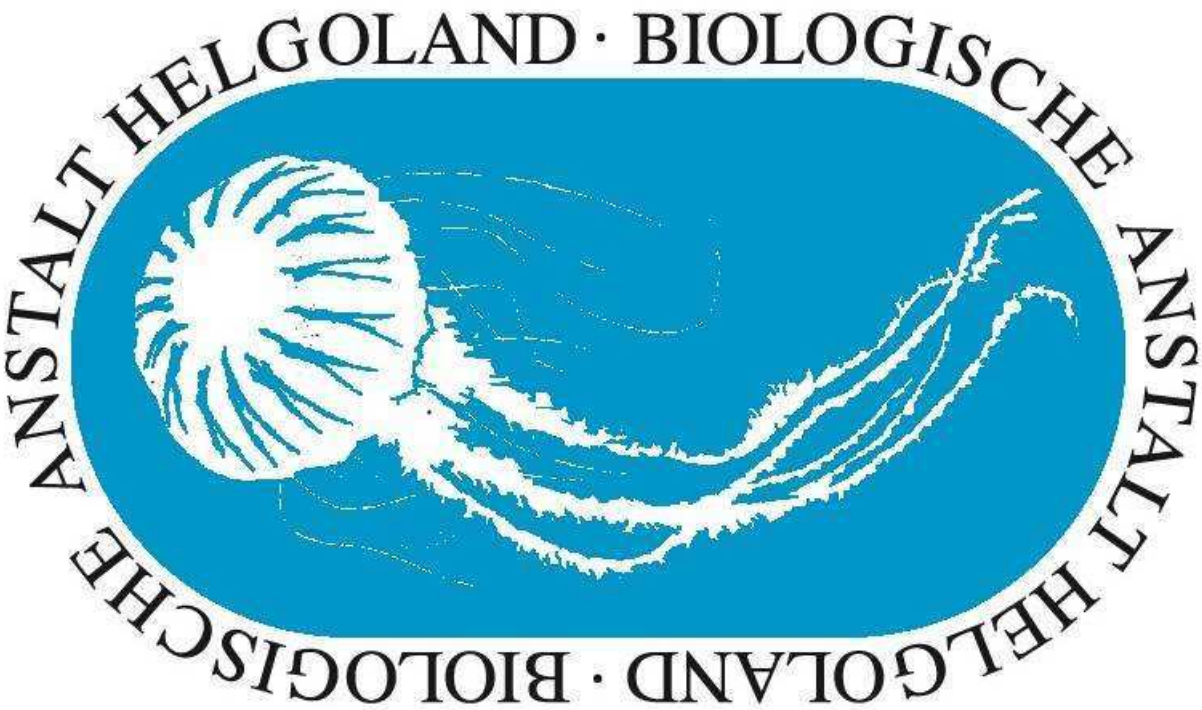
My internship at the Alfred-Wegener-Institute on Heligoland



28th June- 23rd July 2010

by *Roderich Römhild*

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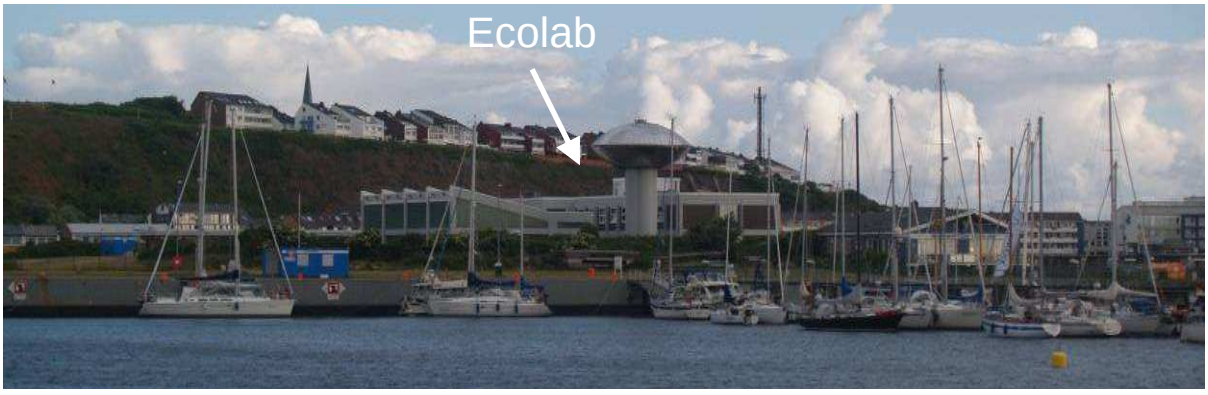
I. Introduction

My name is Roderich Römhild. I am 17 years old and have just completed 11th grade at the *Friedrich- Ebert- Gymnasium* in Harburg. Being in love with science and especially biology, I took part in many science-student-competitions, such as the *International Biology Olympiade [IBO]*. By advancing to the fourth and final national round, I got the opportunity to apply for one of the internships, offered by the *Förderverein der Biologieolympiade e.V*. Because I am very interested in marine ecology and ocean-research I was awarded a four-week internship (28th June- 23rd July 2010) at the biological station on Heligoland [BAH]. This research centre is part of the *Alfred Wegener Institute [AWI]* and mainly researches marine foodwebs and climate-change-impacts on coastal ecosystems. In my time there I hoped to find out, if this is the right future for me, by working in many parts of the station as best I can.

On Heligoland, I worked for Stefanie Schnell, who is a PhD- student at the biological station. Stefanie researches the ocean acidification effects on pelagic community structure and food chains in the ecolab [bottom picture; white arrow]. This is also the place, where I worked most of the time.

My internship at the biological station of Heligoland was clearly structured into three main parts. From the 28th of June until the 1st of July I took part in the POLMAR-program [Helmholtz Graduate School]. The following Friday and the full next week I worked at various AWI stations and programs on Heligoland. During this week I worked onboard a research-trawler, at the aquarium, in the ecolab and with samples from Heligoland. The following two weeks I spent researching the grazing-preferences of marine copepods, as well as food-based competition and predation between *Artemia* and cultured copepods. During this period I also compared different methods for the analysis of population-densities of small marine organisms such as *Rhodomonas salina*. Moreover I supported Stefanie with her copepod-research.

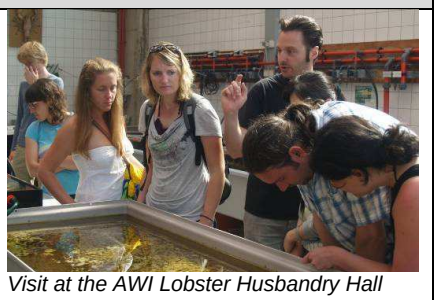


Picture: Southern Port of the German island HELIGOLAND with view on the ECOLAB, where I researched marine copepods

II. My first two weeks at the AWI

1) The POLMAR-program

The Helmholtz Graduate School POLMAR provides a postgraduate program at the AWI for PhD-students. POLMAR is established as a transdisciplinary umbrella organization to combine high level science qualification in the various disciplines of polar and marine research with other key qualifications through joint seminars, lectures and practical training (www.polmar.awi.de). Each participant collects a certain amount of credit points during his time as PhD-student. This project organized a field trip to the AWI station on Heligoland. During this week I was treated as a PhD-student and could take part in the program fully. Along with academic events, such as lectures on current research work at the AWI Heligoland, recreational activities around the island made the program very successful and enjoyable. This week was an excellent opportunity for me to get to know the island, my working community and the AWI.

Monday 28 June, 2010		
12:30 pm	Arrival at Heligoland	 *Guided Tour of the "Upperland" with Mr. Hagmeier and the POLMAR-students*
02:30 pm	***Seminar Room "AlteKaserne":*** Address of welcome (K. Wiltshire, AWI Helgoland)	
03:00 pm	Lecture with walk around the Upperland" (according to weather conditions): Introduction/History of the BAH & Island Geology (E. Hagmeier)	
Evening	BBQ with doctoral students of Heligoland and others (self-organization)	
Tuesday 29 June, 2010		
09:30 am	***Ecolab Building A:*** Guided Tour through Lobster Husbandry and Laboratories for Crab Husbandry (J. Beerman/H.-D. Franke, AWI Helgoland)	 *Visit at the AWI Lobster Husbandry Hall*
11:00 am 11:45 am	***Seminar Room A-227:*** Lecture: Invasive Species under Climate Change (H.-D. Franke, AWI Helgoland) Lecture: Ecological Stoichiometry: From Molecules to Ecosystems (A. Malzahn)	
12:15 pm	Lunchtime (self-organization)	
02:00 pm	***Seminar Room A-227 & Diving Basis/Heincke-Hall:*** Lecture and Guided Tour: Research on Fishery Ecology & Basic Information on Scientific Diving Activities at the BAH (P. Fischer and colleagues, AWI Heligoland)	 *European oystercatcher [Heligoland Dune]*
04:35- 06:30 pm	***Heligoland Dune:*** Guided Seal Tour at the Heligoland Dune in German (R. Blädel)	

Wednesday 30 June, 2010		
09:30 am - 12:00 pm	***Seminar Room "AlteKaserne" & Laboratory Building C:*** Lectures and Guided Tour: Introduction into Microbial Research at the BAH (AG Microbial Ecology)	 *Northern gannet nesting on the cliffs*
12:15 pm	Lunchtime (self-organization)	
02:00- 4:00 pm 04:30- 05:30 pm	***Heligoland Aquarium & Ornithological Station Heligoland:*** Guided Tour: Heligoland Aquarium (E. Hensel, AWI Heligoland) Guided Tour: Ornithological Station Heligoland (Ornithological Station)	 *Guillemot species living in the cliff-crevices*
Evening	another BBQ (self-organization)	
Thursday 1 July, 2010		
09:30 am 10:15 am	***Seminar Room "AlteKaserne":*** Lecture: Microzooplankton at Heligoland Roads (M. Löder, AWI Heligoland) Lecture: Introduction to Long Term Plankton Data (A. Kraberg, AWI Heligoland)	 *View on the southern tip of the island*
11:00 am	Guided Tour "Bunker"	
03:30 pm	Departure of POLMAR students	

2) Alkalinity-investigation of artificial sea water

During my internship on Heligoland, I also worked one day with Evamaria Krause, a PhD-student at the microbiological lab. We wanted to test a newly arrived alkalinity measuring device. The total alkalinity [A_T] of a sea water sample is defined as the number of moles of hydrogen ion equivalent to the excess of proton acceptors over proton donors in one kilogram of sample (Dickson *et al,* 2007). This can be expressed in a short equation [considers most frequent seawater ions]:

$$A_T = [HCO_3^-] + 2[CO_3^{2-}] + [B(OH)_4^-] + [OH^-] + [HPO_4^{2-}] + 2[PO_4^{3-}] + [SiO(OH)_3^-] + [NH_3] + [HS^-] + \ldots - [H^+]_F - [HSO_4^-] - [HF] - [H_3PO_4] - \ldots$$

This parameter along with CO_2-partial-pressure and the pH-value is used to calculate the amount of dissolved CO_2 in sea water.

The total alkalinity was measured with an open-cell titrator [top image next page]. A fixed amount of 50 ml sea water is placed in an open cell where it is titrated with a solution of hydrochloric acid, backed by a sodium chloride solution equivalent to the approximate ionic strength of sea water. This titrant [brown bottle] is a mixture of 0,05 M hydrochloric acid and 35 g NaCl per litre. The sea water sample is titrated in a two-

Picture: Open-cell titrator for alkalinity measurements

stage process. The sample is first acidified to a pH between 3,5 und 4,0. Thereby the evolving CO_2 is allowed to escape by stirring the solution for a short period of time, before continuing until a pH of about 3,0 is achieved. The progress of the titration is monitored with a glass electrode [brownish-yellow due to iodine]. The measured titrant volume is used to compute the total alkalinity of the sample.

I analysed three different samples: a freshly filtered sea water sample [2,7 µm filter] and two different artificial sea water samples, I prepared earlier that day. The first of these artificial sea water samples was a simple mix of 35 g of an aquarium-salt-mix in one litre of deionised water. The second of these self-prepared samples was mixed according to the following recipe.

Artificial Sea Water:		
- 28 g NaCl	- 5 g $MgCl_2 * 6\ H_2O$	- 0,2 g $NaHCO_3$
- 7 g $MgSO_4 * 7\ H_2O$	- 2,4 g $CaCl_2 * 6\ H_2O$	- 985 ml deionised water

Through linear regression, I received the following results. The therefore necessary graphs and tables can be found in the attachment [pages 45- 47].

Sample	Total alkalinity [moles of H^+ equivalents/kg]
1) Natural sea water from Helgoland:	2986 µM
2) Artificial sea water I [ready-mix]:	2971 µM
3) Artificial sea water II [recipe above]:	2984 µM

Evidently the attained results display, that both artificial sea water mixtures have a chemically high resemblance to the real water. Summing up both sets of artificial sea water may therefore be used in aquaria or biological experiments concerning local species.

3) Trip aboard the Uthörn

On Tuesday the 6th of July I had the opportunity to accompany Kristine Carstens and Julia Haafke aboard the research-trawler *Uthörn* [bottom picture]. The mission of the monthly trip is, to get some water samples at different sampling sites off the shore of Heligoland. We travelled to five sampling sites, of which the farthest was 8 nautic miles off the island. Because of heavy seas we were forced to turn back before reaching sampling site six. At each sampling site we measured the same parameters: pH-value, water-clarity (how deep can I see a white dish?), different nutrients, chlorophyll- content and many more [top picture]. During the entire cruise a mobile ferrybox collected additional data automatically. The ferrybox has many sensors bellow the ships hull, constantly measuring different parameters. This technology is also used at the *GKSS [Gesellschaft für Kernenergieverwertung in Schiffbau und Schiffahrt mbH; Research centre in the Helmholtz Community]* near Hamburg and in a stationary ferrybox several yards from the sampling site Helgoland Roads.

Julia and a crew-member taking a water sample from 10 m depth

Picture: The research-trawler UTHÖRN at the pier, about to depart at 8:00 am; Next to the UTHÖRN is the small research-boat AADE. The AADE takes daily samples of the roads water. These samples I worked with later, in my internship at the Biological Station of Helgoland.

4) At the aquarium

On the 7th of July I worked at the aquarium of Heligoland. The day was a very fulfilling part of my internship at the BAH. From 8:00 am until 9:00 am I prepared power-food for various fish of the aquarium. This power-food consisted of especially prepared sprat. The frozen sprat had to be defrosted in saltwater and cut into differently sized pieces: smaller pieces for crabs, anemones, and flounders, bigger pieces and heads for the 50 year old sturgeon, turbots, codfish and dogfish. The stingrays got uncut fish and small plaice received only tiny filet-particles. All snails, mussels and sea stars received plankton. After feeding all species of the aquarium, and thereby learning a lot about them, I washed fresh sea sand to raise the sediment of the king-prawn tank in which also three dead-mans-hands [sponge species] live. After noontime lunch-break, it was time to evacuate a salt-water tank of snails, to make room for a moulding lobster. Following this procedure we went to the seafront to collect various algae. These algae we sorted into bundles and then fastened these into cavities of chalk stones from *Heligoland Dune*. The algae-bundle-leaden stones were placed inside some of the aquariums tanks, as living decoration. The used algae were mainly *Fucus vesiculosus.* After cleaning up, the day was already over.

Me cutting up sprat

Quatered sprat for codfish

Fresh sprat-filet for small plaice

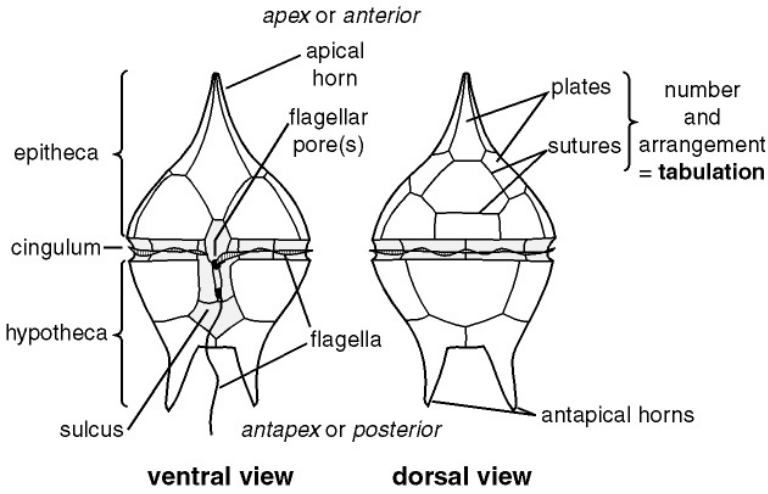
5) Research at Heligoland Roads

On the 8th and 9th of July I had the opportunity to do some plankton- research. I was tutored by Dr. Alexandra Kraberg, who just finished her new book on coastal phytoplankton. With the help of this excellent book I was able to identify and quantify various algae-species in my samples. The samples I worked on, were from the marine-research-site Helgoland Roads. This sampling site is more than 45 years old. As part of a long-term-data-program I analyzed two different seawater-samples.

Before starting with my results, I will give a short very condensed summary on terms and general structure of diatoms and dinoflagellates, the two main components of phytoplankton.

All diatoms have a characteristic basic structure: they are encased within a unique silica cell wall called *frustule*, which houses the two halves or valves of the cell, hence the group name *di*atom. The *hypovalve* is the smaller half, which fits into the slightly larger *epivalve*. The connection between both valves is called *girdle*. Diatoms are traditionally divided into two larger groups, *pennate* and *centric* diatoms. Centric diatoms have a radial symmetry, whilst pennate diatoms are bilaterally symmetric.

Dinoflagellates are divided into two large groups: *Thecate* dinoflagellates ["armoured"], which have an outer covering [theca] consisting of a series of cellulose plates and *athecate* ["naked"] dinoflagellates, which do not have a theca and therefore, often have a more variable shape ("Coastal Phytoplankton" by Dr. Kraberg). Dinoflagellates have two flagella for propulsion. The first flagella forms a tight ribbon and is located in the characteristic transverse groove, called *cingulum*. The other flagellum is straight and lies in the *sulcus*, a longitudinal groove. The general structure of a thecate dinoflagellate is shown in this image (www.geo.ucalgary.ca).



The first plankton sample was filtered through a 80 µm filter, while the second was treated with a 20 µm filter. Unlike in most chemical methods however we didn't use the filtrate, but the particles left on the filter. Because these numbers describe the mesh width of the corresponding filters, the 20 µm sample contained generally more algae and also smaller particles. I took pictures of the plankton I found, to be able to compare my identification-results with Dr. Kraberg's. I worked with a Zeiss microsope and a very


My workplace: Zeiss-microscope and plankton sample

high quality camera [Zeiss AxioCamHRc]. Both my workplace and one of the samples are shown in the picture on the previous page.

Using the stacking-program *picolay*, I was able to improve the quality of some of my snapshots. It is often crucially necessary to create picture-stacks when doing microscopy, because the microscope only focuses in a very thin depth-layer. Because the analyzed objects however are three-dimensional bodies, you can only achieve a picture in which all parts are focused, by shooting many pictures after each other, each in a different focal depth, and the stacking them on top of each other. The program then identifies focused areas and creates a mixed picture showing only these. This method is best described with a short picture series.

Protoperidinium pentagonum [thecate dinoflagellate]

Image 1: focused on the protruding left foot of *P. pentagonum*

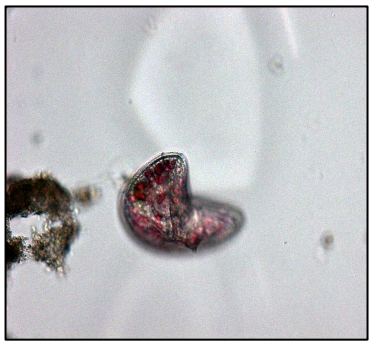


Image 2: focused on the right foot of *P. pentagonum*

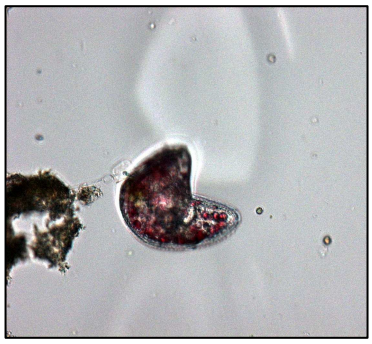
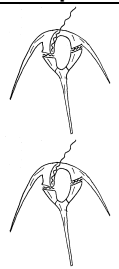
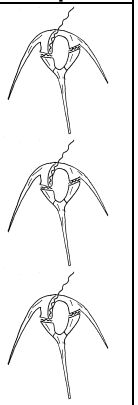
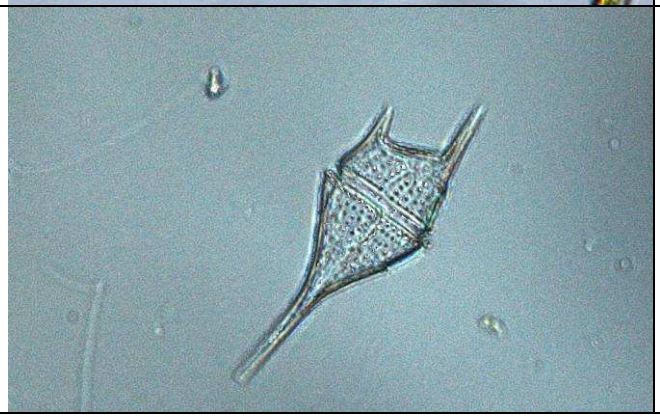


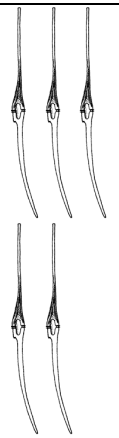
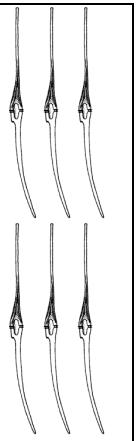
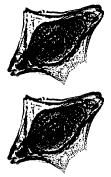
Image 3: stack of two images; focused on both visible feet of *P. pentagonum*

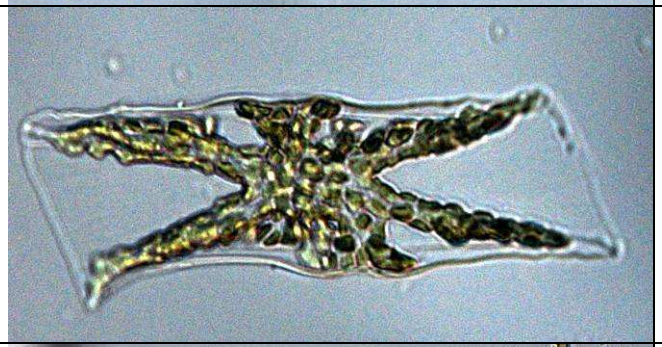
The resulting image is a stack made up of only two images. Usually stacks are made from a much higher number of images [usually 10-20]. The only difficulty, when producing stacked images is, that all pictures need to have the same camera settings and the same position. Motile objects like dinoflagellates therefore are hard to get stacked pictures of.

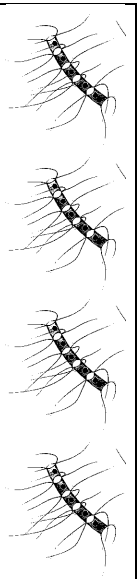
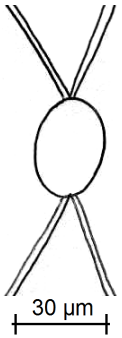
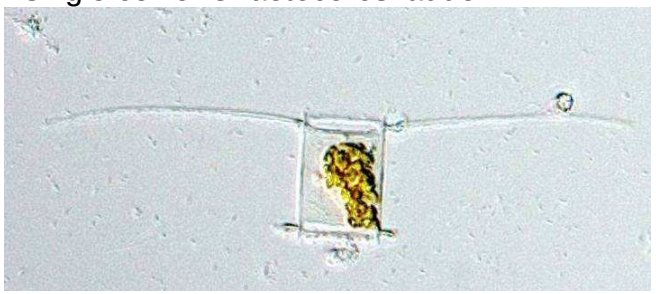
I summarized my main results in the following short table. The last two columns show the relative frequency of occurrence of the species. I analysed four microscope slides of each sample. Each identified organism of a species is represented by an individual symbol.

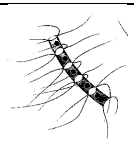
Additional pictures can be found in the attachment.

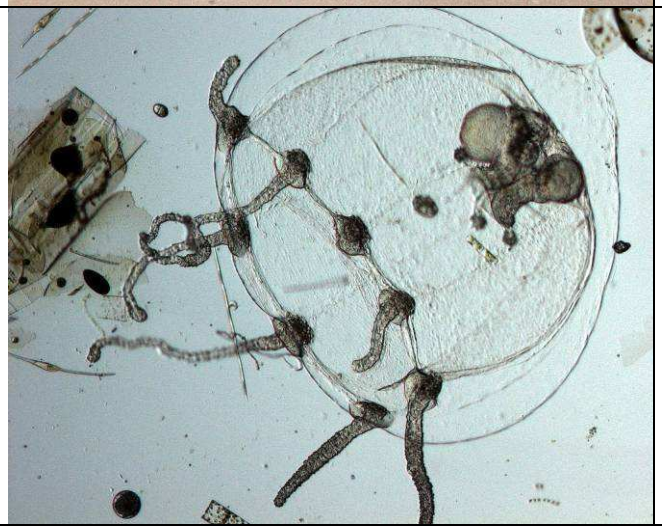
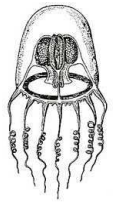
Species	Picture	Information	20 µm	80 µm
Ceratium tripos		- marine thecate dinoflagellate - autotrophic [yellow-brown chloroplasts] - cells dorso-ventrally flattened - epitheca with rounded left and steep right side forming a straight apical horn [not open-ended] - hypotheca extending into two short antapical horns [distal ends closed] - antapical horns arising in a straight line from the antapex of the cell before curving upwards until they are almost parallel to the apical horn - season: all year but peak in autumn - diameter: 50- 80 µm - length: up to 300 µm		
Ceratium lineatum		- marine dinoflagellate - autotrophic [contains numerous yellow-brown chloroplasts] - armoured with cellulose thecal plates - epitheca triangular with long anterior horn - hypotheca trapezoid, extending into a long and a short antapical horn, which are straight and in line with the main cell body - season: summer, autumn - diameter: 22- 41 µm - length: 105-151 µm		

Ceratium fusus		- marine thecate dinoflagellate - mixotrophic - cells needle-shaped and with numerous yellow-brown chloroplasts and sometimes food-vacuoles - long epithecal anterior horn - hypotheca extending into a very long left horn and a right rudimentary tooth-like antapical horn (arrow) - thecal plates delicate and ornamented - season: summer, autumn - diameter: 15- 30 µm - length: 150- 230 µm		
Protoperidinium pentagonum		- marine thecate dinoflagellate - heterotrophic - also found in brackish waters - many reddish inclusions and vacuoles - apical view: kidney shape - produces pale brown cysts - season: summer, autumn - diameter: 75- 100 µm - length: 75- 110 µm		

Protoperidinium depressum		- marine thecate dinoflagellate - heterotrophic [pallium traps prey] - one apical horn - two antapical horns - large number of reddish inclusions [fat droplets] - bioluminescent - season: mainly summer - diameter: 115- 144 µm - length: 116- 200 µm		
Mediopyxis helysia		- marine pennate diatom - cells solitary or in short chains [2- 6 cells] - autotrophic [many green chloroplasts] - cells heteropolar with short round elevation at one end of the valve and a pointed elongated horn at the other - season: spring, summer (peak), autumn - diameter: 27- 78 µm - length: 85-125 µm		
Odontella sinensis		- marine centric diatom - autotrophic [numerous chloroplasts] - elliptical cylinder shape; - cells solitary or in short chains - central position of the nucleus - valve poles form outer slightly curved horns - two processes per valve [longer than horns] - season: summer, autumn - diameter: 80- 260 µm - length: 80- 440 µm		

Chaetoceros lauderi		- marine centric diatom - autotrophic [many chloroplasts per cell] - cells higher than wide in girdle view - cells connected into straight chains with very narrow apertures - elliptic cylinder shape - four setae protruding pairwise from intercalary cells - no chloroplasts in setae - season: summer, autumn - diameter: 18- 50 µm - length: 18- 60 µm		
		- picture and drawing of *C. lauderi* in valve view 	- single cell of *Chaetoceros lauderi* 	

Chaetoceros sp.		- sample not preserved well enough for species identification
		
		- most likely is *Chaetoceros curvisetus*
		- special characteristics, suggesting this species are:
		  - colonies curved and relatively long
		  - apertures between adjacent cells wide and elliptical
		  - setae similar, long and thin
		
		- found during summer
		- diameter: 30 µm
		- length: 200 µm
				

Rathkea octopunctata		- young medusa of hydrozoa - heterotrophic zooplankton - umbrella bell-shaped - short brownish manubrium - 4 radial canals - 8 marginal bulbs with solid tentacles - gonads completely covering manubrium - medusa reproduces asexually at low temperatures (up to 6℃) and sexually at higher temperatures - season: spring		

III. Comparison of different methods for the analysis of population densities of *Rhodomonas salina*

1) Introduction

To identify the size of a population can be fairly easy, when dealing with large species, which can easily be counted by eye or use of the capture-recapture-method [Petersen method]. When the analysed organisms however are too small to be seen with the naked eye, it gets more complicated.

There are various methods

Rhodomonas salina are tiny flagellates about 5-15 µm in size. They are used for the feeding of marine copepods [e.g. *Acartia tonsa*] at the BAH. To enable best possible dosing, it is important to know the population density of the *Rhodomonas* cultures. I analysed this parameter with three different methods. My aim was to identify the method best suited for this task.

2) Project Information

I measured the population density of two *Rhodomonas salina* cultures [culture A and culture C] for two weeks daily. Both cultures were kept at the same conditions in the 18℃ climate chamber. They were supplied with *F/2-medium*, light and sufficient oxygen. The oxygen was brought into the 5 litre glass bottles through glass tubes. The ascending bubbles also created the necessary gentle rotating currents, evenly distributing the cells in the glass bottles.

For measuring I had three different methods: manual counting, use of the FlowCam and measuring with the CASY-apparatus.

Rhodomonas salina cultures A [left] and C [right] in the climate chamber

3) Material and Methods

This project contained four main methods. The main facts and procedures of these methods I have summarized in the following paragraphs.

Preparation of F/2-medium

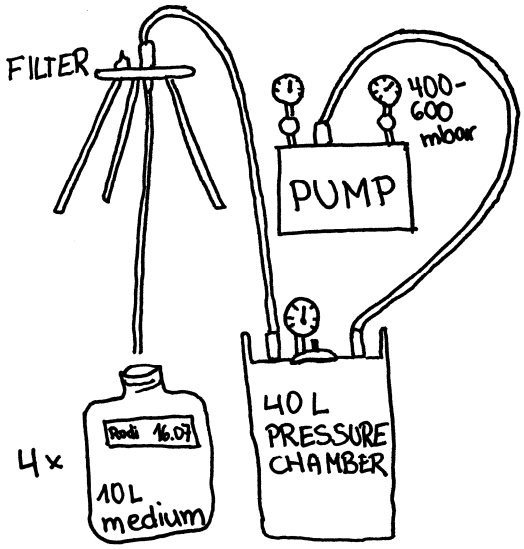
The culturing of the *Rhodomonas* flagellates required sufficient nourishing with minerals and vitamins. These were provided in a nutrient solution. This F/2-medium was needed in large quantities, so it was prepared about two times per week. Each

portion consisted of 40 litres of medium. It was a mix of several nutrient-mixes and filtered seawater. I prepared this mixture several times. The procedure is as follows:

A clean pressure chamber is filled with about 40 litres of freshly filtered sea water. Then exactly 40 ml of *Metal-Mix*, 40 ml of *Vitamin-Mix*, 40 ml of disodium-hydrogen-phosphate [Na_2HPO_4]-solution and 40 ml of sodium-nitrate [$NaNO_3$]-solution are added with help of *Eppendorf* analysis pipettes. Every few weeks these mixtures needed to be freshly prepared according to the recipe in the attachment [page 48].

Single nutrient ingredients of the medium from left to right: Na_2HPO_4, Metal-Mix, $NaNO_3$ and the Vitamin-Mix

Thereafter the pressure chamber is closed tightly and then connected to a filter system and a strong pump. The filter system is made up of two filters, a glass fibre filter [2,7 µm] and a cellulose filter [0,2 µm]. The pump is switched on, when all the hoses are securely connected. The pressure at the pump needs to be held between 400 and 600 mbar above atmospheric pressure. The finished nutrient solution is filled into four 10 litre bottles, which then are properly tagged with name and date. When the pressure chamber is empty, it is cleaned with demineralised water, the filters are exchanged and autoclaved. Now 40 litres of fresh culturing medium are finished and ready for use.



Picture [left] and sketch [right] of the most important step of the procedure: the pressure chamber is attached to the pump and the filter system. The finished medium is filled into four bottles.

Manual counting of *R. salina* cultures with a stereomicroscope

The easiest way of measuring the population density of *Rhodomonas salina* is manual counting through a stereomicroscope. At a magnification of 90x and fixation of the flagellates with Lugol's solution, they can be counted. In order as not to have to count a full millilitre of the *Rhodomonas* culture, which can easily contain about one million cells per millilitre, a counting chamber is used. This device is built like a microscope slide with 1 mm² big squares and a 1 ml high rim surrounding 1000 squares. This enables exactly 1 ml to fit inside the counting vessel. This millilitre is divided into 1000 microlitres. By counting one microlitre of solution [one square] and then multiplying the values by a factor of 1000, one achieves an approximation for the number of organisms per millilitre.

Because flagellates are very motile, due to their two flagellas, and often translucent, the sample needs to be treated with LUGOL's solution. This chemical contains complex I_5^--ions, that bind to starch and thereby to the pellicula of the *Rhodomonas*. Because these ions bind into the amylase-spirals, the pellicula changes colour into dark blue or black. This chemical reaction kills the cells and thereby immobilizes them, fixating the sample.

Because the microscope has a small depth of focus, the sample needs to rest about 10 minutes, before being examined. This allows all cells to evenly sediment on the floor of the counting chamber.

I counted ten squares of each sample every day, to gain a fairly accurate average population density. Counting is eased by the use of a clicker-counter.

Because the number of cells per microlitre is still very big, I diluted the samples one to ten or one to hundred depending on the estimated density of the *Rhodomonas* cultures.

After finishing the count, the counting chamber needed to be cleaned with demineralised water and ethanol.

FlowCam measurement of *R. salina* cultures

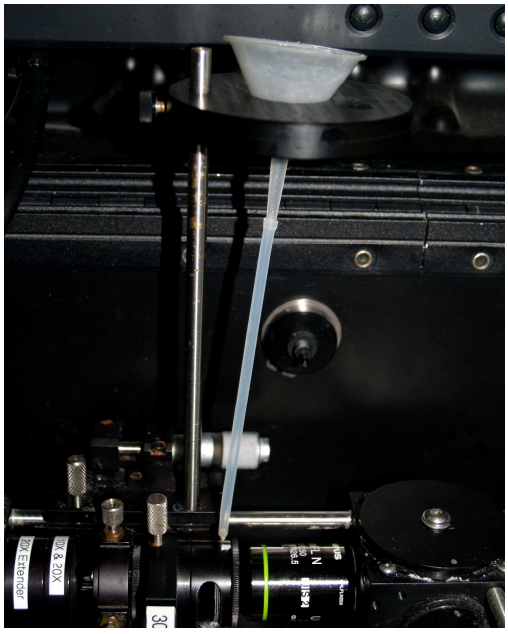
The FlowCam offered a very interesting measuring principle. This device analyzes size and number of particles in a certain volume of a fluid stream.

The sample is filled into a funnel attached to a hose system. With a peristaltic pump the water column is

Completely set up FlowCam

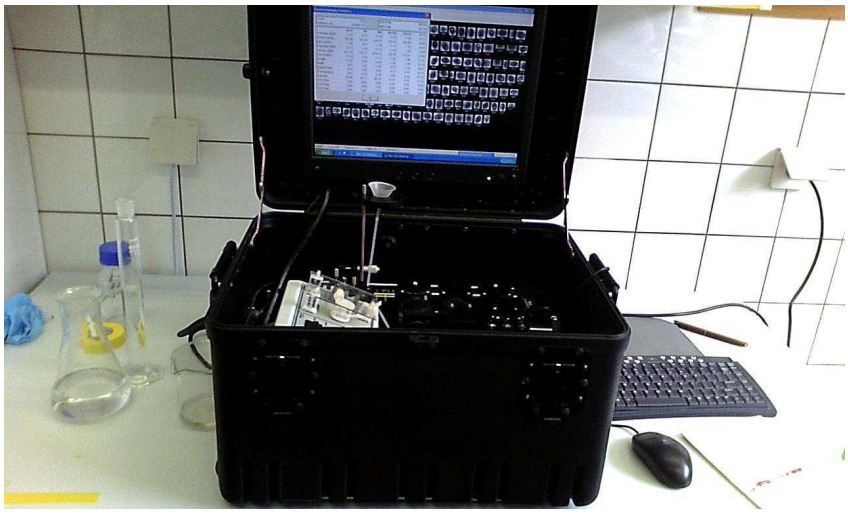
sucked through a thin glass chamber. This glass chamber is so thin, that cells can only pass through in a single layer. A laser beam detects each cell moving with the stream and thereby signals a microscopic camera to take a picture of each passing cell. The program measures the time needed for 1000 cells to pass through the glass chamber at a certain pump speed. Thereby the cell concentration is calculated. The FlowCam determines the size of the passing particles from the pictures.

Before starting the measurement of the samples, the FlowCam needs to be set up properly. First the box containing the measuring setup needs to be opened and a scaffolding for the funnel has to be screwed on top of the camera box. Then the funnel is inserted and connected to the open end of the hose. The pump is strung and set to the speed of 5. Now the power of the computer, monitor and pump are switched on. After entering the correct password, the pump is switched on and the cleaning cycles are prepared. Before doing a first cleaning cycle with demineralised water [about 5 ml], a waste vessel is placed under the end of the hose system. Thereafter a cleaning cycle with filtered sea water is done to create an isotonic environment for the *Rhodomonas* cells in the hose system. Now the first measuring cycles can begin.



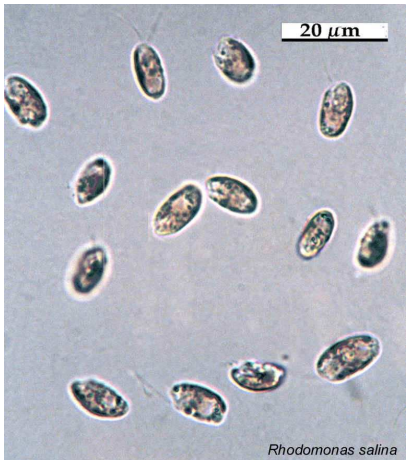
The measuring heart of the FlowCam with funnel, first part of the hose system and camera. The adjustment screws change focus and exposure of the camera.

After adding the first sample, the computer program is started and set up. The camera gain is adjusted to 700 and the computer set only to count cells between four and fourteen micrometers in size. Now the camera capture setting *AutoImageMode* is started and the program told to analyze 1000 cells. When measuring, a live view window of the passing cells is displayed on the



Picture of the FlowCam during the evaluation of the measurements. Popup window shows SummaryStatistics.

computer monitor. This is used by the supervisor to ensure proper focus and cleanliness. After 1000 cells have been counted, measuring is stopped and the pump can now be switched off. After picking the cells in the correct size limits [usually cells between 7 and 14 µm] all pictures shot of the passing cells are presented in a popup window. Because the program can't tell which particles it made pictures of, one has to delete all pictures, which are not supposed to be included in the calculations. All pictures that are insufficiently focussed or show dust particles or simply a empty glass chamber are deleted manually. Under the program setting *SummaryStatistics*, the population density and other measurement results are displayed. These can now be copied, but the results are also stored on the computer. After the first measuring cycle is now over, a second one can be started by switching on the pump and restarting the *AutoImageMode*. Five measuring cycles per sample are done before flushing the FlowCam with one cycle of sea water and then analyzing the second sample.



Picture showing Rhodomonas passing through the glass chamber of the FlowCam. Clearly visible are the two flagella of the uppermost cell.

When all measurements are done, the system needs to be cleaned. This is done with three cleaning cycles, one each with sea water, mucasol and demineralised water. Mucasol is a solvent for unpolar and fatty impurities. After this is accomplished the FlowCam maybe shut down, the funnel removed and the waste vessel cleaned. Now the box is closed and the current supply removed.

Measurement of *R. salina* cultures with the CASY-apparatus

CASY technology is an electric field multi-channel cell counting system. This apparatus measures cell number, size distribution, and cell viability quickly and reliably (www.roche-applied-science.com). These parameters are determined with the following method. Intact plasma membranes of cells [in this case *Rhodomonas salina*] act as electric isolators. A weak electric field is applied to a fraction of the analyzed sample. Because living cells exclude the electric

CASY cell-counting system

current, CASY can generate a three dimensional map of the sample. The holes in this map show the *Rhodomonas* cells. Size, volume and number of cells can now be determined by the machine. The system automatically calculates the population density per millilitre. The human influence on this apparatus is fairly low compared to the other methods. When measuring is finished, one only has to tell the device, which size of particles are supposed to be included in the calculations. Too small or too big particles naturally occurring in sea water thereby don't influence the results.

The measuring procedure is as follows:

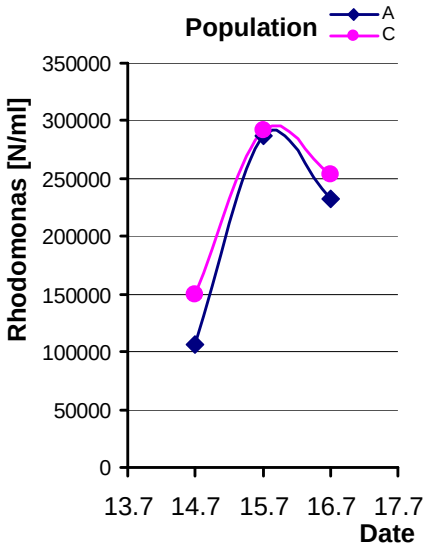
1. Switch on the machine and enter password.
2. Three cleaning cycles with CASYTON [isotonic salt water solution].
3. Fill measuring vessel with 100 µl of sample.
4. Add 4950 µl of CASYTON swiftly, thereby evenly distributing the cells throughout the measuring vessel.
5. Repeat step 4, as to achieve a 10 ml sample in the correct dilution.
6. One cleaning cycle.
7. Five measuring cycles of which the CASY system generates an average.
8. Determining of the to be counted particle sizes [adjustment of the vertical red bars visible in the upper picture].
9. Copy all achieved measuring results into the measuring book.
10. Repeat steps 7-9 two more times.
11. Start five cleaning cycles with a new measuring vessel containing 10 ml of pure CASYTON.
12. Shut down CASY and clean all used measuring vessels three times with tab water.

4) Results

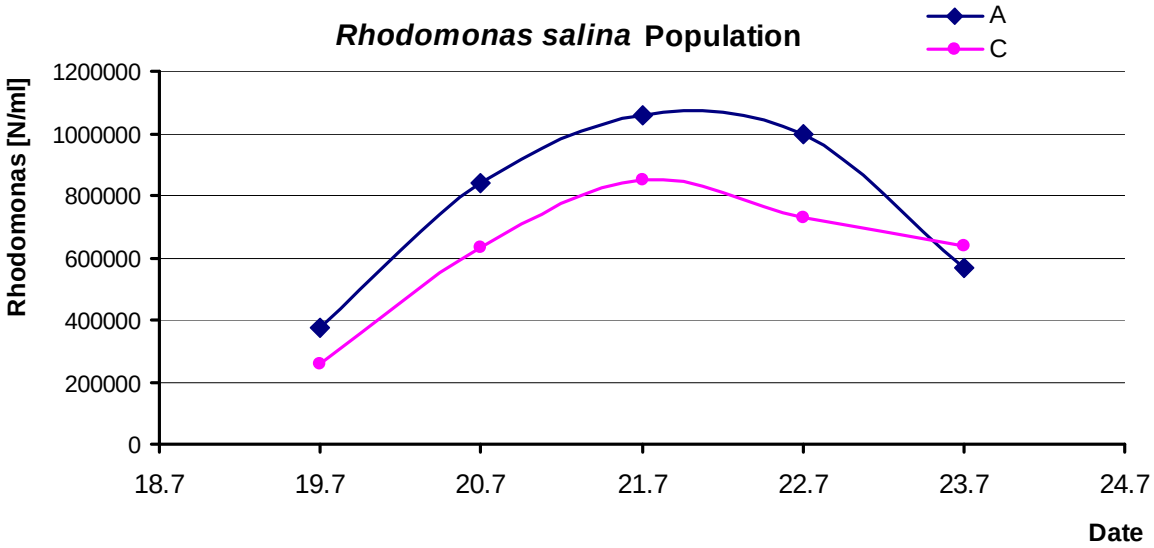
The measurements of two *Rhodomonas salina* cultures over a time span of two weeks are summarized in the next few pages.

Results: Stereomicroscope [Counting-Chamber]

I	14.07.2010		15.07.2010		16.07.2010	
	A	C	A	C	A	C
1	84	150	206	22	18	24
2	76	183	354	28	21	26
3	54	108	233	23	25	22
4	103	173	287	30	27	17
5	141	166	315	39	35	25
6	100	139	197	20	32	26
7	145	197	329	35	16	20
8	130	146	456	35	21	32
9	162	121	251	27	24	30
10	66	109	247	33	14	32
ø	106,1	149,2	287,5	29,2	23,3	25,4
N/ml	106100	149200	287500	292000	233000	254000

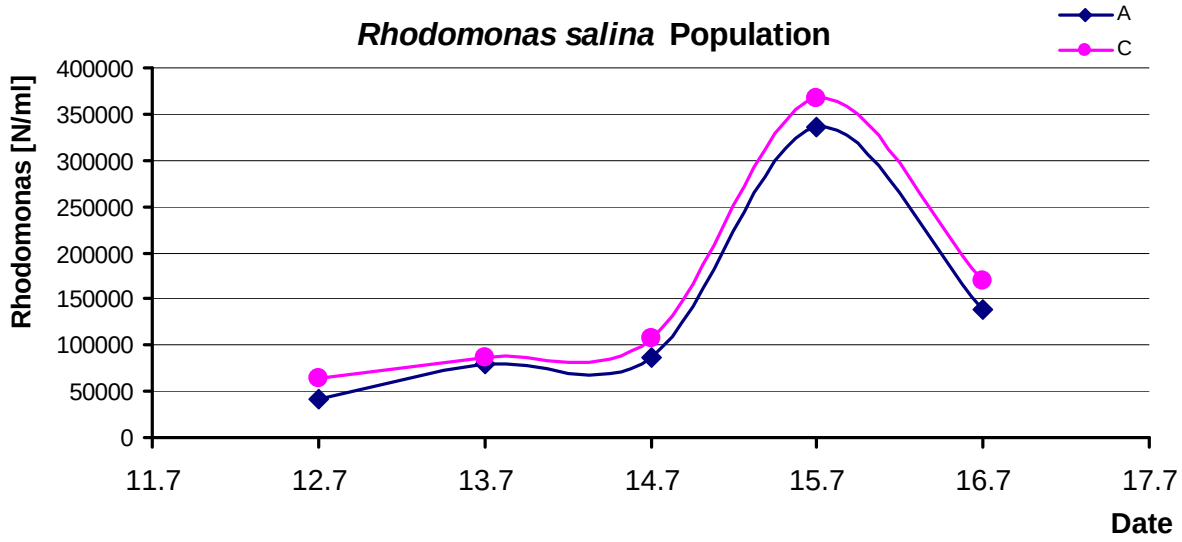


II	19.07.2010		20.07.2010		21.07.2010		22.07.2010		23.07.2010	
	A	C	A	C	A	C	A	C	A	C
1	26	13	86	62	103	69	119	73	38	62
2	15	36	80	68	94	92	91	65	62	67
3	56	21	58	48	124	88	125	65	56	71
4	51	32	95	62	87	86	87	82	53	79
5	45	21	105	56	128	102	102	89	66	76
6	38	42	75	50	98	87	135	69	48	66
7	44	20	47	68	109	111	98	76	46	34
8	27	36	116	64	107	80	79	62	77	65
9	37	18	85	84	132	59	74	77	51	50
10	36	20	95	72	76	75	86	73	68	67
ø	37,5	25,9	84,2	63,4	105,8	84,9	99,6	73,1	56,5	63,7
N/ml	375000	259000	842000	634000	1058000	849000	996000	731000	565000	637000

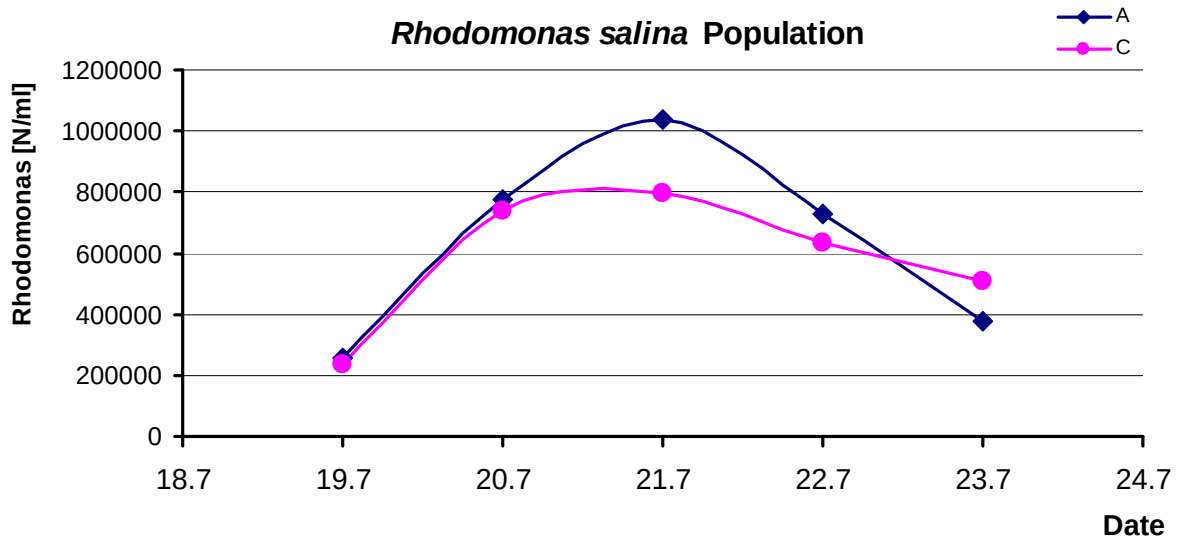


Results: FlowCam

I	12.07.2010		13.07.2010		14.07.2010		15.07.2010		16.07.2010	
	A	C	A	C	A	C	A	C	A	C
1	37461	64465	79973	91177	100762	97375	351786	421891	193385	167906
2	44499	69253	77657	95404	90080	99980	310310	367581	109956	165468
3		57857		70645	85044	103859	379249	366447	138594	151727
4					80729	111968	322982	322299	127279	174308
5					75837	126269	315368	353050	121399	185877
ø	40980	63858	78815	85742	86490	107890	335939	366254	138123	169057



II	19.07.2010		20.07.2010		21.07.2010		22.07.2010		23.07.2010	
	A	C	A	C	A	C	A	C	A	C
1	271339	198646	783971	728803	1198510	977268	944202	805998	377191	443959
2	267810	227254	776165	819782	1032132	783951	836107	800515	342901	505779
3	255265	244517	847971	679819	1006659	770830	635491	572309	367798	694109
4	248630	245302	652083	820593	985126	733752	643631	447417	379508	457508
5	245976	251282	820750	642000	966309	724931	586527	547009	419013	444621
ø	257804	233400	776188	738199	1037747	798146	729192	634650	377282	509195

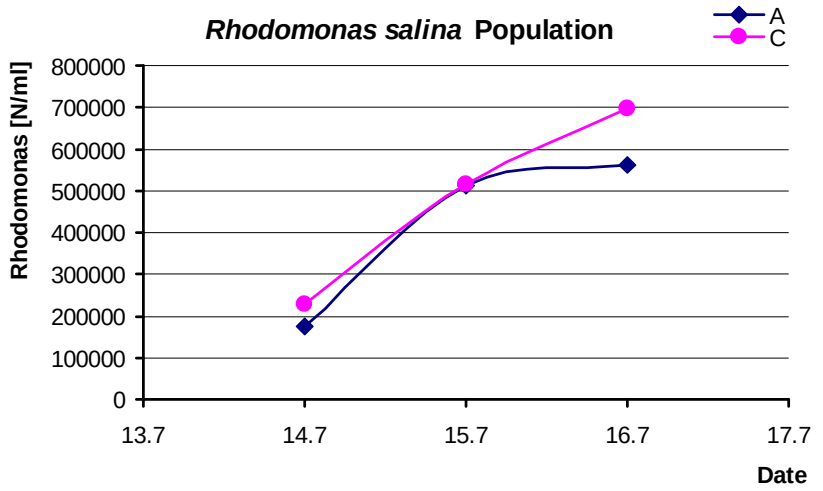


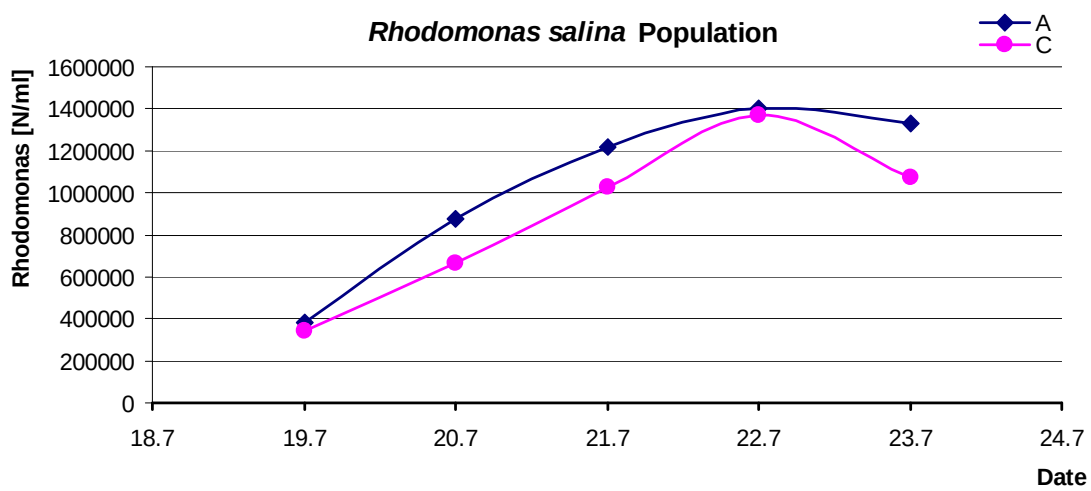
Results: CASY-apparatus

	14.07.2010		15.07.2010		16.07.2010		19.07.2010		20.07.2010		21.07.2010		22.07.2010		23.07.2010	
Sample	**A1**	**C1**	**A1**	**C1**	**A1**	**C1**	**A1**	**C1**	**A1**	**C1**	**A1**	**C1**	**A1**	**C1**	**A1**	**C1**
Counts	1656	2153	5494	4718	5044	6459	3894	3367	8564	6081	10972	9783	12619	11981	12074	9364
Counts/ml	175200	227800	581200	499100	533600	683300	411900	356200	906000	643300	1161000	1035000	1335000	1267000	1277000	990600
Total Counts/ml	737400	813300	1208000	1620000	1901000	2146000	1548000	1372000	2522000	2280000	3722000	3894000	3258000	3960000	4036000	3135000
% Counts	23,80%	28%	48,10%	30,80%	28,10%	31,80%	26,60%	26,00%	35,90%	28,20%	31,20%	26,60%	42,30%	32,00%	31,70%	31,60%
Volume/mL [fl]	136300000	163400000	364400000	324000000	284500000	329400000	165000000	141700000	380100000	263800000	594100000	453400000	740200000	573700000	676400000	462200000
Mean Diameter [µm]	11,31	11,04	10,49	10,68	10,02	9,63	9,04	9,04	9,20	9,20	9,82	9,36	10,10	9,43	9,98	9,55
Peak Diameter [µm]	11,34	10,87	10,20	10,74	9,98	9,25	8,90	8,74	9,01	9,10	9,61	9,18	9,81	9,38	9,90	9,41
Mean Volume [fl]	778,30	717,30	626,90	649,10	533,20	482,10	400,40	397,80	419,60	417,00	511,90	438,10	554,50	452,70	529,60	466,60
Peak Volume [fl]	763,80	673,40	555,00	648,80	520,10	415,00	369,00	349,60	383,20	394,20	465,10	405,20	493,70	432,30	508,00	422,20
Sample			**A2**	**C2**	**A2**	**C2**	**A2**	**C2**	**A2**	**C2**	**A2**	**C2**	**A2**	**C2**	**A2**	**C2**
Counts			3771	4892	5241	6859	3708	3106	8099	6275	11581	9901	13849	13091	13263	9887
Counts/ml			398900	517500	554400	725600	392300	328600	856300	663800	1225000	1047000	1465000	1385000	1403000	1046000
Total Counts/ml			1079000	1631000	1825000	2139000	14440000	1378000	2583000	2431000	3860000	3740000	3128000	3579000	4041000	3525000
% Counts			37%	31,70%	30,40%	33,90%	27,20%	23,80%	33,20%	27,30%	31,70%	28,00%	46,80%	38,70%	34,70%	29,70%
Volume/mL [fl]			244600000	336400000	284100000	349200000	163000000	133800000	364500000	275500000	693200000	443600000	810800000	626800000	715400000	466800000
Mean Diameter [µm]			10,45	10,64	9,86	9,63	9,16	9,12	9,24	9,20	10,18	9,24	10,10	9,43	9,82	9,39
Peak Diameter [µm]			10,18	10,66	9,82	9,26	8,91	8,87	9,15	9,01	9,88	9,03	9,92	9,38	9,73	9,45
Mean Volume [fl]			613,10	650,00	512,50	481,20	415,60	407,30	425,40	415,00	565,80	423,50	553,40	452,60	509,90	446,30
Peak Volume [fl]			552,80	634,90	495,60	416,30	369,80	365,60	401,40	483,30	505,10	386,00	511,10	431,40	482,00	441,40
Sample			**A3**	**C3**	**A3**	**C3**	**A3**	**C3**	**A3**	**C3**	**A3**	**C3**	**A3**	**C3**	**A3**	**C3**
Counts			5307	5043	5617	6483	3274	3183	8222	6502	11970	9361	13319	13769	12421	11130
Counts/ml			561400	533500	594200	685800	343500	336700	869800	687800	1266000	990300	1409000	1457000	1314000	1177000
Total Counts/ml			1204000	1648000	1793000	2143000	1591000	1408000	2707000	2432000	3852000	3666000	3031000	3898000	4058000	3360000
% Counts			46,60%	32,40%	33,10%	32%	21,60%	23,90%	32,10%	28,30%	32,90%	27,00%	46,50%	37,40%	32,40%	35,00%
Volume/mL [fl]			327800000	345300000	310700000	325900000	139300000	135500000	365500000	272000000	702700000	427200000	773900000	683800000	69480000	595400000
Mean Diameter [µm]			10,25	10,64	9,94	9,59	9,08	9,08	9,20	9,04	10,10	9,32	10,06	9,55	9,94	9,82
Peak Diameter [µm]			9,98	10,55	9,92	9,21	8,78	8,80	9,14	9,01	9,85	9,04	9,92	9,49	9,77	9,81
Mean Volume [fl]			584,00	647,20	522,90	475,10	405,40	402,30	420,20	395,40	555,00	431,40	549,20	469,40	528,70	505,60
Peak Volume [fl]			520,40	615,60	510,50	409,10	345,50	356,40	399,50	382,60	500,80	386,60	511,00	447,50	488,60	494,10

AVERAGES	14.07.2010		15.07.2010		16.07.2010		19.07.2010		20.07.2010		21.07.2010		22.07.2010		23.07.2010	
Sample	A1	C1	A1	C1	A1	C1	A1	C1	A1	C1	A1	C1	A1	C1	A1	C1
Counts	1656	2153	4857	4884	5301	6600	3625	3219	8295	6286	11508	9682	13262	12947	12586	10127
Counts/ml	175200	227800	513833	516700	560733	698233	382567	340500	877367	664967	1217333	1024100	1403000	1369667	1331333	1071200
Total Counts/ml	737400	813300	1163667	1633000	1839667	2142667	5859667	1386000	2604000	2381000	3811333	3766667	3139000	3812333	4045000	3340000
% Counts	23,80%	28%	0,44	0,32	0,31	0,33	0,25	0,25	0,34	0,28	0,32	0,27	0,45	0,36	0,33	0,32
Volume/mL [fl]	136300000	163400000	312266667	335233333	293100000	334833333	155766667	137000000	370033333	270433333	663333333	441400000	774966667	628100000	487093333	508133333
Mean Diameter [µm]	11,31	11,04	10,40	10,65	9,94	9,62	9,09	9,08	9,21	9,15	10,03	9,31	10,09	9,47	9,91	9,59
Peak Diameter [µm]	11,34	10,87	10,12	10,65	9,91	9,24	8,86	8,80	9,10	9,04	9,78	9,08	9,88	9,42	9,80	9,56
Mean Volume [fl]	778,30	717,30	608,00	648,77	522,87	479,47	407,13	402,47	421,73	409,13	544,23	431,00	552,37	458,23	522,73	472,83
Peak Volume [fl]	763,80	673,40	542,73	633,10	508,73	413,47	361,43	357,20	394,70	420,03	490,33	392,60	505,27	437,07	492,87	452,57

Diagrams of the development of the population density of two *Rhodomonas salina* cultures



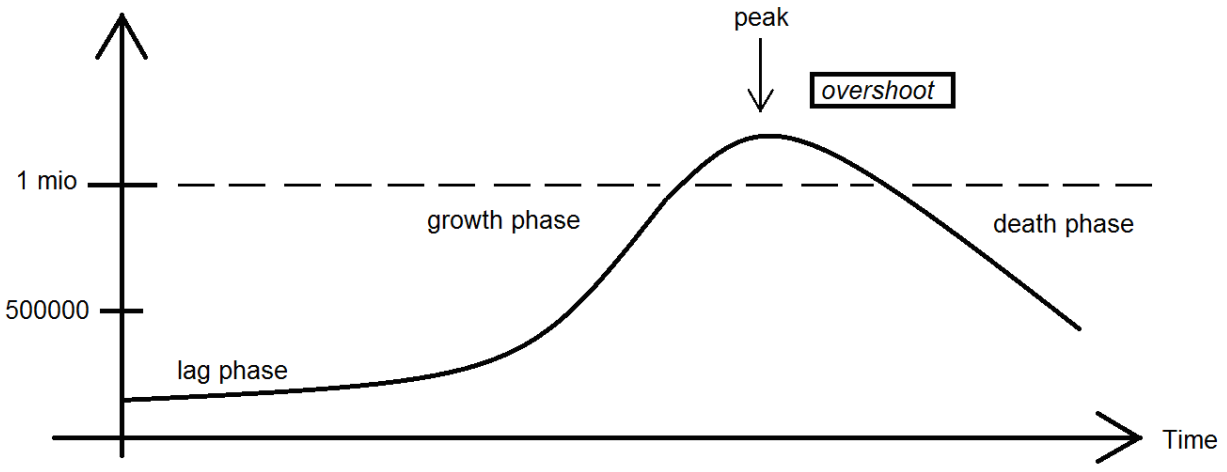


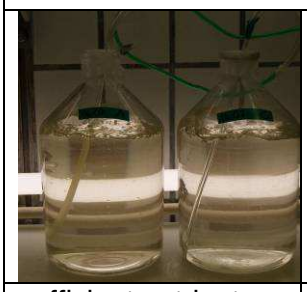
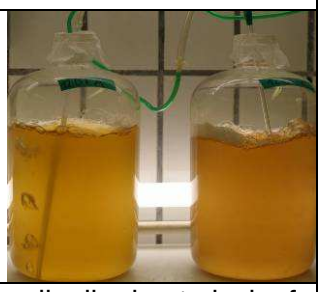
Explanation of these development graphs of *Rhodomonas salina* populations

Rhodomonas salina are biological r-strategists, producing large amounts of daughter cells in short time periods through binary fission. This exponential population growth is halted, when the growth environment, in this the medium runs out of nutrients or turns toxic. In this case the dinoflagellate population keeled over, due to undersupply with nitrogen [colour changes from dark red to green-yellow]. This usually happens in these cultures. Normally the nutrient supply of the medium suffices for population growth ending with a population density of one million cells per ml. But sometimes the population also keels over earlier. It also takes some generations, until the population totally recovers. Therefore it is best to use the dinoflagellates before the population keels over due to nitrogen undersupply. It however isn't advisable to add more sodium-nitrate to the medium in advance, because a too high concentration of sodium-ions can negatively effect enzyme reactions or otherwise disturb the comparability of achieved measurements.

When the culture is restarted, either because the dinoflagellates were used for experiments, or because the population keeled over, some remaining cells of the old culture [about 250 ml] are used as starter cells. This amount of cells is poured into new 5L-glass-bottles. Then these new bottles are tagged with the name of the culture and the starting date. Now the bottles are filled with about 5L of fresh medium, sealed with a small square of *PARAFILM* [sturdy stretchable plastic foil] and then reattached to the oxygen supply hose system. Old bottles are cleaned with fresh water, aqua dest. and autoclaved.

In conclusion, the ideal growth curve should look like the schematic drawing next page: With a short starting phase followed by a longer exponential [$N_{new}=N_{old}*2^{x*t}{}_{fission}$] phase, slacking at the approach of the population density of one million cells per ml [or 5 000 000 000 cells in 5L], and then suddenly keeling over and descending rapidly, as the nitrogen supply runs out [limit of one million cells per ml applies only to this medium and a volume of five litres].



			
sufficient nutrients; starting cells recover and grow to full size before binary fission	fastest growth rate; cells multiply and nutrients diminish	growth rate almost zero; first nutrient [nitrate-ions] used up	cells die due to lack of nitrogen → culture *keels over*

5) Conclusions

According to the achieved results I evaluated the different measuring methods. Each method has its advantages and disadvantages.

One of the criteria considered is the required time for each measurement. This criteria is important because scientists always works on a tight schedule. The earlier a project is finished, the less costs are incurred, or more research done. The method requiring the most time is the FlowCam-measurement. This measuring method took an average of 75 minutes [2x20 min flushing, 10x3min measuring, 5 min flushing between different samples]. The manual counting through the stereomicroscope required 15 minutes for the preparation, fixation, dilution and sedimentation per sample. The counting of 1 µl took an average of one minute. This makes a total of 50 minutes. Using the CASY-apparatus was even shorter. Flushing and one measurement take 5 minutes. One additional minute for copying the results needs to be added. With 2x3 measurements and two additional cleaning cycles this makes a

sum total of 35 minutes. In conclusion rank number one goes to CASY, second place to manual counting and third rank to the FlowCam.

The second criteria I evaluated by, is the accuracy of the measurement. For good research results accuracy of measurements is vitally important. Accurate results form the basis of every scientific work. Comparing the measured results to the colour-intensity of the measured samples, and also the measured results among each other, I arrived at the following conclusions.

Manual counting is very inaccurate, because it requires working with dilutions and therefore only achieves results with an accuracy of the thousands digit. Another problem is, that the cells rarely are evenly distributed in the counting chamber, and that cells can easily be overlooked when counting. Moreover although 15 minutes are left for the sample to settle in the counting chamber not all cells sediment, and therefore not all can be counted. Another difficulty of the trustworthiness is the fact, that single counts vary greatly between in one case 206 and 456 cells in one µl. That is more than double the first value! Amazingly however the achieved results generally tend to fit to the FlowCam's results. Nevertheless the accuracy is poor, but sufficient for this purpose, because only the general magnitude plays a role in the surveillance of the cultures.

FlowCam results have an astounding accuracy, because each counted object is photographed and therefore can be verified by the user. Thereby making it possible to exclude, that dust particles, air bubble or bigger salt particles from the calculations. This method also fulfils a higher surveillance quality, because through the photographic evaluation researchers can check, if any other organisms such as diatoms have managed to infiltrate the culture. High cell-numbers however tend to give problems to this apparatus, because too many cells pass through the counting chamber, whereby not all can be registered by the laser. However all measurements are close together with no real breakaway-values.

The CASY apparatus is supposed to be very accurate due to it's sophisticated measuring technology, enabling to tell alive and dead cells apart. Moreover CASY also offers interesting information on size development of the cells as well as the standard mistake of the measurement. Why the achieved results vary from the other two methods, is unexplainable. The measurements tend to be rather bigger than the real values. One reason for this could be that the majority of the cells are located at the bottom of the counting vessel, from where the measured sample is extracted.

Even by adding the CAYSTON with ample pressure and speed doesn't completely solve the problem of even cell distribution. Yet this almost fully-automated measuring method is more accurate than manual counting, because for one value, the machine measures five times and calculates an accurate average. In total the overall average is generated from 15 values. The other strong point in accuracy is, that CASY has an integrated quality control, that registers air bubbles or other mistake-sources automatically and then repeats the affected measurement, thereby avoiding values differing too far from reality. This is also the main reason, why all measured values are very close together, thereby having a small standard mistake.

Moreover the money spent on each method also plays a role in daily research. Money often is the limiting factor in institutes and universities. The least expensive method is the manual counting method with counting chamber, LUGOL's solution and a stereomicroscope. All these requirements are usually openly available at every university or research institute. While this method only costs about 1€ per measurement [chemicals for fixation and cleaning], the other two are a bit more pricy. A ten year old FlowCam [estimated age] is worth $40000 (www.mainebiz.biz). A new device from *Fluid Imaging* costs $85000. Moreover for the daily cleaning cycle 5 ml of mucasol are used up. With an estimated life-time of 30 years and daily measuring [not counting weekends], one measurement costs: 5€ [$40000/1,27 = 31500€; 31500€/(260*30) + 1€ for mucasol]. That is five times more expensive the manual counting. The CASY apparatus costs £4500 [4 year old used bargain (www.helixtechnologies.co.uk)]. With a life time of 20 years and daily measuring [not counting weekends] one measurement of the *Rhodomonas salina* cultures costs 3€ [4500/0,82 = 5500€; 5500€/(260*20) + 2€ for CASYTON]. This is unexpectedly cheap for this excellent machine.

I have summarized these conclusions in this small table, to achieve a good overview of the advantages and disadvantages of the different methods. Three stars symbolize good results, two average and one star poor evaluation.

	Manual Counting		FlowCam		CASY	
Time/Measurement	50 min	**	75 min	*	35 min	***
Cost/Measurement	1 €	***	5 €	*	3 €	**
Accuracy	poor	*	very high	***	high	**
Total	6/9	******	5/9	*****	7/9	***** **

Apparently the CASY apparatus is the best suited solution. Although the FlowCam has a longer life time, more accurate results and can be repaired easily due to simple mechanical concepts, it takes irrationally long. Manual counting isn't very entertaining, quite inaccurate and only advisable for low-budget project and education. In the long term it apparently really pays off to invest into modern technology.

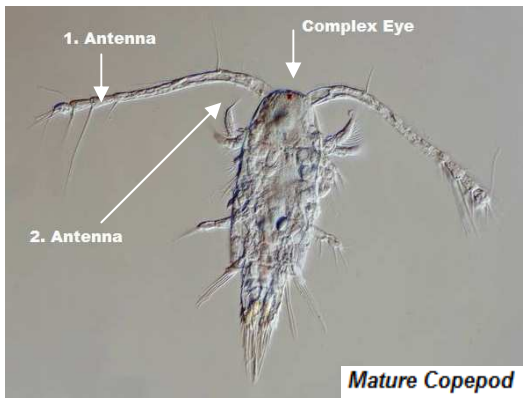
IV. My own project: predator-prey interactions between *Acartia tonsa*, *Artemia salina* and *Rhodomonas salina*

1) Introduction: The importance of copepod research

Ecologists often describe interactions between different species. The most common of these linkages is predation, where one species [predator-species] feeds on the other [prey-species], making it a "+/-" interaction, only profiting the predator-population. These predator-prey linkages are the prime movers of energy through food chains and are an important factor in the ecology of populations, determining mortality of prey and birth of new predators.

In marine food-webs copepods and algae are key elements. Representing the step from primary production [autotrophic] to secondary production [heterotrophic], these organisms provide the energy-basis for all other marine animals such as fish, crabs and jellyfish. Because of this copepods are often used as life food in aquariums.

Copepods [picture on the right (Franz Neidl)] are small crustaceans and are often believed to be the most abundant group of metazoans in the world, thereby constituting the biggest source of protein in the oceans. *Copepoda* consists of 10 orders including about 14 000 described species. In average copepods are between 0,2 and 2 mm long. Their main characteristics are two pairs of antenna, of which the first is larger and has bristles, a single complex eye [red] and of course their "oar feet", giving the group its name, which is especially recognizable in the German term "Ruderfußkrebs" meaning as much as "rowing-feet-crustaceans". This key element of copepod-structure enables these crustaceans to move at speeds up to 90 meters [or 45 000

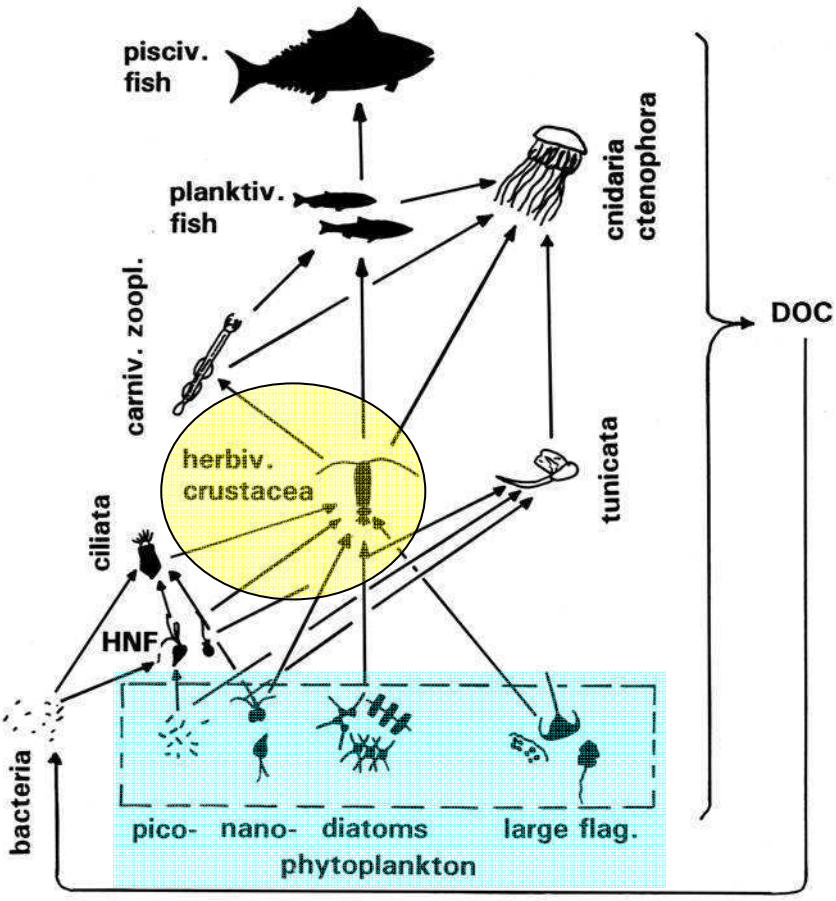


body lengths] per hour. However, these crustaceans don't only eat algae, but also zooplankton. In the lab marine copepods sometimes prey on smaller crustaceans such as brine shrimps [*Artemia*].

Copepods also have another very important feature: They produce countless faecal pellets contributing greatly to the marine snow and therefore accelerating the flow of nutrients and minerals from surface waters to the bottom of the seas.

Another interesting current research topic is the fact, that copepods often carry bacteria, such as *Vibrio*-species like *Vibrio parahaemolyticus* or *Vibrio cholera*, that can function as waterborne pathogens. This transport mechanism is an interesting research topic being worked on all over the world, because it enables dangerous bacteria to spread over huge distances. One of the prime researchers of this topic is Dr. Jaime Martinez-Urtaza from the *University of Santiago de Compostela*. Dr. Martinez-Urtaza presented his current research results at the BAH on the 7th of July.

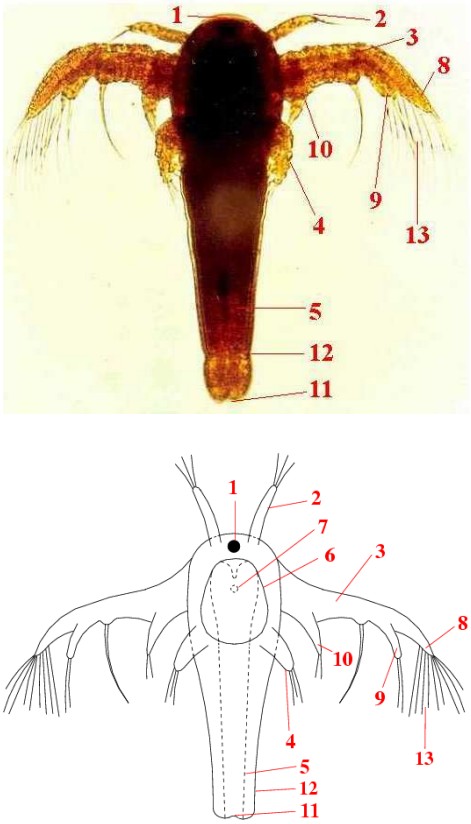
In conclusion there are various important reasons for the research of marine copepods and especially their interactions in food webs [image below].



Marine foodweb interactions; copepods mostly function as herbivores
DOC: disolved organic carbon HNF: heterotrophic nanoflagellates

Brine shrimp such as *Artemia salina* also play an important role in nature and aquaria. This crustacean zooplankton species has been around long before the dinosaurs age. This long survival is mainly due to their great tolerance of salinity and their ability to lay long-term cysts. These eggs differ from the normal thin-shelled eggs, normally produced in life-friendly habitats. Cysts are thick-shelled eggs, that can remain in a dormant stage for several years and thereby survive dry and oxygen-poor habitats, before hatching when placed in aquatic habitats again. These eggs are only produced when the salinity increases rapidly, thereby indicating the drying out of the habitat. Artemia salina cysts are sold in almost every aquarist to provide life food [nauplii stage] for fish larvae. Mature *Artemia* can withstand salinities as high as 340 g/l, approximately 10 times the salinity of sea water, and a very low dissolved oxygen level as low as 1,5 ml/l, being less than half the 5 ml/l typical of surface seawater (Abatzopoulos *et al* 2002). Artemia salina have three eyes and the ability to change colour according to the salinity. Whilst being dark red in high salinity waters, Artemia salina are pale-greenish in low salinity areas such as river mouths (www.bachflohkrebse.de).

Young brine shrimp larvae are about 0,5 mm long and usually feed on planktonic algae, bacteria and detritus in wild habitats. In captivity the nauplii are cultured with yeast powder.



Basic anatomic structure of *Artemia salina* meta-nauplius [3 days old]

Picture of an Artemia salina copepod nauplius [top] and schematic drawing of the most important characteristics [bottom]. Scientific terms explained in the table below.

#	organ	additional information
1	naupliar eye	dark, red or black median eye; consists of 3 pigment cup ocelli; the brain surrounds the eye
2	antenna 1	also called: antennulae
3	antenna 2	primary swimming organs; equipped with swimming setae
4	mandible	the mandibles possess peripheral incisors and blunt-crowned teeth used for mechanical processing of filtered food particles; they move laterally to grind food
5	gut	digestive tract
6	labrum	used for holding food in position for mastication and swallowing
7	mouth	
8	exopod	part of antennae 2; equiped with chief swimming setae
9	endopod	part of antennae 2
10	endite	part of antennae 2
11	anus	
12	telson	region where new body segments are produced as the nauplius matures
13	swimming setae	

pictures from www.captain.at

2) Project Information

Due to the vital importance of copepod research, I had the idea to find out, if copepods rather feed on phytoplankton or zooplankton. Phytoplankton is hereby represented by the cryptophyceae dinoflagellate *Rhodomonas salina*, while small *Artemia salina* nauplii represent the zooplankton diet. The predator representing marine copepods is *Acartia tonsa*. To answer this question I performed several independent experiments.

On the 16th of July I fed different numbers of *Acartia tonsa* with *Rhodomonas salina* [R-series], *Artemia salina* [A-series] and a combination of both [W-series]. The copepods were given 5 hours to feed on their prey [10:00 am- 03:00 pm] at 18℃. The W-series was given an additional hour. All organisms were held in small Petri dish filled with 5 ml of artificial sea water. Each Petri dish had an identical twin to verify results. One ml of *Artemia salina* culture were added to the A-series Petri dish. R-series Petri dish were supplied with one ml of *Rhodomonas salina* culture. All remaining Petri dish were given one ml of each food source.

Five ml of artificial sea water are filled into the different Petri dish with an Eppendorf *analysis pipette.*

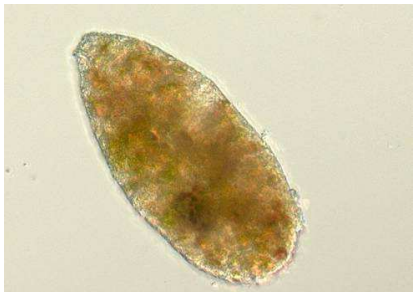
Unexpectedly I observed an interesting scientific phenomenon, which I researched in further experiments on the 19th and on the 21st of July. These experiments are described on page 36.

3) Material and Methods

This scientific project did not need many different methods. Besides counting marine copepods, dinoflagellates and brine shrimp and the proper use of a stereomicroscope with corresponding camera adaptation, the only methodical challenge was the production of small Petri dish to hold the zooplankton.

Counting zooplankton accurately in large numbers inside pipettes

A large part of three days I spent counting mature marine *Acartia tonsa* copepods for Stefanie's research project. The task was to count and fill 1, 2, 4, 8, 16, 32, 64, 128, 256 and 512 copepods into vessels of different sizing. They would then be fed with 1 ml *R. salina* per copepod and thereafter let one hour to produce faecal



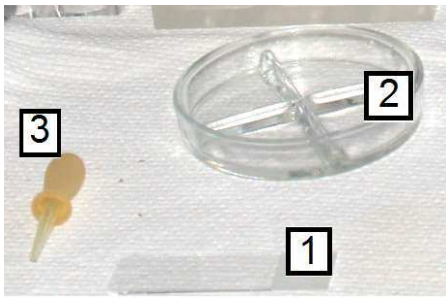
Copepod faecal pellet [stack of 16 pictures].

pellets which amount would then be measured in the seawater.

Copepod tank in the climate chamber. These marine crustaceans are fed with Rhodomonas salina. *Fully grown, they are fished out with a 150 µm filter [on rack above tank].*

The first step was to get the mature copepods out of their culturing tanks. This is best done with a 150 µm filter. This filter is moved in figure-eights through the tank, thereby collecting about 2000 to 3000 copepods on its thin mesh. These copepods would then be rinsed into a big beaker with freshly prepared artificial seawater. After cleaning the filter with tab water, the beaker is placed in front of a strong light source. Because *Acartia tonsa* swim toward light, the majority of the small crustaceans is collected on one side of the beaker. From this concentrated zooplankton solution, a small amount is transferred into a flat dish. When the target vessels are fully prepared according to the table below, the counting can begin.

Number	*Acartia tonsa*	Volume artificial sea water in ml	Volume of concentrated *Rhodomonas salina* solution in ml
1	1	0,5	1
2	2	0,5	2
3	4	1	4
4	8	1	8
5	16	2	16
6	32	2	32
7	64	3	64
8	128	3	128
9	256	5	256
10	512	5	512



Counting equipment: [1] pipette for counting up to 10 copepods; [2] flat dish for concentrated copepod-solution; [3] microscope slide for separation of too big copepod numbers into small groups

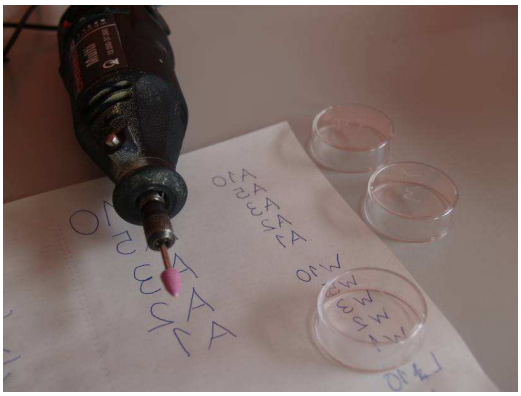
Copepods can be counted inside a small pipette. Due to their high jumping motility however, larger groups need to be separated into smaller, easily countable groups on a microscope slide.

As not to forget the current number of moved copepods, a cash-register like clicker is used to keep count. Counting the sum total of 1025 copepods alone takes about 4 hours of concentrated work. This work can't be paused, as to avoid the starvation of the copepods inside the vessels [*Rhodomonas salina* added after all are counted].

This method was also necessary for my own research work, although I worked with smaller numbers. Brine shrimp are counted the same way. They however are even smaller than the 1 mm sized *Acartia tonsa*.

Preparation of special zooplankton Petri dish

The experiment took part in small Petri dish. I labelled these with a special technique making it possible to tell the different Petri dish apart. For this purpose I engraved an inscription in to the bottom of the Petri dish. As not to cause rough surfaces on the inside which would possibly influence the zooplankton, I drilled into the outwards facing bottom. This engraving therefore needed to be done in mirror-writing. To ease this work, I prepared a template. In this fashion I prepared similar culture dish for a fresh jellyfish medusa culture, using the corresponding Petri dish tops.



Pictures on the right: tools and template for the inscription of the Petri dish [top]; finished Petri dish ready for use seen from above [bottom]

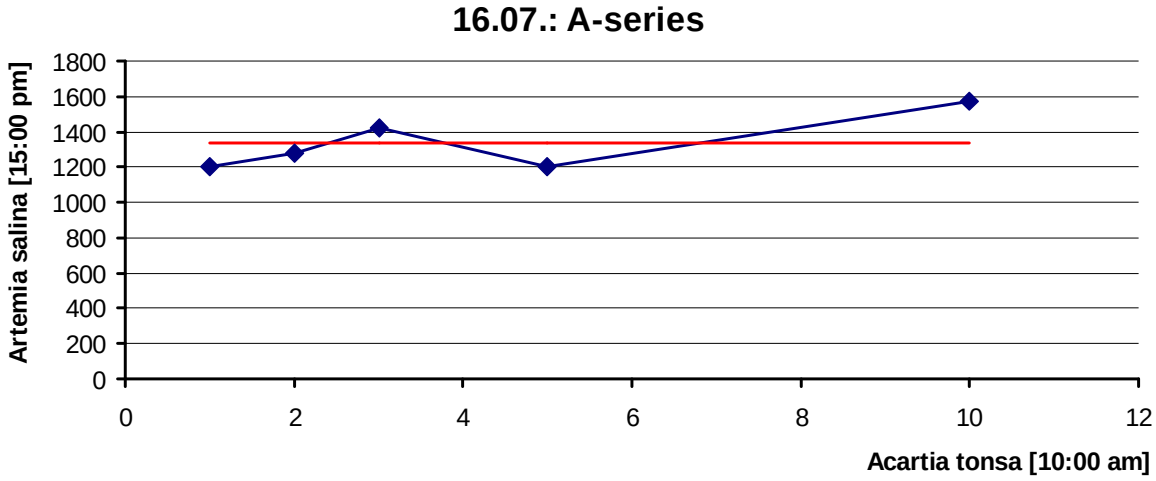
4) Results

All observed and measured data are summarized in the following pages.

Experiment I: grazing preferences of *Acartia tonsa* [16th July]

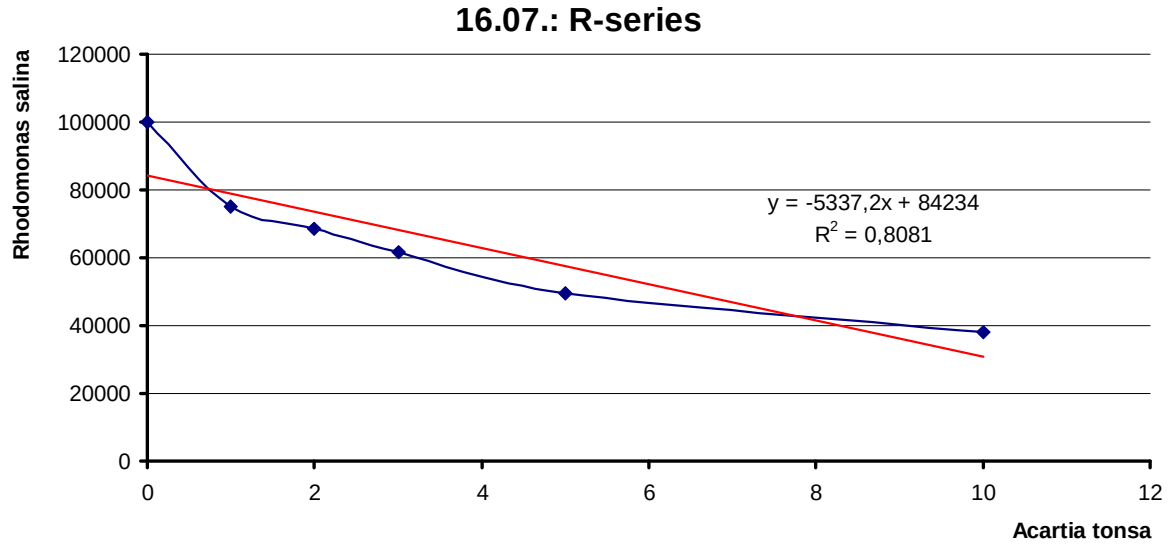
A		Start 10:00 am		Results 03:00 pm									
		Acartia tonsa	*Artemia salina*	*Acartia tonsa*		*Artemia salina*							
				alive	dead	1	2	3	4	⌀ N/µl	⌀ N/µl	⌀ N/ml*	1 ml → 6 ml
A1	**1**	1	1 ml	0	1	2	0	2	3	1,75			
	2	1	1 ml	0	1	2	2	4	1	2,25	2,00	200	1200
A2	**1**	2	1 ml	0	2	2	4	0	3	2,25			
	2	2	1 ml	0	2	3	0	4	1	2,00	2,13	212	1275
A3	**1**	3	1 ml	0	3	1	2	3	4	2,50			
	2	3	1 ml	0	3	3	2	4	0	2,25	2,38	237	1425
A5	**1**	5	1 ml	2	3	2	3	0	2	1,75			
	2	5	1 ml	0	5	2	4	2	1	2,25	2,00	200	1200
A10	**1**	10	1 ml	1	9	5	2	0	4	2,75			
	2	10	1 ml	2	8	1	4	3	2	2,50	2,63	262	1575

Note: All A-series Petri dish were provided with 1 ml of *Artemia salina* solution containing in average 1500 cells. The results vary, because not every Petri dish got exactly the same number of cells. Average is shown in the diagram [red line]. Dilution calculation indicated by *.



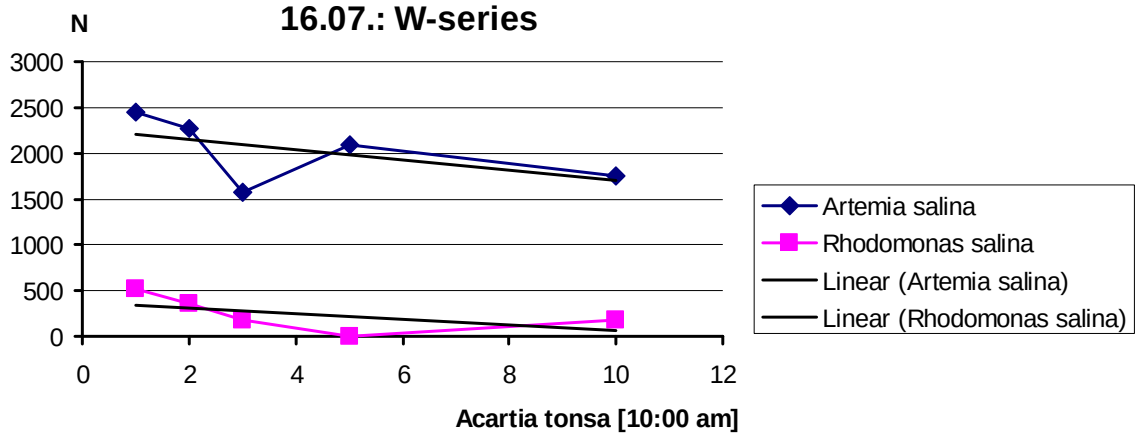
R		Start 10:00 am		Results 03:00 pm									
		Acartia tonsa	*Rhodo. salina*	*Acartia tonsa*		*Rhodomonas salina*							
				alive	dead	1	2	3	4	⌀ N/µl	⌀ N/µl	⌀ N/ml*	1 ml → 6 ml
A1	**1**	1	1 ml	1	0	139	124	128	101	123,00			
	2	1	1 ml	1	0	118	131	137	124	127,50	125,25	12525,0	75150
A2	**1**	2	1 ml	2	0	110	137	102	115	116,00			
	2	2	1 ml	2	0	124	89	128	110	112,75	114,38	11437,5	68625
A3	**1**	3	1 ml	3	0	114	108	90	112	106,00			
	2	3	1 ml	3	0	107	110	87	96	100,00	103,00	10300,0	61800
A5	**1**	5	1 ml	5	0	56	99	92	86	83,25			
	2	5	1 ml	4	1	62	78	90	99	82,25	82,75	8275,0	49650
A10	**1**	10	1 ml	9	1	74	70	55	65	66,00			
	2	10	1 ml	10	0	54	60	70	60	61,00	63,50	6350,0	38100

Note: All R-series Petri dish were provided with 1 ml of *Rhodomonas salina* solution containing in average 100000 cells. These however are more equally distributed because, they don't move as quickly as *Artemia* and also don't usually form clusters. Linear trend line is shown in the diagram. Dilution calculation indicated by *.



W		Start 10:00 am			Results 04:00 pm								
		Acartia tonsa	*Rhodo. salina*	*Artemia salina*	*Acartia t.*		*Artemia salina*						
					alive	dead	1	2	3	4	⌀ N/µl	⌀ N/ml*	1 ml → 7 ml
W1	**1**	1	1 ml	1 ml	1	0	4	3	2	5	3,50	350	2450
W2	**1**	2	1 ml	1 ml	2	0	4	3	2	4	3,25	325	2275
W3	**1**	3	1 ml	1 ml	3	0	2	0	4	3	2,25	225	1575
W5	**1**	5	1 ml	1 ml	5	0	4	5	2	1	3,00	300	2100
W10	**1**	10	1 ml	1 ml	9	1	0	5	2	3	2,50	250	1750
		Acartia tonsa	*Rhodo. salina*	*Artemia salina*	*Acartia t.*		*Rhodomonas salina*						
					alive	dead	1	2	3	4	⌀ N/µl	⌀ N/ml*	1 ml → 7 ml
W1	**1**	1	1 ml	1 ml	1	0	0	1	2	0	0,75	75,0	525
W2	**1**	2	1 ml	1 ml	2	0	1	0	0	1	0,50	50,0	350
W3	**1**	3	1 ml	1 ml	3	0	0	0	0	1	0,25	25,0	175
W5	**1**	5	1 ml	1 ml	5	0	0	0	0	0	0,00	0,0	0
W10	**1**	10	1 ml	1 ml	9	1	0	0	0	1	0,25	25,0	175

Note: All W-series Petri dish were provided with 1 ml of *Rhodomonas salina* solution containing in average 100000 cells and 1 ml of *Artemia salina* solution containing in average 2000 cells [New solution because old one didn't suffice; therefore new counting and different density]. Linear trend lines are shown in the diagram. Dilution calculation indicated by *.



		Additional observations that can't be expressed in numbers
A2	**1**	*Acartia tonsa* remains are devoured by three *Artemia salina* [sketch in 'discussion']
A5	**1**	Two *Acartia tonsa* aid each other to kill *Artemia salina*
A10	**2**	Two *Artemia salina* nibble on dead *Acartia tonsa* remains
R1	**1**	*Acartia tonsa* very active; many jerky movements
R2	**1**	*Acartia tonsa* dark bellied
R3	**2**	One faecal pellet
R5	**2**	Hind legs of three copepods hold *Acartia tonsa* remains such as antenna
R10	**1**	*Acartia tonsa* hard to count due to high motility
R10	**2**	Two faecal pellets

Amazingly almost all copepods died in the A-series experiments, although many brine shrimp survived. This suggests, that *Artemia salina* attacks *Acartia tonsa*. I wanted to research this possibility further.

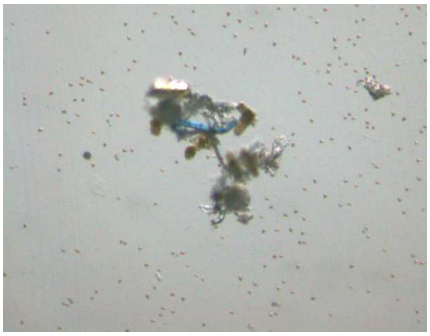
Experiment II: interactions between *Acartia t.* and *Artemia s.* [19th July]

The possibility of number-dependent role switching in predator-prey interactions between Acartia tonsa and Artemia salina offered an interesting research topic. I followed up this idea with the following experiments.

Over a span of only two hours [02:45 pm- 04:45 pm; 03:00 pm- 05:00 pm] this time, I let a fixed number of *Acartia tonsa* cope with a different number of *Artemia salina*. In both series these organisms didn't have any other food source than each other. The experiment also took place at 18℃ and with 5 ml of artificial sea water in each Petri dish.

Note: Pictures below partly show copepod remains from the end of the experiment.

R		Start 02:45 pm		Results 04:45 pm			
		Acartia tonsa	***Artemia salina***	***Acartia tonsa***		***Artemia salina***	
				alive	**dead**	**alive**	**dead**
R3	**1**	3	20	1	2	20	0
	2	3	20	2	1	16	4
R5	**1**	5	10	3	2	9	1
	2	5	10	2	3	8	2
R10	**1**	10	5	7	3	5	0
	2	10	5	5	5	5	0



L		Start 03:00 pm		Results 05:00 pm			
		Acartia tonsa	***Artemia salina***	***Acartia tonsa***		***Artemia salina***	
				alive	**dead**	**alive**	**dead**
L3	**1**	10	20	9	1	9	11
	2	10	20	10	0	15	5
L5	**1**	10	10	9	1	10	0
	2	10	10	10	0	7	3
L10	**1**	10	5	10	0	3	2
	2	10	5	10	0	4	1

		Additional observations that can't be expressed in numbers
R3	**2**	*Acartia tonsa* white bellied [also applies to most other Petri dish]
L3	**1**	Copepods keep to the outermost edge of vessel
L3	**1**	Many *Artemia salina* dead but untouched [carcasses]
L10	**2**	One *Artemia salina* eaten completely [no traces, carcass]

I was not yet fully satisfied with my results. And still wanted to check, if the situation changed if Artemia salina were in great minority.

Experiment III: interactions between *Acartia t.* and *Artemia s.* [21st July]

W		Start 10:15 am		Results 01:15 pm			
		Acartia tonsa	***Artemia salina***	***Acartia tonsa***		***Artemia salina***	
				alive	**dead**	**alive**	**dead**
W1	**1**	5	1	5	0	0	1
	2	5	1	5	0	0	1
W2	**1**	5	2	5	0	1	1
	2	5	2	4	1	2	0
W3	**1**	5	3	5	0	2	1
	2	5	3	5	0	3	0
W5	**1**	5	5	5	0	3	2
	2	5	5	5	0	4	1
W10	**1**	5	10	5	0	4	6
	2	5	10	5	0	3	7
	3	5	10	4	1	10	0

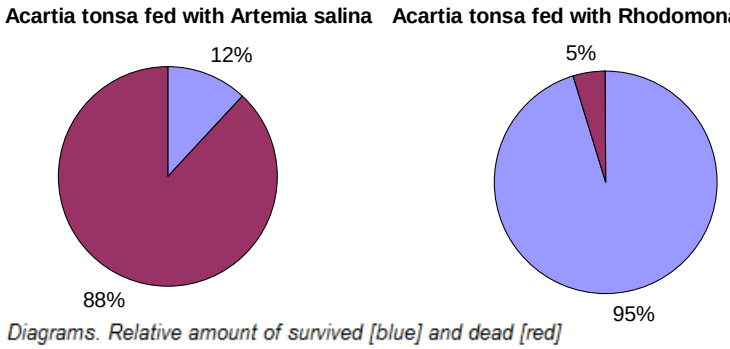
All additional collected data not displayed here, is shown in the attachment.

5) Conclusions

Evaluation of the experiment results on the next pages.

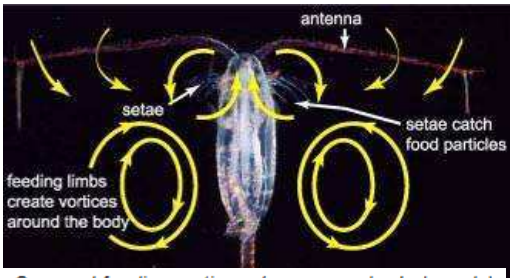
Grazing-preferences of *Acartia tonsa* [experiment I]

95% of *Rhodomonas salina* fed *Acartia tonsa* survived, whereas only 11% of *Artemia salina* fed copepods survived the feeding experiment.



Diagrams. Relative amount of survived [blue] and dead [red] Acartia tonsa.

In conclusion *A. tonsa* prefers "vegetarian" phytoplankton food such as *R. salina* over bigger zooplankton. One mature *A. tonsa* copepod needs about 5350 *R. salina* cells to survive five hours, or about 1070 cells per hour. These huge cell amounts are eaten by constant grazing. Copepods take up these large cell numbers by generating rotating currents [vortices] around themselves (www.ucmp.berkeley.edu). The generation of faecal pellets also indicates health and sufficient food supply.



Copepod feeding-vortices (www.ucmp.berkeley.edu).

Although *A. tonsa* also is able to eat *A. salina*, they prefer *R. salina*, because they are easier prey, due to small size and low speed. *A.* salina are either too fast or too

dangerous for most copepods, thereby explaining the high death rate of *A. tonsa* in the A-series experiments.

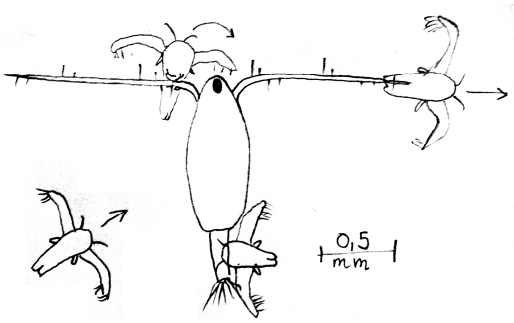
Interactions between *Acartia tonsa* and *Artemia salina* [all experiments]

These observations can be explained by two completely different hypothesises. The basic idea of the first hypothesis is, that in certain numbers, brine shrimps work together to hunt down their predators and eat them. This suggests number-dependent predator-prey interactions with switching roles. The second possible explanation refers to *A. salina* as completely peaceful creatures only eating bacteria, detritus and algae. The copepods carry these food sources on their body and after having died are therefore attacked by hungry *A. salina* crowds, thereby disassembling the already dead corpses.

Discussion: Hypothesis I versus Hypothesis II

There are observations backing each of the two hypothesis. But which one is closer to truth?

Hypothesis I is mainly backed by the A-series [experiment I] observations. Apparently almost all copepods died [95%], whereas almost all brine shrimp survived. Moreover *A. salina* completely eat the copepod remains, without leaving traces. This experiment suggest, that the *Artemia* being in incredible majority, actively hunt down the copepods for feeding. The mangled *Acartia tonsa* remains, as well as the observed situation in A2/1 [experiment I] strengthen this hypothesis. With sufficient nutrition from hunted down *A. tonsa*, the *A. salina* nauplii could survive much longer, as can be seen in all experiment series. According to this hypothesis *Artemia* hunt in groups, because most copepods die, when *A. salina* are in big majority. This is backed by data of the A-series, where the brine shrimp were in a huge majority of 1500:1 [A1] to 150:1 [A10]. To sum up, the original predator-prey roles are switched according to population sizes. Therefore the copepods are forced to keep to the uttermost edges of the Petri dish, as observed in L3/1 [experiment II] because these strategic points offer protection on one side. The threshold value for this change in predator-prey linkages, would be around, 6:1 *A. salina* to *A. tonsa*. As discovered in the W-series



Situation in A2/1 [experiment I]: sketch of Acartia tonsa *being broken apart by three* Artemia salina

however, *Artemia* are only forced to eat *A. tonsa*, when no other food source such as *R. salina* are available.

Hypothesis II also offers fact based explanations. First of all hunting is an untypical behaviour for undersized nauplii larvae [0,5 mm], which aren't fully grown and under-developed. The larger copepods [1-2 mm] are faster and would therefore be in the advantage. Another fact strengthening these ideas is, that *A. salina* swim underside up, with their legs directed towards the light source. Thereby not being able to see what they reach for with their hairy legs (Abatzopoulos *et al* 2002). Because of this they often also try eating sand particles or other indigestible particles. This strongly suggests, that *A. salina* aren't able to hunt down highly motile *A. tonsa*, even in a small Petri dish. Moreover *A. salina* are very resistant towards different ecological factors such as temperature changes and oxygen concentration in the water. *A. tonsa* however need fairly high oxygen concentrations to survive [they are planktonic copepods inhabiting surface waters]. If the oxygen concentration drops too low in a time span of e.g. 5 hours, as in the A-series investigations, *A. tonsa* would die of its own accord, whilst the *Artemia* could survive. Another interesting phenomenon was observed in R5/2 [experiment I]. In this Petri dish, three surviving copepods seemed to hold remains of a dead Acartia tonsa. This can only be explained by the fact, that this one died of its own accord, because *A. tonsa* never attack each other in such healthy situations, with loads of dinoflagellates as food. Therefore the dead copepod must have died because of bad health and oxygen-undersupply. This bad health could have been caused by the pipette ahead of the experiment. Apparently the copepod was not able to recover. Its remains would thereby not willingly be held on to by the other three copepods, but cling to them because of a sticky sugary liquid excreted by the decaying remains. This hypothesis is also backed by my later experiments with shorter time frames, in which relatively more copepods survived, as in the R-series and L-series of the second experiment. These two experiment series ran through a time span of three hours instead of five. Moreover they included fewer organisms and thereby oxygen-consumers. Of the R-series 56% of all *Acartia* survived, whilst 97% of the L-series didn't end up dead. In comparison to the already mentioned survivors-rate of the A-series [5%], this clearly shows the significance of this reasoning. Also the W-series only has 4% mortality-rate in a time span of three hours.

On the one hand hypothesis one offers an interesting explanation, based on the power of groups in comparison to single organisms, as often can be observed in nature. However on the other hand the results of L3 [experiment II] clearly state, that although the *A. salina* are in a 2:1 majority, they can't really reduce the *A. tonsa* population. In conclusion I believe that hypothesis II offers the most plausible explanation. Summarizing the observed phenomenon, Artemia salina are ecologically a lot more tolerant than *A. tonsa*, therefore surviving longer in the small Petri dish. Whilst most copepods perish after 4 hours without food, or too little space, the brine shrimp can survive more than 24 hours without serious problems [check attachment for collected data dealing with this topic]. After dying the copepods secrete a sticky sugary liquid. Looking for food such as copepod associated bacteria or detritus, the peaceful *Artemia* nauplii take the copepod carcass apart, scattering the remains. The *Artemia* only dispose of decayed material, and don't attack the copepods.

V. Résumé

I really enjoyed my internship at the BAH. It was very interesting and entertaining to peek into the work of marine scientists. The work in the lab was rich in variety and full of completely new experiences. I especially enjoyed the work on the phytoplankton samples and in the aquarium. Having worked with scientists for 4 weeks, I believe I have learned a lot about the life as a scientist today. To be a good scientist, one has to work hard and be determined to succeed even when it doesn't work out the first, second, third, fourth or fifth time. Often scientists need to generate creative solutions, to solve technical problems. The most important message I learned probably was, that you need to be open for new ideas, and criticism from other researchers. These usually help to improve one's own work, no matter where they come from.

I believe, that my stay on Heligoland has truly helped me in terms of career choices. I want to be a marine biologist, because I love the sea and I believe that it has many answers to modern global problems. I hope to help uncover some of these mysteries and make the world a better place.

The BAH was a very welcoming institute, and I was integrated into the research community very well, probably especially due to the POLMAR week. I worked in the foodweb group of the BAH, and want to thank all scientists involved with my internship. Special thanks go towards Dr. Boersma, head of the foodweb group, for making this internship possible and integrating me into the BAH community. I am

also very grateful that Dr. Kraberg, Evamaria Krause, Ulrich Alexander, Helgo Block, Kristine Caarstens, Julia Haafke and Axel Orban for taking care of me during my internship and the trust they extended towards me. Moreover I want to thank the *Förderverein der Biologieolympiade e.V*. for giving my the possibility of such an amazing internship, and for their good work to lead young students into biology. But most of all I want to thank Stefanie Schnell for accepting me as apprentice, teaching me in the lab and caring for me during my entire stay on Heligoland and editing this report.

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Picture (objective 10x, projection-eyepiece 2,5x, stack with 7 pictures)
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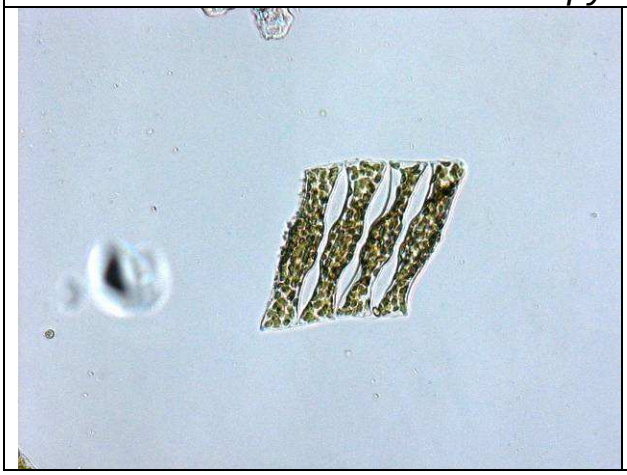
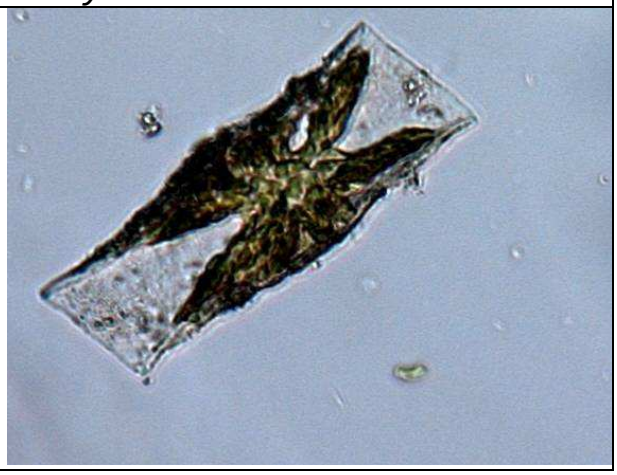
VII. Attachment

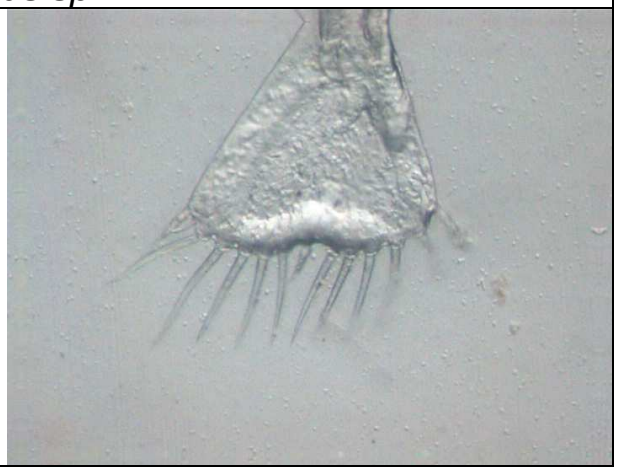
1) Additional phytoplankton pictures

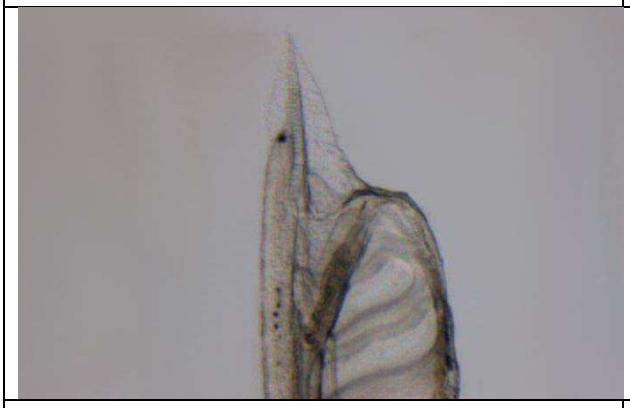
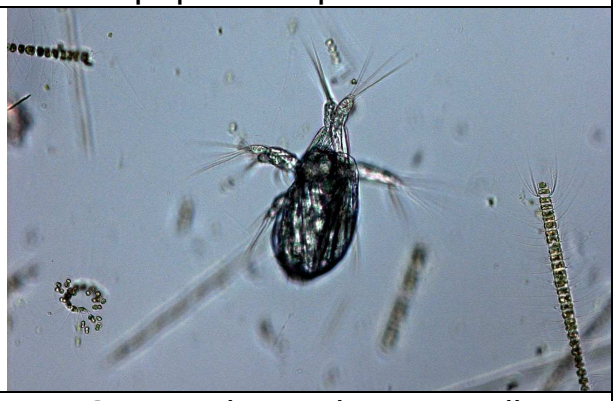
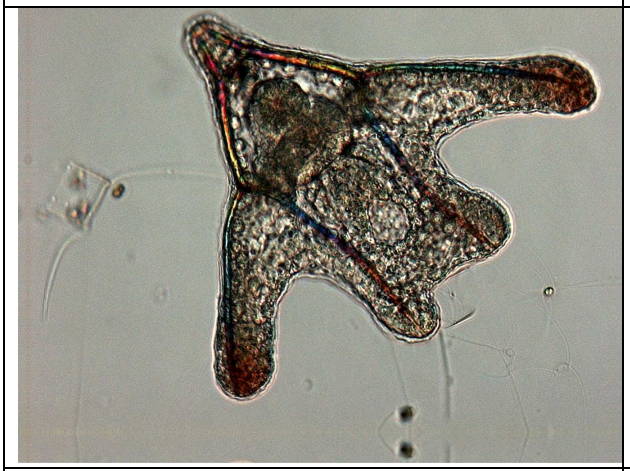
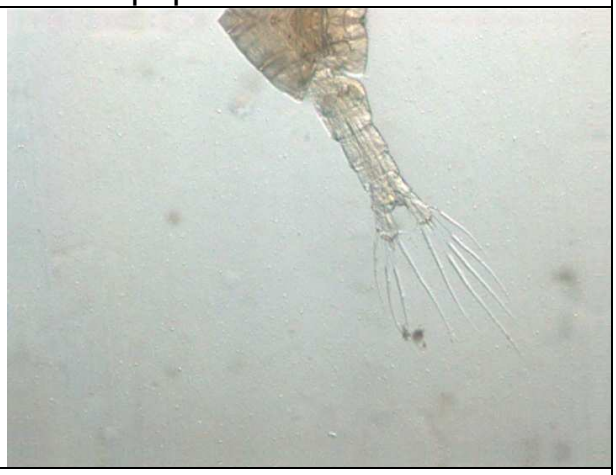
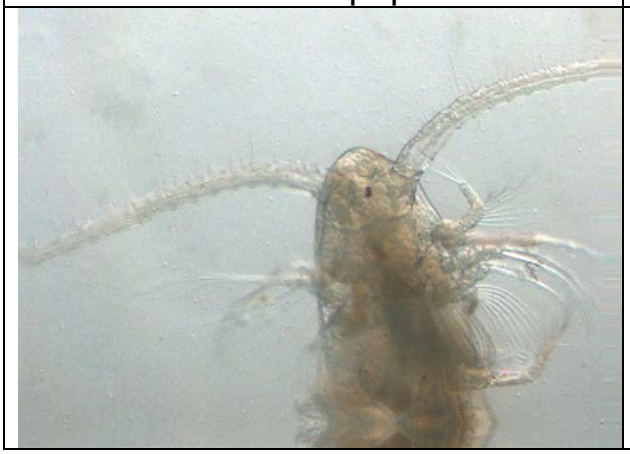
Ceratium fusus

Mediopyxis helysia	
	

2) Zooplankton pictures taken on the 22nd and 23rd of July

Pictures of zooplankton from 150 µm sample [Heligoland Roads].

Polychaeta	*Pandalus sp.*
	
Pandalus sp.	
	

Lancelet Fish Larvae	*Corymorpha nutans*
	
Megalopa Crustacea Larvae	Copepod Nauplius Larvae
	
Echinodermata Larvae	Copepod *Acartia tonsa* tail
	
Mature Copepod	Copepod Nauplius Larvae
	

3) Alkalinity investigation: graphs and tables

The raw-data is listed in the tables below. Linear regression and calculations done in Excel 2003. Colour key, and procedure aid are valid for all data sets.

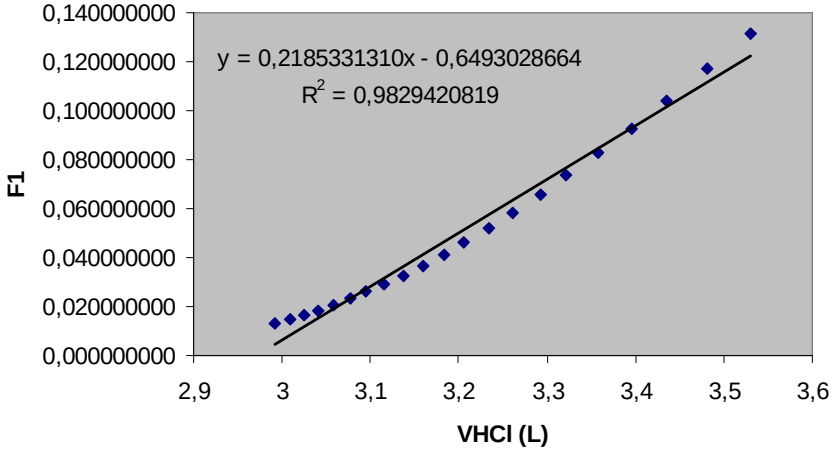
Colour key and procedure aid
known
sample volume (V0) and concentration of acid [HCl]
titration output
acid added (VHCl) and pH
directly calculated from titration output
Total Volume (V0+VHCl), proton concentration [H+], and absolute number of protons (F1)
calculated from linear regression
slope (a) and y-axis intercept (b)
calculated from known and regression
total alkalinity (TA)

05.07.2010 artificial sea water mix I **ALKALINITY**

VHCl (ml)	pH	[HCl] (M)	V0 + VHCl (ml)	[H+] (M)	F1
3,814	2,41	**0,05**	53,814	0,003890451	0,209360754
3,725	2,46	**V0 (ml)**	53,725	0,003467369	0,186284373
3,65	2,51	**50**	53,650	0,003090295	0,165794350
3,587	2,56		53,587	0,002754229	0,147590854
3,531	2,61		53,531	0,002454709	0,131403023
3,481	2,66		53,481	0,002187762	0,117003679
3,436	2,71		53,436	0,001949845	0,104191896
3,395	2,76		53,395	0,001737801	0,092789875
3,358	2,81		53,358	0,001548817	0,082641757
3,322	2,86		53,322	0,001380384	0,073604850
3,292	2,91		53,292	0,001230269	0,065563483
3,261	2,96		53,261	0,001096478	0,058399525
3,234	3,01		53,234	0,000977237	0,052022246
3,206	3,06		53,206	0,000870964	0,046340489
3,183	3,11		53,183	0,000776247	0,041283150
3,159	3,16		53,159	0,000691831	0,036777043
3,137	3,21		53,137	0,000616595	0,032764009
3,116	3,26		53,116	0,000549541	0,029189413
3,095	3,31		53,095	0,000489779	0,026004806
3,078	3,36		53,078	0,000436516	0,023169387
3,059	3,41		53,059	0,000389045	0,020642346
3,041	3,46		53,041	0,000346737	0,018391269
3,025	3,51		53,025	0,000309030	0,016386292
3,01	3,56		53,010	0,000275423	0,014600166
2,992	3,61		52,992	0,000245471	0,013007993

b
0,649302866
a
0,218533131
b/a
2,971187313

TA (M)
0,002971187
TA (µM)
2971

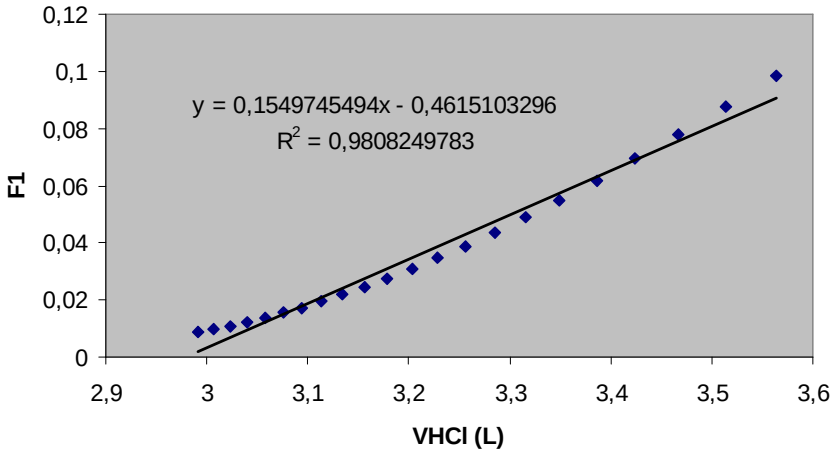


05.07.2010 artificial sea water mix II **ALKALINITY**

VHCl (ml)	pH	[HCl] (M)	V0 + VHCl (ml)	[H+] (M)	F1
3,848	2,536	**0,05**	53,848	0,002910717	0,156736295
3,761	2,586	**V0 (ml)**	53,761	0,002594179	0,139465677
3,686	2,636	**50**	53,686	0,002312065	0,124125510
3,621	2,686		53,621	0,002060630	0,110493037
3,564	2,736		53,564	0,001836538	0,098372340
3,514	2,786		53,514	0,001636817	0,087592599
3,467	2,836		53,467	0,001458814	0,077998422
3,424	2,886		53,424	0,001300170	0,069460260
3,386	2,936		53,386	0,001158777	0,061862488
3,349	2,986		53,349	0,001032761	0,055096788
3,316	3,036		53,316	0,00092045	0,049074689
3,285	3,086		53,285	0,000820352	0,043712432
3,256	3,136		53,256	0,000731139	0,038937543
3,229	3,186		53,229	0,000651628	0,034685528
3,204	3,236		53,204	0,000580764	0,030898990
3,178	3,286		53,178	0,000517607	0,027525296
3,156	3,336		53,156	0,000461318	0,024521797
3,134	3,386		53,134	0,000411150	0,021846029
3,114	3,436		53,114	0,000366438	0,019462965
3,094	3,486		53,094	0,000326588	0,017339854
3,076	3,536		53,076	0,000291072	0,015448922
3,058	3,586		53,058	0,000259418	0,013764197
3,04	3,636		53,040	0,000231206	0,012263192
3,023	3,686		53,023	0,000206063	0,010926078
3,007	3,736		53,007	0,000183654	0,009734939
2,992	3,786		52,992	0,000163682	0,008673818

b
0,470708158
a
0,157724558
b/a
2,984368213

TA (M)
0,002984368
TA (µM)
2984

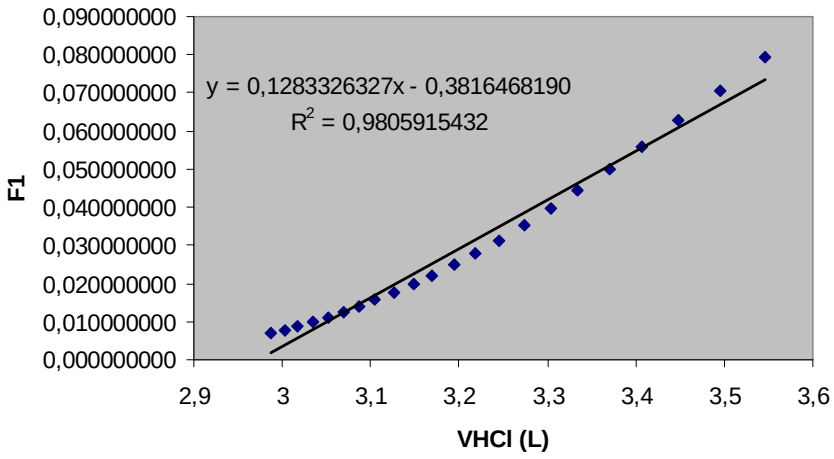


05.07.2010 sea water Heligoland **ALKALINITY**

VHCl (ml)	pH	[HCl] (M)	V0 + VHCl (ml)	[H+] (M)	F1
3,831	2,6295	**0,05**	53,831	0,002346929	0,126337549
3,742	2,6795	**V0 (ml)**	53,742	0,002091703	0,112412298
3,666	2,7295	**50**	53,666	0,001864232	0,100045884
3,602	2,7795		53,602	0,001661499	0,089059652
3,546	2,8295		53,546	0,001480812	0,079291573
3,495	2,8795		53,495	0,001319775	0,070601380
3,448	2,9295		53,448	0,001176251	0,062868263
3,407	2,9795		53,407	0,001048335	0,055988416
3,371	3,0295		53,371	0,000934329	0,049866093
3,334	3,0795		53,334	0,000832722	0,044412391
3,304	3,1295		53,304	0,000742164	0,039560320
3,274	3,1795		53,274	0,000661455	0,035238329
3,245	3,2295		53,245	0,000589522	0,031389098
3,218	3,2795		53,218	0,000525412	0,027961377
3,194	3,3295		53,194	0,000468274	0,024909365
3,17	3,3795		53,170	0,000417350	0,022190478
3,148	3,4295		53,148	0,000371963	0,019769101
3,126	3,4795		53,126	0,000331513	0,017611937
3,105	3,5295		53,105	0,000295461	0,015690451
3,087	3,5795		53,087	0,000263330	0,013979389
3,069	3,6295		53,069	0,000234693	0,012454919
3,052	3,6795		53,052	0,000209170	0,011096902
3,034	3,7295		53,034	0,000186423	0,009886769
3,017	3,7795		53,017	0,000166150	0,008808768
3,003	3,8295		53,003	0,000148081	0,007848749
2,987	3,8795		52,987	0,000131978	0,006993093

b
0,389359435
a
0,130648398
b/a
2,980208253

TA (M)
0,002980208
TA (µM)
2980



4) Recipe for F/2-medium [in German]

Rezept für f/2-Medium

Stammlösungen:

1. 75 g $NaNO_3$[1] in 1000 mL Aqua dest. lösen
2. 5 g Na_2HPO_4[2] in 1000 mL Aqua dest. lösen
3. 15 g $Na_2SiO_3 \times 9H_2O$[3] in 1000 mL Aqua dest. lösen;
4. 200 mL HCl_{cc}[4] auf 1000 mL mit Aqua dest. auffüllen; mit dieser Lösung werden dann 10 mL der Lösung 3. (Silikat) auf einen pH-Wert von 7,1 eingestellt und so ermittelt, wieviel mL des HCl-Wasser-Gemischs zur Stammkultur benötigt werden
5. Metall-Mix
   a) 150 mg $ZnSO_4 \times H_2O$[5],
   100 mg $CuSO_4 \times 5H_2O$,
   120 mg $CoSO_4 \times 7H_2O$ und
   2000 mg $MnSO_4 \times H_2O$ in 100 mL Aqua dest. lösen
   b) 5000 mg $FeCl_3 \times 6H_2O$ in 100 mL Aqua dest. lösen
   c) 65 mg $Na_2MoO_4 \times 2H_2O$ in 100 mL Aqua dest. lösen
   d) 5000 mg $Na_2EDTA \times 2H_2O$ in 100 mL Aqua dest. lösen
   10 mL Lösung a),
   10 mL Lösung b),
   10 mL Lösung c) und
   100 mL Lösung d) mischen;
   anschließend auf ca. 800 mL mit Aqua dest. auffüllen und mit ca. 0,25N NaOH auf pH 7,5 einstellen; zuletzt auf 1000 mL mit Aqua dest. auffüllen.
6. Vitamin-Lösung
   1 mg Vitamin B_{12}, 1 mL
   1 mg Biotin und 10 mL
   200 mg Thiamin x HCl in 1000 mL Aqua dest. lösen 100 mL

Herstellen des Kulturmediums:

Natürliches Seewasser wird mit einem Membranfilter (0,2 oder 0,45 µm) filtriert. In das Wasser werden pro 1 L Seewasser je 1 mL der Lösungen 1., 2., 3., 5. und 6. sowie die für 4. ermittelte Menge gegeben.

Das Rezept gibt die Herstellung von f/2 + Si an. Zur Kultivierung von z. B. *Rhodomonas* genügt f/2 – Si, wobei einfach Lösung 3 nicht ins Seewasser hinzugegeben wird.

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1 alternativ können auch 89,1 g KNO_3 verwendet werden
2 alternativ können auch 9,44 g $Na_2HPO_4 \times 7H_2O$, 12,61 g $Na_2HPO_4 \times 12H_2O$ oder 13,39 g $Na_3PO_4 \times 12H_2O$ verwendet werden
3 alternativ können auch 11,2 g $Na_2SiO_3 \times 5H_2O$ verwendet werden
4 konzentrierte Salzsäure
5 alternativ können auch 267,12 mg $ZnSO_4 \times 7H_2O$ verwendet werden

5) Additional data on the copepod research

21st-22nd of July

W		Start 10:15 am	Results 01:15 pm		Results 02:15 pm		Results 04:30 pm		Results 09:15 am	
		Artemia salina	**Artemia salina**		**Artemia salina**		**Artemia salina**		**Artemia salina**	
			alive	**dead**	**alive**	**dead**	**alive**	**dead**	**alive**	**dead**
W2	**1**	10	10	0	10	0	9	1	8	2
W3	**1**	10	9	1	9	1	8	2	7	3
W5	**1**	10	10	0	10	0	10	0	8	2
W10	**1**	10	10	0	10	0	9	1	8	2