



Grade 9

Nitrogen and the Alhagen Wetland

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Preface

Being one of Sweden's largest wetlands used for wastewater treatment, the Alhagen Wetland is an excellent place to study nature and biological processes. In grade six, pupils get to review the plant and animal life of the wetland. This instructor's guide shows how to build on this experience and expand the understanding of the wetland for grade 9 pupils. The central topic of the field study is how nitrogen is transported through air, soil and water. This is to spur to a discussion on recycling, nitrification, denitrification and bio-indicators. The field study should also contribute to reaching the goals stated in the national curriculum (Lpo 94) for biology for grade nine pupils.

Pupils are to:

- Know of some of our planet's eco-systems.
- Be able to give examples of the cycles in which nature transports substances through air, soil and water.
- Know the requirements for and importance of biological diversity.
- Be able to perform field observations and laboratory work.
- Be able to perform and interpret simple environmental measurements.
- Be able to use natural sciences, aesthetics and ethics in order find standpoints in questions concerning the preservation of biotopes.

The field studies at Alhagen could also be used as part of an educational theme where ordinary school work and teaching material is included. By integrating different school subjects and backing up the field studies with relevant class-room lessons, pupils have the opportunity to put their knowledge in context and gain a deeper understanding of what they have learnt. The *Toilet Etiquette* material, for instance, provides ample connections to the national curriculum's (Lpo 94) goals for social sciences for grade nine pupils.

Pupils are to:

- Understand and apply ecological thinking and demonstrate the consequences of different courses of action in questions regarding environment, life and society.

The chemical analysis that the pupils are to perform at the wetland, especially those parts concerned with the processing of nitrogen, have been preceded by two candidate theses written by students at the Swedish University of Agricultural Sciences (SLU) in Uppsala. These two papers, both dealing with nitrification in the wetland, were written by Anna-Stina Påledal and Ebba af Petersens.

If you have any questions regarding this material, copies, etc; please contact the Nature School of Nynäshamn on the phone number or address below.

We would like to thank the Water and Sewage Service Administration whose cooperation makes field studies at the Alhagen Wetland possible.

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Robert Lättman & Mats Wejdmark

Programme

Assembly at the parking lots	8.30
Introduction, biological cycles, methods	around 8.30
Morning snack	around 9.15
Sampling, netting and reviewing	9.30-11.30
Lunch	11.30-12.30
Determination of species, analysis	12.30-13.00
Termination of day out	13.00-13.30



The Alhagen Wetland, Grade 9

Eutrophication

During the last decades, the eutrophication of the Baltic Sea has become a question taken more and more seriously. Depleted bladder wrack banks and sea beds void of oxygen are indirect indicators of an excessive input of nutrients. This in turn affects the reproduction of many species which use the bladder wrack as a “nursery”. The reproduction of the cod is also in danger since the roe, which needs salt levels only found at great sea depths, dies from lack of oxygen. This knowledge affected the parliament which in 1991 decided that all large, coastal wastewater treatment facilities were to reduce their nitrogen discharge by 50%.

Alhagen – An Artificial Wetland

Nynäshamn’s wastewater treatment facilities lacked the necessary nitrogen reduction stage. Thus it was ordered to expand its facilities to meet the new demands before 1999. The municipality of Nynäshamn decided to construct a wetland to trap nitrogen. Alhagen is situated in a valley that stretches 2.5 kilometres in a south-west / north-east direction. Before the wetland was constructed, there was a natural swamp in the north part of the valley, closest to the Baltic Sea. The south part of the valley consisted of overgrown fields, and it was here that, in 1997, a number of shallow dams were built.

Trapping Nitrogen in a Wetland

At Alhagen, biological processes convert nitrogen in the water into ordinary air nitrogen. The ambition is that half the nitrogen arriving in the cleaned sewage water, mainly in the form of ammonium, is to be converted this way. From the clarifying basins at the south part of the wetland, the nitrogen rich water is emptied into the east and west inlet dams. These two dams are alternately (every other day) filled and emptied to increase the level of oxygen in the water. This benefits the bacteria that convert ammonium, via nitrite, to nitrate in an oxygen demanding process called nitrification. Aeration is also improved through the overland flow as the water runs down to “Puddle Hollow”.

From the oxygen rich dams, the water is emptied into “Sedge Marsh” and “Reed Marsh”, located in lower parts of the wetland. Via photosynthesis, additional oxygen is provided by vegetation. However, since there is little movement of water and water levels are stable in this part of the wetland, conditions for an anaerobic environment are created in the deeper waters. This favours bacteria that can use the oxygen in bound up in nitrate (NO_3). Thus nitrate is converted, via nitrite (NO_2), to air nitrogen (N_2) through denitrification. Vegetation facilitates this process by acting as an oxygen producer, as a place for growth, and a source of carbon for the bacteria.

The New SBR-facility

In 2003, a new biological treatment facility was introduced as an additional part of the treatment process before the wastewater reaches the wetland. All municipalities in Sweden have been made responsible for taking care of their own separate sludge and it is mainly this material that is received by the new facility. Separate sludge is collected from houses with septic tanks, summer houses, etc. The new facility will also receive wastewater from the municipality’s central sewage system when there is little separate sludge and during the winter month due to reduced efficiency of the wetland.

SBR stands for Sequencing Batch Reactor and is basically a nitrification dam but also works as a degrader of organic material (BOD). Oxygen is pumped into the system and ammonium is converted to nitrate. This means that the large quantity of ammonium that previously arrived at the wetland is now replaced with nitrate.

Nitrification and Denitrification

Nitrification is a process that converts ammonium to nitrate. Furthermore it is aerobic, i.e. it can only take place if there is oxygen in the water. It is a two stage process with nitrite as an intermediate form. Both stages are performed by bacteria (Nitrosomas and Nitrobacteria respectively).

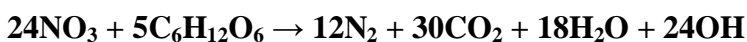
Chemical formula:



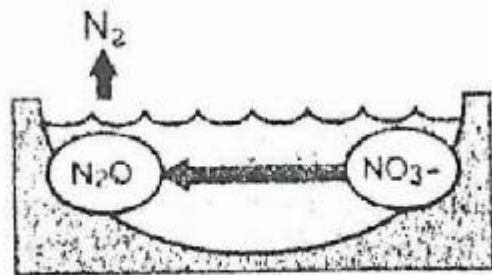
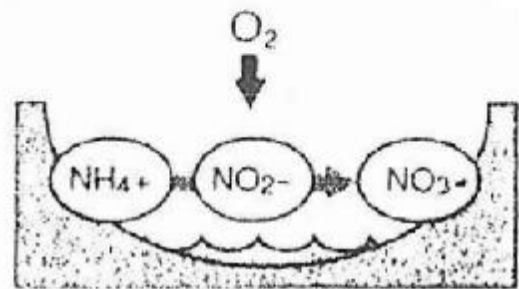
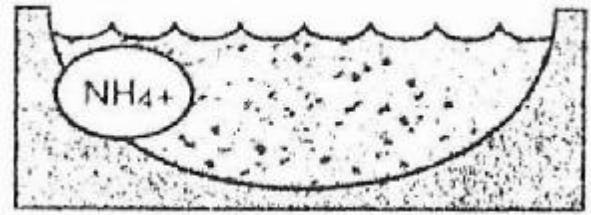
The bacteria use NH_4 and NO_2 as their source of energy and need no organic carbons. Carbon dioxide, on the other hand, is needed as a carbon source when building up the cells. The necessary oxygen is added at several places down the wastewater treatment process. In the SBR-facility, before the wastewater reaches the wetland, oxygen is pumped into the water. In the inlet dams the water is aerated through the continuous filling and emptying of the dams. At the overland flow, additional oxygen is diffused into the water. Lastly, in the lower parts of the wetland, where, to a large extent, oxygen deficiency is desirable, some oxygen is added through the photosynthesis of water living plants. When the wastewater reaches the inlet dams, the positively charged ammonium ions will stick to negatively charged soil particles. When the dams are emptied, oxygen increases and makes it possible for the bacteria to nitrify the ammonium. Since hydrogen ions (H) are released, the process has a somewhat acidifying effect.

Denitrification also has nitrite as an intermediate form. Dinitrogen oxide (laughing gas) is also an intermediate form before the end product N_2 (air nitrogen) is released into the atmosphere. For this process the bacteria require a source of energy. The source of carbon in this case is the plants that live in the wetland. In the formula below, glucose is the energy source. Glucose is one of the sugars built up by the plants in the wetland through photosynthesis. When the plants die, the bacteria's metabolism breaks the sugar down to carbon dioxide and water (comparable to what happens in a compost).

Chemical formula:



Anaerobic conditions are required for the reaction to take place. If pure oxygen is available, the bacteria will use this instead of the nitrite. Since the bacteria use vegetation to obtain carbon (the energy source) there is no need to harvest plants since they are decomposed according to the formula above. The reaction releases hydroxide ions (OH) that compensate for the acidification caused by the nitrification earlier in the treatment process.



Assignments for grade 9

Common assignments for all five groups:

- Chemical analysis.
- Netting for benthic animals.
- Review of plant life.
- Review of bird life.



Chemical Analysis

To find out if a wetland such as Alhagen is functioning properly, a number chemical analysis can be performed. The wetland was constructed in order to reduce nitrogen discharge by 50% which means nitrogen levels are the most relevant measure. The nitrogen will be found as a part of different chemical substances depending on where in the wetland measurements are made. Thus it becomes interesting to measure the levels of different forms of nitrogen, most notably ammonium, nitrite and nitrate, for the respective dams. From the pupils' point of view, the nitrite measurements will prove that nitrification takes place in two stages. Nitrification, which is most efficient between pH 7 and 8, has an acidifying effect as hydrogen ions are released from the ammonium (NH_4). Thus the waters pH-value is also of interest.

In the wetland, there is a close to inexhaustible supply of nitrogen. Phosphor, however, is removed at the wastewater treatment facility and thus phosphor deficiency could limit biological production, impairing the wetland's ability to reduce nitrogen levels. Thus it is also of value to measure phosphor levels. Usually the soil itself will contain enough phosphor, but this phosphor might not be available for plants to use. From the pupils' point of view, the phosphor measurements will prove that the chemical treatment at the wastewater treatment facility is effective.

The wastewater treatment facility is allowed a maximum discharge of 15 mg total nitrogen per litre of water (annual average) from the wetland directly into Mysingen. The water laboratory of Nynäshamn's municipality continuously measures levels of ammonium, total phosphor and pH at two sample points in the wetland. The values in the incoming columns (In) below refer to water leaving the wastewater treatment facility and entering the wetland. The values in the outgoing columns (Out) refer to water leaving the wetland and entering the Baltic Sea (sample point at the resting spot just before the outlet into the Baltic Sea).

Date	Ammonium mg/l		Nitrate mg/l		Nitrite mg/l		Total Phosphor mg/l		pH
	In	Out	In	Out.	In	Out	In	Out.	
99.09.02	44	12	---	0,5	(98.08.06) 0,11	0,11	0,21	0,09	7,2
99.09.15	47	12	---	0,5	---	---	0,15	0,11	7,2
2000 average	34	8,8	---	0,5	---	---	0,43	0,15	7,1
2001 average	36	9,9	---	---	---	---	0,41	0,08	7,1
2002 average	28	9,8	---	---	---	---	0,38	0,11	7,0
03.06.12	10	5	13	0,04	---	---	---	---	---
03.08.28	11	1,5	13	1,0	---	---	0,28	0,08	7,1

The class will have six sample points (for five groups) in the wetland. In order to make measurements in all the dams, the group working at the main dam must also take samples the inlet dam (see the end-of-day protocol). It is important that samples are taken at the same position each year in order to attain comparable results. The locations of the sample points are clearly marked on the map.

Sampling procedures

Each group will use a test kit containing equipment for measuring ammonium, nitrate, nitrite, phosphate and pH-levels. The test kit consists of a bag with a number of test tubes, reagents and a legend for colour matching. Written instructions translated to Swedish are included in the pupil's guide. Test tubes and reagent containers for ammonium are green and are therefore simple to single

out from the others which have other colours. The results are entered onto each group's field protocol. Direct sunlight should be avoided when performing the tests and normal safety precautions undertaken when handling chemicals. Used chemical solutions should be poured into the containers for chemical residues.

Benthic animal life as an indicator of oxygen deficiency

Reviewing the water's biological life is one way of discovering a potential oxygen deficiency. The advantage of measuring the water's contents of for example benthic (bottom living) animal life is that it doesn't fluctuate from day to day as a chemical parameter could. Animal life is thus a relatively stable indicator of what condition the water is in.



When using indicators to discover an oxygen deficiency, no information on the level of oxygen is obtained. A comparison with other dams and subsequent grading is, however, possible.

Pupils can test if the oxygen level is suitable for nitrification or denitrification by running a landing net through the bottom sediment to catch benthic animals. This netting is a test of function that can be compared to the results of the chemical analysis. Hopefully, the combination will prove that the oxygen level is the deciding factor for which of the two processes that is active. The assignment also lets pupils learn and practise standardized netting procedure.

Benthic animal life is interesting to study since some groups of animals are more sensitive to oxygen deficiency than others. When oxygen levels decrease, the first animals to die out will be those that are very oxygen demanding. The extremely hardy group of animals less affected by low oxygen levels will die out last. Since the hardy group is prevalent in oxygen rich environments also, its presence alone can not be used as an indicator of oxygen deficiency. In combination with the absence of more sensitive species, however, it can be used as an indicator oxygen deficiency.

The bottom five species or groups on the field protocol are hardy; sludge worms (Tubifex), isopods, midge larva, alderfly larva and molluscs. The top three species on the filed protocol are sensitive; freshwater shrimp, mayfly larva and caddis fly larva.

Standard Netting Procedure

Netting for benthic animals is to be done using a standardized method. The procedure for netting using a hand held landing net in flowing water is described by SIS (Swedish Standards Institute). The general principle for netting in slowly flowing or still waters is to first stir the waterbed and then run the landing net (in this case a sieve with a lengthened handle) through the water, vegetation or soft bottom sediment. The waterbed can be stirred by driving the landing net or some other tool quickly through the water close to the bottom.



Stirring and netting are done alternately for one minute. Thereafter the landing net is carefully emptied of its contents onto a plastic tray. Pebbles and small sticks are thrown away after any small animals attached to them have been removed. The animals are sorted into an ice tray.

This procedure is repeated three times. Thereafter the sorted animals are counted and each group of pupils uses containers to bring a number of the benthic animals they found back to the pavilion. A few animals are to be brought back even if the group has made notes of what they have found. Stereo loupes and books are available for determining species in order to sort and count the animals specified on the field protocol.

Plant life

In a wetland rich on nutrition, there is a risk that large plants displace smaller, less competitive species. This increases total plant production but decreases biological diversity. One way of keeping competitive species, such as reed and cattail, from becoming too dominant is mowing or other mechanical processing. A rich spectrum of wetland plants provide for a broad range of wetland

animals thereby achieving the desirable biological diversity. In order to map changes in the wetland's plant life over time, each group of pupils is to identify and determine as many plant species as possible.

Reviewing procedures

Within a 20 x 12 meter square (10 m from the sampling point along the bank in each direction, 2 m out into the water and 10 m inland) the total number of species is to be established and as many species as possible determined. Plants that the pupils do not manage to determine are to be entered onto the field protocol, as unknown species 1, 2, etc. The protocol can be supplemented by a herbarium of plants typical for the investigated area. Additional documentation can be done using a digital camera.

Birds

During the 19th and 20th century, most wetlands in the south of Sweden were trenched out in order to make more land useful for farming. The populations of many birds dependent on wetland areas for survival decreased drastically during the same period. The Alhagen Wetland stands as a good example to show that these birds will rebound quite quickly when areas fulfilling their requirements for food, nesting and breeding become available. In order to map changes in number and types of bird species over time, the pupils are to count the number of observed species and use books to determine as many species as possible. Theoretically, changes in bird populations and species could be compared with changes in plant life in order to try to find any dependencies between them. Birds that the pupils do not know the name of are to be entered into the field protocol as unknown species 1, 2, etc.

Reviewing procedures

Observations are done using binoculars from the sampling point of each group. Birds in the dam where the sampling point is located and any other birds visible from the sampling point are to be counted and identified. The names of species determined and the total number of species are to be entered onto the field protocol.

Preparations

Many pupils will have been introduced to topics of this field study when visiting Alhagen in grade 6. During that visit they reviewed birds, plants, land living and water living bugs in the wetland. It is important, however, that school time is set aside for pupils to prepare for the field studies.

Things to do before the field study:

- Divide the class into five groups (all groups will be assigned the same tasks but different dams).
- Make copies of and distribute the assignment paper (see pages 15-18), field protocol (see page 19) and end-of-day protocol (see page 14). Let each group read through their assignment.
- Provide each group with a copy of the map (see page 11). Study the map in class and explain how the water flows through the system of dams on its way to the Baltic Sea.
- Discuss how the sewage system works; from flushing the toilet to the wastewater treatment facility to the wetland and out into the Baltic Sea. How is the water processed at the wastewater treatment facility? What does water leaving the facility contain when it enters the wetland? It is also important to understand the processes of nitrification and denitrification.
- Explain how benthic animal life can be used as an indicator of oxygen deficiency.
- Let each group form their own hypothesis regarding the levels of oxygen, nitrate and phosphate in the each dam. Also make a hypothesis on where animals sensitive to oxygen deficiency are to be found.
- Learn about the benthic animals, plants and birds you expect to encounter on your day out. This will make determination of species during the field study easier.
- Work with the educational theme *Toilet Etiquette* (see page 8).



Toilet Etiquette

The campaign *Toilet Etiquette* was initiated in spring 2003 as a way to increase public knowledge on what can and cannot be flushed down the toilet. The long-term aim of the campaign is that the sludge produced at the wastewater treatment facility is to become so pure it can be used as an agricultural fertilizer, thereby sparing seas and lakes from environmentally harmful substances. The parties involved in producing the campaign were:

The Nature School



The Water and Sewage
Service Administration



The Agenda 21 coordinator



Sewage and Sludge

Today, households dispose of more chemicals than the industrial sector. Many heavy metals that are harmful to humans are flushed down the drain. A few examples are cadmium, found in paints and tobacco (don't empty ashtrays into the toilet), and quicksilver, e.g. from tooth fillings. Some substances impair wastewater treatment since they affect micro organisms. Examples of these are petrol, oils, degreasing agents, drugs and cleaning agents. Objects such as tops, tampons, pads, etc. can cause blockages in pipes and pump stations. All of the above mentioned contaminate the sludge produced at the wastewater treatment facility and render it unusable.

The large content of nutrients is what makes the sludge suitable as a fertilizer. It is especially important to take care of phosphor that is a non-renewable natural resource that according to the Swedish Environmental Protection Agency's estimates will be depleted in 100 to 400 years.

To avoid that environmentally harmful substances end up in the wastewater, consumers must have the possibility to choose environmentally friendly products at gas stations and mechanics' shops. The *Car Care Watchers* activity for grade nine pupils helps see to it that such products are available. *Car Care Watchers* is done in cooperation with the municipality's environmental department and the results of the pupils' shop investigations are sent there. This work is important since every year the quality of the sludge is re-examined, i.e. the sludge must continuously be of high quality to continue being used. The pupils' yearly review is good way to put pressure on companies to reduce their range of environmentally harmful products that end up in the sludge, reducing its quality.



The range of educational material distributed during the campaign can be viewed on page 12. Instructor's guides for working with the *Toilet Etiquette* theme or the *Car Care Watchers* activity are available on the Nature School's First Class homepage. On page 13 you will find a home investigation that the pupils can do as part of a discussion on what chemicals a normal household flushes down the toilet.

Field Study at the Alhagen Wetland

We will begin the day by going through how nitrogen flows through air, water and soil. Then we will present the methods and procedures to be used for studying the wetland. After a short snack break, each group will begin sampling, netting and reviewing the wetland. The afternoon will be used for determination of species and compiling field results.



Important Note! All work will be performed out of doors. The pavilion, the only rain shelter available, is not large enough to accommodate all pupils and has no heating. Dress in warm clothes appropriate to the weather. Don't forget to bring both lunch and snack packs. Since the sampling points lie at some distance from each other, a bicycle for the class teacher might be useful.

Programme

Assembly at the parking lots	8.30
Introduction, biological cycles, methods	around 8.30
Morning snack	around 9.15
Sampling, netting and reviewing	9.30-11.30
Lunch	11.30-12.30
Determination of species, analysis	12.30-13.00
Termination of day out	13.00-13.30



List of materials for each group:

- Assignment paper, map, field protocol and end-of-day protocol (copied and distributed by the class teacher)
- Colour coded and plastic coated versions of the field protocol and sampling instructions (see page 17 and 18). These are included in each groups test kit along with the materials below.
- Test kit for chemical analysis. Each group must also have a watch, not included in the kit.
- Landing net (a sieve with lengthened handle) and a tray.
- Ice tray and a number of containers for bugs.
- Binoculars.
- One loupe per person and one stereo loupe per group.
- The booklet "Life in and About a Wetland", the book "What I Find in Lake and Stream", a book of birds, and a book of plants.

List of tasks to be performed by each group:

- Chemical analysis using the test kit.
- Netting for benthic animals.
- Review of bird and plant life.
- Determination of species and number of species.
- All results are to be entered onto the field protocol and the end-of-day protocol.

Exercises to Do After the Field Study

Analysis of the results found in the end-of-day protocol (reasons and possible sources of errors):

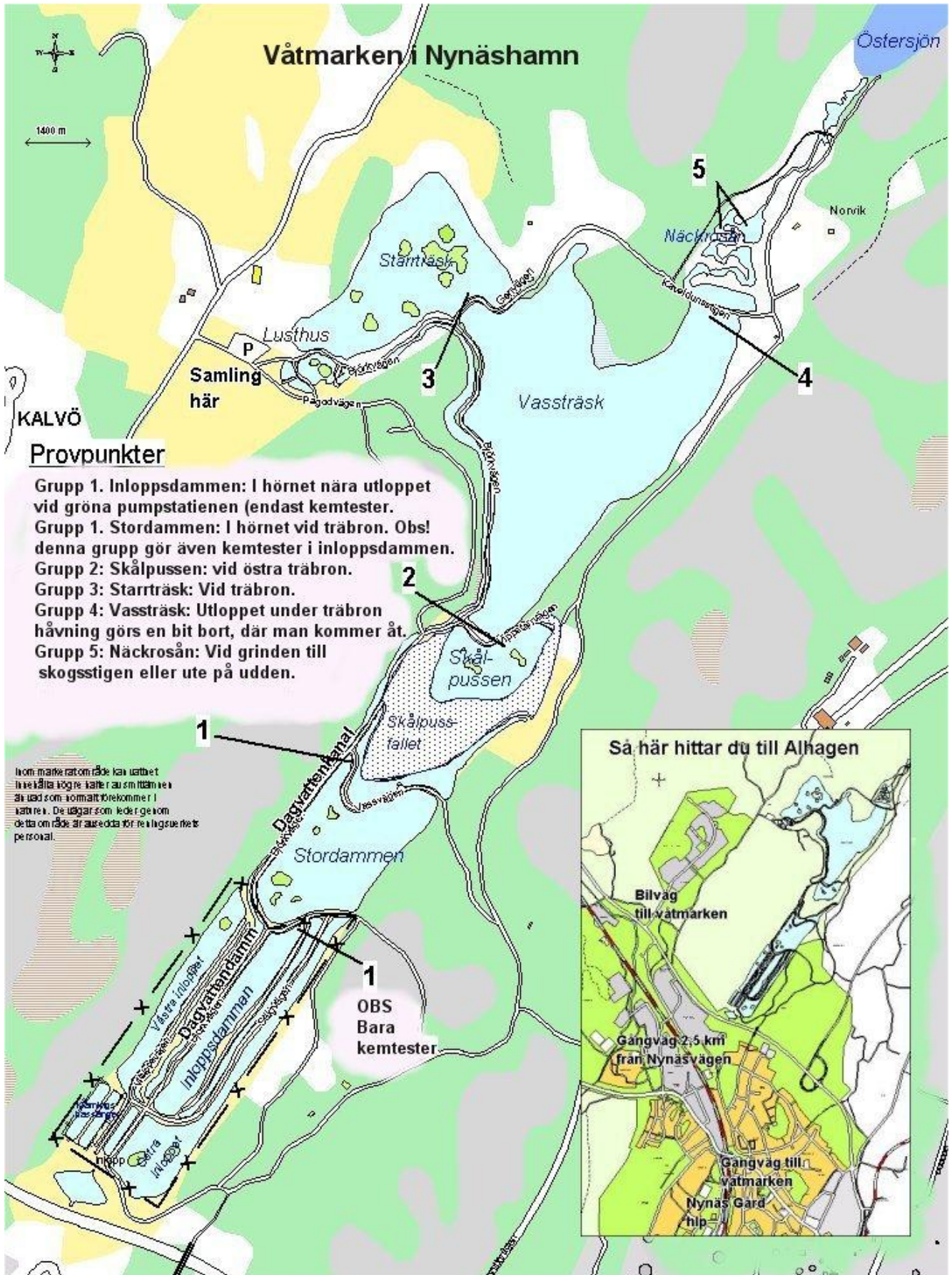
- Was the group's hypothesis correct?
- Some examples of sources of errors:
 - The bottom of dam at the location where netting was performed was not representative for the whole dam.
 - Heavy rains the previous day meant that the rainwater affected the wastewaters chemistry.
 - Those performing tests did not stick to the stipulated time or did not shake the test tube well enough for the reagent to dissolve.

Discussion:

- Where does nitrogen come from and where does it end up?
- How does the transportation of nitrogen in wastewater differ from how nitrogen normally flows through air, water and soil without human interference?
- What do we mean when we say that our food is made from oil? (Oil is burnt when producing nitrogen fertilizers).
- What alternatives to artificial fertilizers are available? (e.g. ecological fertilization using trefoil vegetation)
- Are there other ways to prevent nitrogen from being discharged into the Baltic Sea? (yes, e.g. urine separation)
- Where does phosphor come from and where does it end up? (It is mined and ends up in sludge that, if pure enough, can be reused in agriculture).
- How do the natural resources and nutrients phosphor and nitrogen differ when it comes to supply and handling?
- How long will our natural resources of phosphor last and what would the consequences for future generations be if these resources were exhausted?
- What ecological explanations (competition between species, etc) are there for some benthic animals being more common than others?
- How many fewer species of birds and plants would there be at Alhagen, had the wetland not been constructed.

Conclusion:

- Does the wetland function as planned?
- Has Nynäshamn managed to meet the demands on reduction of the municipality's nitrogen discharge?
- Has biological diversity increased since the dams were built?





The Waterbook (for pupils in grade 6, can be borrowed from the Nature School)



The Waterbook, Teachers Manual (for all grade 6 teachers)



My Friend Molly (for all grade 1 teachers)



Available at the Nature School

Environmentally Friendly Cleaning Tips (for pupils in grades 1-9)



Sticker (for pupils in grades 1-9)



Available at the Nature School

Things That Shouldn't Be Flushed (for pupils in grades 1-9)



Available at the Nature School

As Long as There's Life – There's Water and Sewage (video, 26 minutes, can be borrowed from the Nature School, for grades 4-9)



Available at the Nature School

Sludge – No Dirty Business (video, 12 minutes, can be borrowed from the Nature School, grades 1-3)



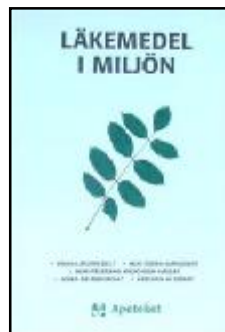
The Nature School and the Alhagen Wetland Area, Year 2000 (video, 30 minutes, available at schools and libraries)



www.scienceacross.org Exchange knowledge and ideas globally on this homepage devoted to environmental issues



The Swedish Chemical Inspectorate's Observation List. One copy distributed to each school's environmental group representative.






Drugs and the Environment (for all grade 4-9 teachers).



Choosing Environmentally Friendly Washing Powder and Detergents (one copy distributed to each school's environmental group representative).

Home Investigation

How many eco-labelled products can you find in your cleaning cabinet and bathroom?
Write the name of the product below the corresponding symbol.

Good Environmental Choice	The Swan	The EU-Flower
		

Examine the products you found in your cleaning cabinet and bathroom. Which of the following chemical substances can you find on the declaration of contents? Write the name of the product next to the corresponding chemical substance.

Chemical Substance	Products
Sodium hypochlorite	
Sodium metasilicate	
Optical whiteners	
Triclosan	
Ammonium	
Anionic Tensides	
Cationic Tensides	
Phosphoric or other acid	

Descriptions of the substances above can be found in the book "Choosing Environmentally Friendly Washing Powder and Detergents" (see page 12).

End-of-day Protocol, the Alhagen Wetland

Date: _____ Class, School & Teacher: _____

Number of Benthic Animals, Plant Species and Bird Species

	Main Dam Group 1	Puddle Hollow Group 2	Sedge Marsh Group 3	Reed Marsh Group 4	Water Lily Stream Group 5
Freshwater shrimp					
Mayfly larva					
Caddis fly larva					
Molluscs					
Isopods					
Alderfly larva					
Sludge worms					
Midge larva					
Number of plant species					
Number of bird species					

Chemical Analysis

<i>mg/litre</i>	Inlet Dam Group 1	Main Dam Group 1	Puddle Hollow Group 2	Sedge Marsh Group 3	Reed Marsh Group 4	Water Lily Stream Group 5
Ammonium						
Nitrate						
Nitrite						
Phosphate						
pH						

Weather (temperature, precipitation, wind): _____

Other circumstances of interest: _____



Assignment paper

The Alhagen Wetland, Grade 9

Preparations (to be done at school)

- The class is divided into five groups (all groups will perform the same task but in different dams).
- Each receives a field protocol and an assignment paper.
- Study the map of Alhagen and the sampling points in order to know where you are positioned relative to the other groups.
- Read through the instructions for netting, chemical water analysis and reviewing plant and bird life.
- Learn about the benthic (bottom living) animals you are to find and count.
- Practise determining the species of plants and birds.
- Learn about how netting for benthic animals can indicate oxygen deficiency in the water.
- Learn about the processes of nitrification and denitrification.
- What results do you expect from your chemical analysis relative to the other groups? E.g. will you nitrate values be the highest, the lowest or in a medium range compared to groups sampling other dams? What benthic animals do you expect to find in your dam? Agree on and write down a hypothesis.

Field Studies at Alhagen

List of materials for each group:

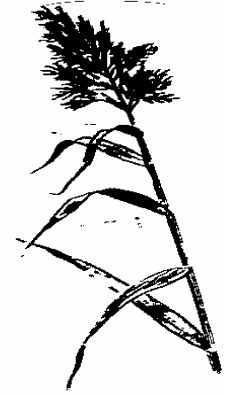
- A backpack.
- Assignment paper, map, end-of-day protocol and field protocol.
- A test kit for chemical analysis (each group must also have a watch, not included in the test kit).
- Landing net (a sieve with lengthened handle) and a tray.
- Ice tray and a number of containers for bugs.
- Binoculars.
- One loupe per person and one stereo loupe per group.
- The booklet “Life in and About a Wetland”, the book “What I Find in Lake and Stream”, a book of birds and a book of plants.

List of tasks to be performed by each group:

- Chemical analysis using test kit (ammonium, nitrite, nitrate, phosphate and pH).
- Netting for benthic animals.
- Review of bird and plant life.
- Determination of species and number of species.
- All results are to be entered onto the field protocol and the end-of-day protocol

Programme

Assembly at the parking lots	8.30
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Determination of species, analysis	12.30-13.00
Termination of day out	13.00-13.30



Important Note! All work will be performed out of doors. The pavilion, where stereo loupes will be available, is not large enough to accommodate everyone and has no heating. Dress in warm clothes appropriate to the weather. Don't forget to bring both lunch and snack packs.

Methods to be used

Netting

Netting for benthic animals will be done using a standardized procedure described by SIS (Swedish Standardization Institute). The procedure is as follows:

1. Stir the waterbed by driving the landing net or some other tool quickly through the water close to the bottom of the dam. Now run the landing net (in this case a sieve with a lengthened handle) through the water.
2. Alternate between stirring the waterbed and netting for one minute.
3. Carefully empty the contents of the landing net onto a tray.
4. Pebbles and small sticks are thrown away after any small animals attached to them have been removed.
5. Sort the benthic animals into the ice tray which has been filled with water. Put a few of the animals into containers and bring them back to the pavilion for closer examination.
6. Repeat steps 1 to 5 three times.
7. Those animals that are specified on the field protocol are sorted and counted (stereo loupes are available at the pavilion by the parking lots).

Benthic Animals

By counting the numbers of some benthic animals, it is possible to get an indication of the oxygen level in the water. This procedure relies on the fact that some benthic animals are more sensitive than others to oxygen deficiency. If many animals sensitive to oxygen deficiency are found, there is a lot of oxygen in the water. If only hardy animals, i.e. those insensitive to oxygen deficiency, are found, there is little oxygen in the water.

The benthic animals you are to count are described with names and pictures on the field protocol. The top three animals on the protocol are sensitive to oxygen deficiency and the bottom five are hardy or insensitive. If you only find the bottom five benthic animals and no freshwater shrimp, mayfly larva or caddis fly larva, the water is oxygen deficient.



Chemical Analysis

Each group will be given a test kit consisting of a bag with test tubes, reagents and a colour matching legend. A plastic coated version of the instructions on the following page will also be included. Since there are six sample points but only five groups, the group working at the main dam must also sample the inlet dam. Avoid direct sunlight when performing the chemical analysis since this can result in faulty colours emerging. The measurements take approximately 45 minutes to perform.

Reviewing plant life

When reviewing plant life, you are to start from the sampling point where netting and chemical analysis is performed. You will review a rectangular area of 20 x 12 m (10 m from the sampling point along the bank in each direction, 2 m out into the water and 10 m inland). Within this area you will write down which plant species are common and tick them off on the field protocol.

1. Mark up the area to review according to the instructions above.
2. Start by looking in the booklet "Life in and About a Wetland". Write down which of these species you find.
3. Try to determine as many species as possible, even those not found in the book. Those species which cannot be determined are entered onto the field protocol as unknown species 1, 2, etc.
4. Enter the total number of species found onto the field protocol (also count species you have not been able to determine).

Reviewing birds

Observations are done using binoculars from the groups sampling point.

1. Count and determine the species of any birds visible from the sampling point.
2. Count and determine the species of any birds in the dam where the sampling point is located.
3. Enter the name and number of birds of each species onto the field protocol. Species that cannot be identified are entered onto the protocol as unknown species 1, 2, etc.
4. Enter the total number of species found onto the field protocol (also count species you have not been able to determine).

Compilation of results

- All groups are to enter their findings onto the shared end-of-day protocol.
- All groups are to enter the findings of other groups onto their own end-of-day protocol.

Exercises to Do After the Field Study

- **Analysis of results** (reasons and possible sources of error)
- **Discussion** (e.g. how on how nitrogen flows through air, soil and water).
- **Conclusions** (does the wetland function as planned?).



Bird and Plant Species

Birds and plants are described in the booklet "Life in and About a Wetland", the book of plants, and in the book of birds.

Number of bird species determined	Number of unknown bird species	Number of plant species determined	Number of unknown plant species

Chemical Analysis

Nitrate NO ₃ (yellow captions and caps)	Ammonium NH ₄ (green captions and caps)	Nitrite NO ₂ (red captions and caps)	Phosphate PO ₄ (blue captions and caps)	pH (black captions and caps)
<p>1. Fill the test tube with water up to the mark.</p> <p>2. Add 2 spoons of reagent nr 1 and shake until completely dissolved. The spoon is attached to the lid.</p> <p>3. Add 1 spoon of reagent nr 2 and shake for 1 minute.</p> <p>4. After 10 minutes; compare the colour of the sample with the colour matching legend (place the test tube without a lid on the round white field and look at the fluid from above).</p>	<p>1. Fill the test tube with water up to the mark.</p> <p>2. Add 10 drops of reagent nr 1 and shake gently.</p> <p>3. Add 1 spoon of reagent nr 2 (the spoon is attached to the lid), shake, and leave for 5 minutes.</p> <p>4. Add 15 drops of reagent nr 3 and shake gently.</p> <p>5. After 7 minutes; compare the colour of the sample with colour matching legend.</p>	<p>1. Fill the test tube with water up to the mark.</p> <p>2. Add 2 spoons of the reagent and shake. The spoon is attached to the lid.</p> <p>3. After 3 minutes; compare the colour of the sample with the colour matching legend (place the test tube without a lid on the round white field and look at the fluid from above).</p>	<p>1. Fill the test tube with water up to the mark.</p> <p>2. Add 10 drops of reagent nr 1 and shake gently.</p> <p>3. Add 1 drop of reagent nr 2 and shake gently.</p> <p>4. After 5 minutes; compare the colour of the sample with the colour matching legend (place the test tube without a lid on the round white field and look at the fluid from above).</p>	<p>1. Fill the test tube with water up to the mark.</p> <p>2. Add 3 drops of reagent nr 1 and shake gently.</p> <p>3. Compare the colour of the sample with the colour matching legend (place the test tube without a lid on the round white field and look at the fluid from above).</p>
Result NO₃	Result NH₄	Result NO₂	Result PO₄	Result pH
mg/litre	mg/litre	mg/litre	mg/litre	









Important Note! Always take care when handling chemicals. Avoid contact with skin or eyes. Used solutions are poured into the containers for chemical residue.

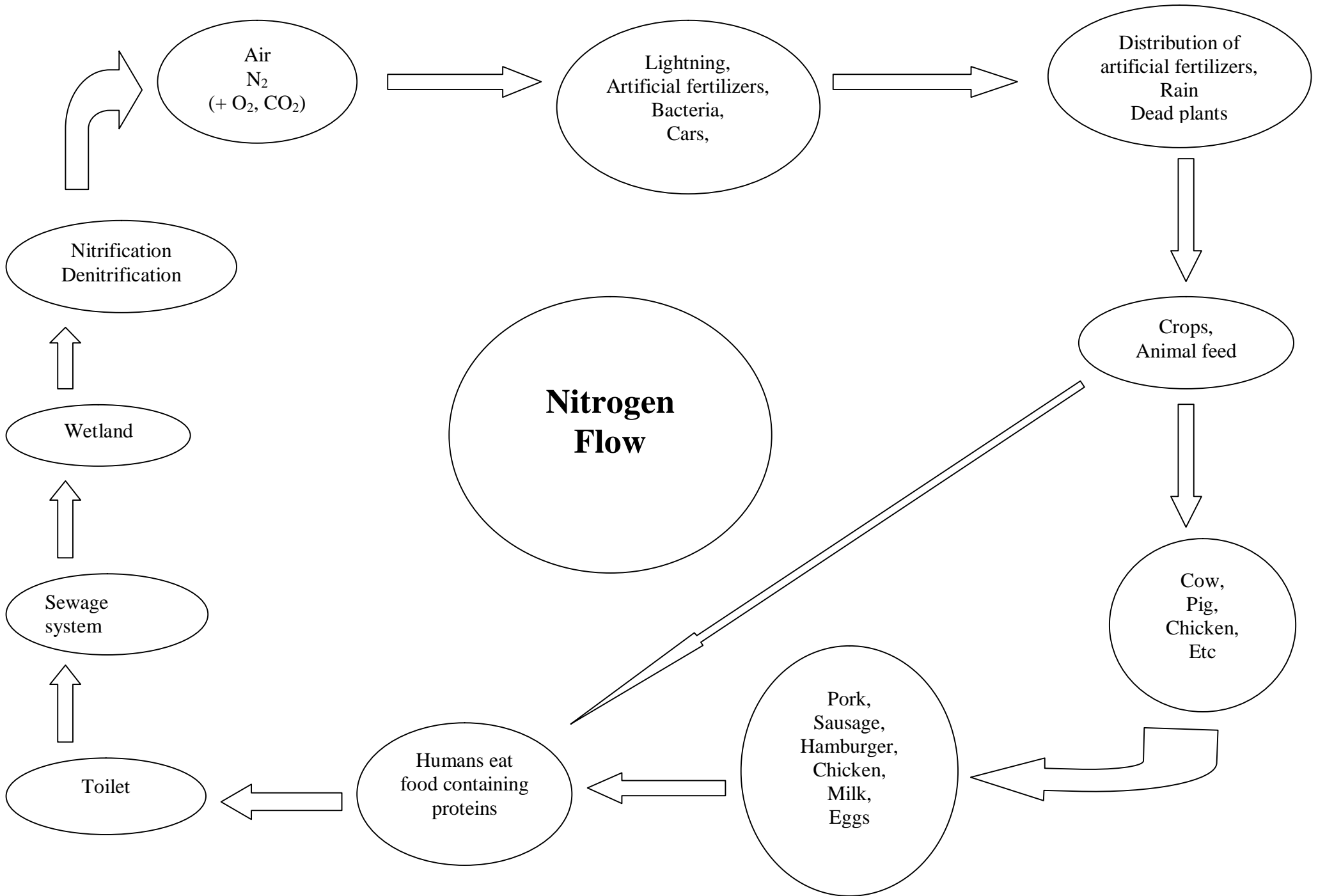
Field Protocol
The Alhagen Wetland



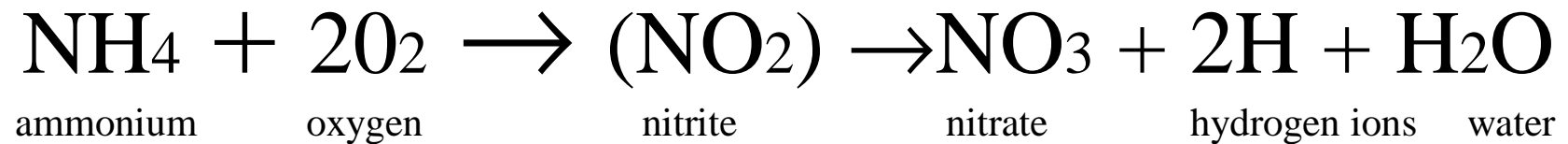
Number of Benthic Animals

Detailed descriptions of the animals can be found in the book “What I Find in Lake and Stream” and the booklet ”Life in and About a Wetland” which you will find in your backpack.

sensitive to oxygen deficiency		Freshwater Shrimp (2-2.5 cm). Looks flat when viewed from the side. Resembles a shrimp. Quantity found:
		Mayfly larva (0.5-2.5 cm). Has gills on its back and three antennae. Can be very small and resemble other fly larva. Quantity found:
		Caddis fly larva (Pipe 1-5 cm). Lives in a pipe built from parts of plants. The larva lies hidden within the pipe. Quantity found:
hardy sensitive to oxygen deficiency		Molluscs Quantity found:
		Isopod (1.5-2 cm). The body is flat and resembles land living wood-louse. Quantity found:
		Alderfly larva (2 cm). Quantity found:
		Sludge worms, Tubifex (3-8 cm) Quantity found:
		Midge larva (max 2 cm) . Are bright red and cannot be confused with any other creature. Quantity found:



Nitrification



During the first phase of nitrification, nitrite (NO₂) is created.
The nitrite is however quickly converted to nitrate (NO₃).

Nitrification is performed by bacteria in an aerobic environment.

Denitrification



Denitrification is performed by bacteria in an anaerobic environment.

*Important note! the formula is not balanced!
Where does the carbon in CO₂ come from?*

Balanced formula: $24 \text{NO}_3 + 5 \text{C}_6\text{H}_{12}\text{O}_6 \text{ equals } 12 \text{N}_2 + 30 \text{CO}_2 + 24 \text{OH}$