NeuLog

Experiment B-45
Ammonium and Nitrate in an Aquarium

Objectives

- To learn about the nitrogen cycle in an aquarium with fish and in a pond.
- To examine the presence of ammonium and nitrate ions in an aquarium with fish, in different stages of the nitrogen cycle.

Introduction

Every new set up of an aquarium goes through a process of establishing bacterial colonies. These colonies also fluctuate during some periods of an older aquarium. When this process is not understood, it can lead to a loss of fish.

An aquarium is a closed environment. A lot of waste exerted from the fish, from unconsumed food and decaying plants stays inside. Fortunately bacteria capable of converting wastes into safer by products begin to grow in the aquarium when the fish are added; still, for a period of several weeks the fish are at risk.

First stage: Ammonia is excreted from the fish which is highly toxic to them. Its ionized form, Ammonium is present when the pH is below 7 and is not toxic to fish.

Second stage: Nitrosomonas bacteria oxidize the ammonia into nitrite, which is also highly toxic to fish. They can begin rising by the end of the first week.

Third stage: Nitrobacter bacteria convert nitrites into nitrates which are not toxic to fish in moderate levels. Partial water changes keep the nitrate level low enough.

When testing the aquarium water, you will first see ammonia levels rising. A few weeks later you should see the nitrite levels rising and the ammonia level dropping. After another few weeks you should see the nitrate level rising and the nitrite levels dropping. On this stage it is safe to add to the aquarium tropical fish.

In this activity we will test an aquarium for ammonium and nitrate on different stages. Sample results for an aquarium that has already got to the last stage are included. A measurement of ammonium and nitrate in a pond or a lake is also recommended.

Prepare equipment and experiment materials

*** <u>Equipment and Sensor</u>**

- PC + NeuLog application
- USB-200 USB module  (or BLT-202 Bluetooth module )
- NUL-240 Ammonium logger sensor 
- NUL-241 Nitrate logger sensor 
- NUL-206 pH logger sensor 

*** <u>Tools and experiment materials</u>**

▪ Utility stand	1
▪ Right angle clamp	1
▪ Extension clamp	1
▪ 50ml beaker	4
▪ Wash bottle	1
▪ Plastic container	1
▪ Pasteur pipette	4

The items above are included in the Utility kit, UTL-KIT.

For the electrode preparation process you may need more beakers.

- **For electrode preparation and offset:**

▪ 200ml distilled water
▪ Ammonium electrode 1000 ppm standard solution
▪ Nitrate electrode 1000 ppm standard solution
▪ Ammonium electrode ISA bottle
▪ Nitrate electrode ISA bottle

- **For the experiment:**

▪ 30 ml of water from a fish aquarium
▪ 30 ml of tap water
▪ pH 4 buffer (not obligatory)
▪ Deionized water or distilled water

Caution!

It is recommended to wear personal protective equipment.Material Safety Data Sheets (MSDS) are available online.

Experimental procedure

* **Experiment setup**

1. A water sample from an aquarium can be taken from each stage described in the introduction and the same experiment can be repeated for each of them in order to observe the nitrogen cycle in an aquarium.

2. For the sample experiment, a sample from an old aquarium (an aquarium that reached the third stage) was taken.

* **Electrode preparation:**

(**It is recommended that the teacher will do it beforehand)**

3. Remove the protective cap encasing the nitrate probe's electrode.

Caution:

Do not touch the PVC membrane at the end of the probe with your fingers.

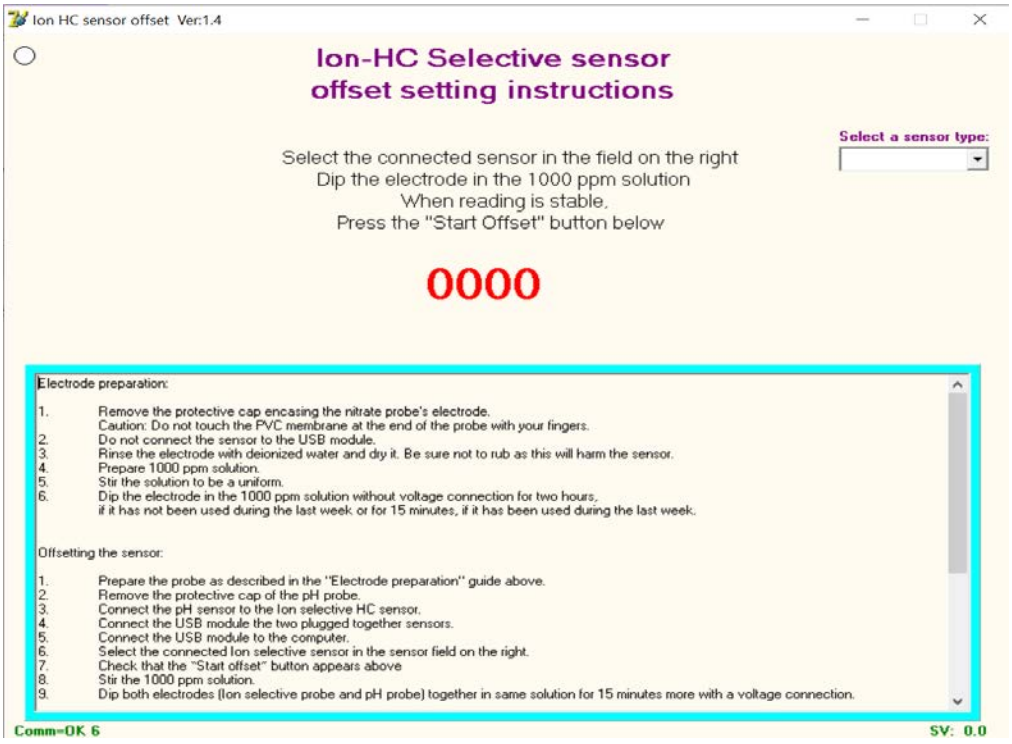
4. Do not connect the sensor to the USB module.

5. Rinse the electrode with deionized water and dry it. Be sure not to rub as this will harm the sensor.

6. Dip the electrode in the 1000 ppm solution without voltage connection for two hours, if it has not been used during the last week; or for 15 minutes, if it has been used during the last week.

* **Offsetting the sensor:**

7. Run the "**Ion_selective_HC_sensor_offset_Vx.y.exe**" file (inside the folder) and follow the instructions.



Note:

The following instructions should be performed separately for each Ion selective sensor.

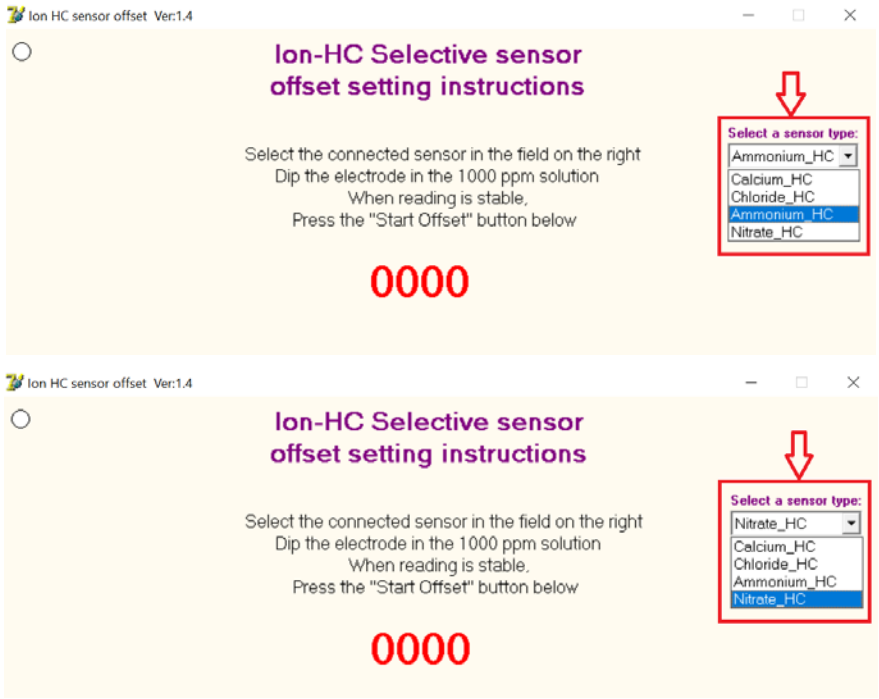
8. Prepare the probe as described in the "**Electrode preparation**" guide above.

9. Remove the pH probe's protective cap.

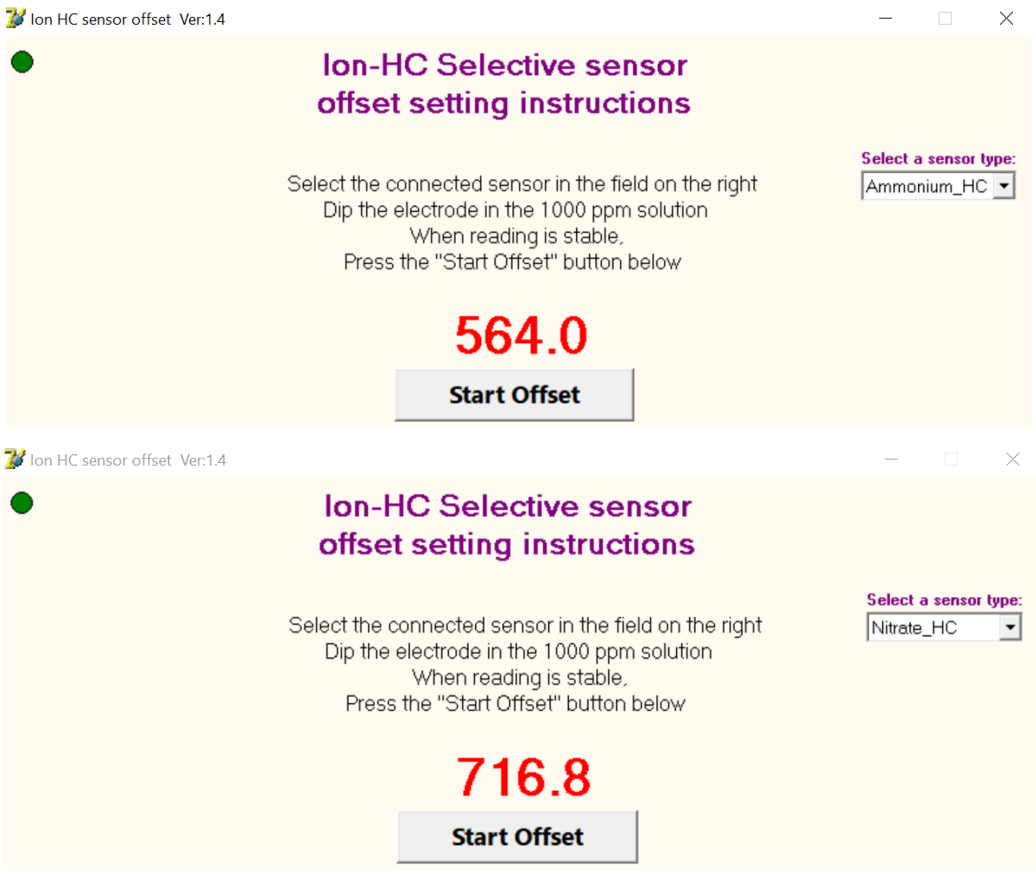
10. Connect the pH sensor to the Ion selective HC sensor.

11. Connect the USB module the two plugged together sensors.

12. Connect the USB module to the computer.
13. Select the connected Ion selective sensor in the sensor field on the right.



14. The upper screen will be changed to the following one:



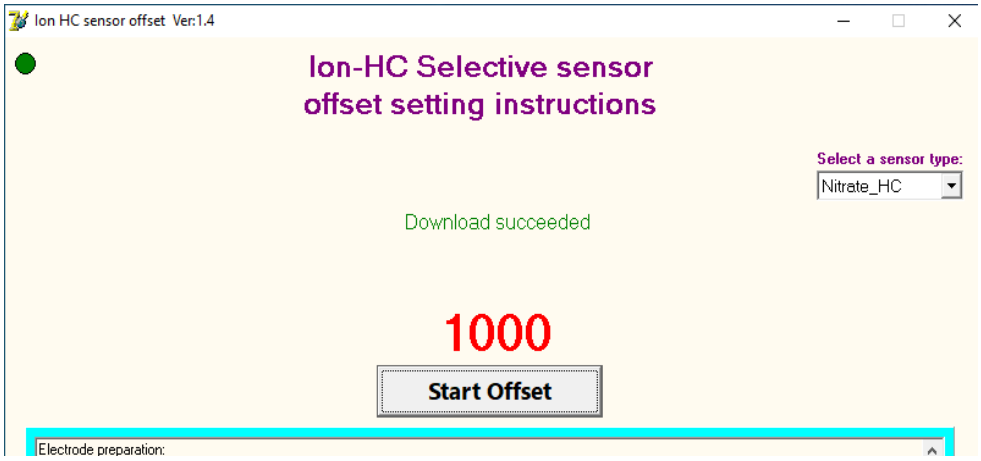
15. Check that the "**Start offset**" button appears above.

16. Stir the 1000 ppm solution.

17. Dip both electrodes (Ion selective probe and pH probe), with a voltage connection, in the same solution for another 15 minutes.

18. The current reading of the sensor will appear on the screen.

19. After a stable reading has been reached, press the **"Start Offset"** button and wait for the process to complete.



20. Rinse the electrode once more with deionized water and dry it.

21. The sensor is now ready to be used.

22. Close the bottle of the 1000 ppm calibration solution.

*** <u>Sensor setup</u>**

23. Connect the ammonium NH_4^+, the nitrate NO_3^- and the pH sensors to the USB-200 module in a chain.

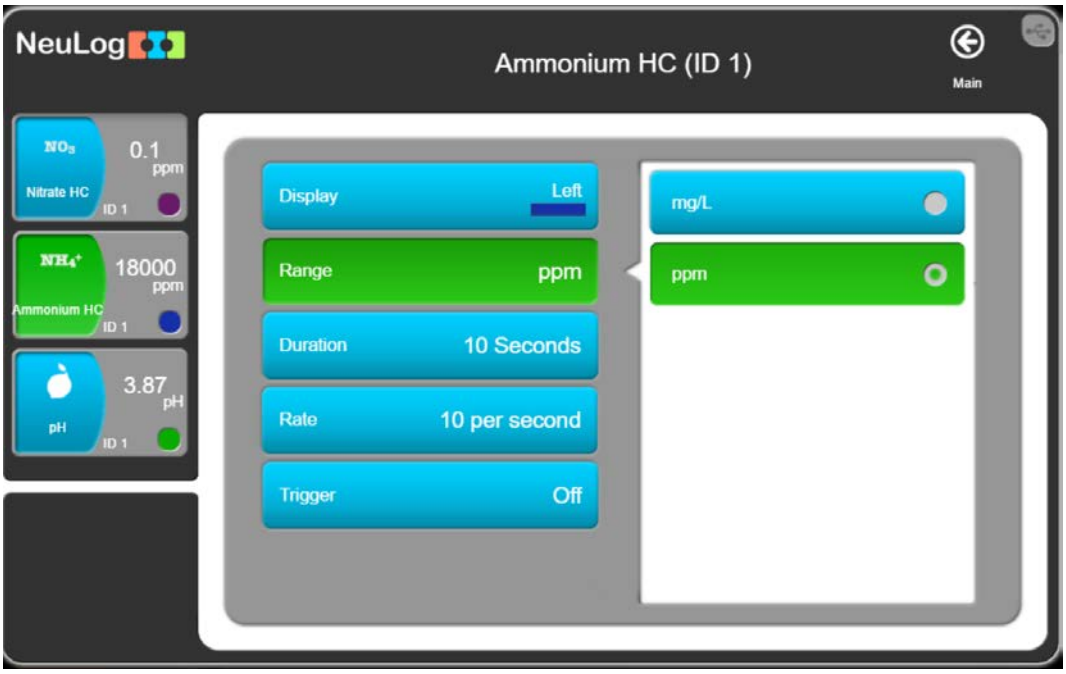
24. Connect the USB-200 module to the PC.

25. Run the NeuLog application and check that the sensors are identified.

*** <u>Experimental parameter setup</u>**

26. Click on each **Sensor's Module** box of the Ion selective sensors at a turn.

27. Select the "ppm" button to change the sensors' ranges.



28. This experiment is done in single step mode so the experiment duration and sample rate will not be set.

Add ionic strength adjustment solution (ISA) to the solution samples to be measured (at a ratio of 1:50) to stabilize the ionic strength in the solution sample to be measured and increase the measurement accuracy.

*** <u>Testing and measurements</u>**

29. Attach the two electrodes together to the extension clamp connected to the utility stand. This is for measuring ammonium and nitrate together. Another option is to connect first the nitrate electrode, make the measurements and then connect the ammonium electrode.

30. Ammonium can be detected better at a low pH. Adding some acidic buffer (not lower than pH 4) to the sample may help detect the ammonium better.

In case the sample solution to be measured has a lot of chloride, it is necessary to add an interference reduction solution (ISISA) to the nitrate electrode to remove interfering ions.

31. Place the beaker with the tap water underneath the electrodes and insert them into the beaker.

32. Click on the **Single step** icon and then on the **Table** icon .

33. Rinse the electrodes with distilled water and then replace the tap water beaker with the sample from the aquarium.

34. Click on the **Single step** icon .

35. The following table includes measurements of tap water and an old aquarium sample.

36. Click on the **Export** icon  and then on the **Save value table (.CSV)** button to save your graph.

37. We can see that the aquarium has higher levels of ammonium and nitrate compared to tap water. Ammonium levels are very low, and stayed low even after the pH 4 buffer was added (not shown). This means the ammonia levels in the aquarium are very low and the fish are at a good condition.