Stefan Sjöholm

Measuring Nitrate in Brackish Water:
A Spectroscopy Technique Comparison

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Abstract

Author:	Stefan Sjöholm
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Nitrate levels are often used as a key indicator when assessing the state of eutrophication of water bodies. Several parameters need to be considered when choosing the appropriate technique for the measurement. This thesis compared a uv spectrometry probe with a test cuvette kit to assess the probe´s suitability for laboratory research involving nitrate. The work was done as a part of a larger project lead by Origin by Ocean´s ongoing research on the nitrate intake rate of *Fucus vesiculosus*. A total of 33 paired samples were collected from brackish water samples from different sources. In addition to the overall comparison, a series of smaller experiments were performed. The thesis project ended with a three-way comparison with the addition of an accredited laboratory spectroscopy analysis in the form of a calibration curve. These samples were measured using both techniques and the acquired data was analyzed for statistical relevance as well with the Bland Altman method. The results show that the probe did not only give results with agreement to the other techniques but also due to its high temporal frequency and automation, produced no anomalies during the entire time and was therefore the best performing technique.

Keywords: nitrate, photospectrometry, method comparison, Bland Altman, water quality

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Nitraattipitoisuutta käytetään usein indikaattorina vesistön rehevöitymisen tason arvioinnissa. Mittausmenetelmää valitessa pitää ottaa useampi tekijä huomioon. Tämä opinnäytetyö vertaili jatkuvatoimisen, spektrometrialla toimivan mittaria ja valmisputkitestiä. Tämän työn tarkoitus oli tarkistaa jatkuvatoimisen mittarin soveltavuutta nitraattiin liittyvään laboratoriotyöhön. Työ tehtiin osana isompaa tutkimustyötä Origin by Oceanin toimesta, joka tutkii rakkohaurun kykyä poistamaa nitraattia vedestä. Työn aikana tutkittiin yhteensä 33 näytettä eri alkuperistä. Kokonaisyhtenäisyyden lisäksi tehtiin pienempi koesarja, jossa otettiin näytteitä tiheämmällä välillä. Työn viimeinen osa oli kalibrointisuoran tekeminen, jossa molempien menetelmien rinnakaisnäytteet vietiin akreditoituun laboratorioon analysoitavaksi. Data analysoitiin mm Bland Altman menetelmällä ja lineaarisella regressioanalyysillä. Tulokset osoittavat, että mittari antoi hyvin vastaavanlaisia tuloksia kuin muut menetelmät. Automaation ja tiheän mittausvälin ansiosta se oli vertailun luotettavin menetelmä.

Avainsanat: nitraatti, photospektrometria, menetelmä vertailu, Bland Altman, veden laatu

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List of abbreviations

CDOM	Colored dissolved organic matter. Water quality parameter. Consist mainly of tannins from the breakdown of plant material.
CRM	Certified reference material. Materials used for quantification reference in validation and calibration.
DOC	Dissolved organic carbon. A water quality parameter that quantifies the amount of dissolved organic matter.
FIA	Flow injection analysis. A method for rapid analysis of several samples in for example spectroscopy or chromatography
IOT	Internet of things. Term used to describe hardware that is connected to the internet for data download or upload.
LOA	Limits of agreement. Used a measure for interval between the difference between two samples occur.
mg/l	Milligrams per liter. Unit of concentration.
PAR	Photosynthetically active radiation. The amount of light radiation usable by photosynthetic plants. Measured in micromoles per seconds or Einsteins
PSU	Practical salinity units. Unit to express the salinity, or conductivity, of water. Equivalent to parts per thousand or g/kg.
UV	Ultraviolet. Refers to the ultraviolet range of light between 315 and 400 nanometers

1 Introduction

Nitrogen is one of the essential elements for life. Nearly 80% of Earth's atmosphere consists of nitrogen in its basic molecular form, as a double atom molecule, N_2. Molecular nitrogen is turned into inorganic nitrogen compounds via various chemical and biological processes. These compounds are then used by algae, plants, fish and other animals to create organic nitrogen compounds like amino acids by nitrogen assimilation. However, and excess of nitrogen in its different forms have been linked to a wide array of ecological problems like eutrophication, especially in the Baltic Sea. In an extensive paper by Galloway *et al.* (2004), the rise in signs of eutrophication can be linked to the explosive increased use of artificial fertilizers containing high amounts of ammonium (NH_4^+), ammonium nitrate (NH_4NO_3) and nitrate (NO_3^-) in the 1960´s. (Galloway 2004; Howarth 2006)

Identifying nitrogen and its most common inorganic form, nitrate (NO_3^-), in aquatic systems is possible by many means. Care should be taken in selecting the appropriate form of measurement. Factors like measuring range, temporal resolution and financial and labor resources all play a vital role in determining the suitable approach to any monitoring study.

This thesis compared 3 different techniques of nitrate determination in a varying set of brackish waters and in doing so determines the agreement between the methods. One method was an OPUS continuously monitoring photospectrometer probe. That data was then compared to other spectrophotometric technique using colorimetric reaction to determine nitrate. The comparison techniques were the HACH Lange LCK 339 cuvette sample test and water sample analysis services provided by the accredited laboratory at Länsi-Uudenmaan vesi ja ympäristö Ry.

The work took place between May and November 2021 and is divided into three separate forms. The first and largest portion was discrete samples taken

throughout the research period. Secondly, single experiments with higher temporal resolution involving the brown algae *Fucus vesiculosus* and its ability to intake nitrate in solution. At the end of the period, all three analysis techniques were employed in creating a calibration curve. The aim of this thesis was to determine if the different techniques showed advantages or disadvantages. By comparing the techniques, this thesis aims to answer the following questions:

- Is the data obtained by the OPUS probe reliable and comparable to other spectroscopy techniques?

- Does the OPUS probe bring advantages over other techniques to studies regarding nutrient flux in water?

2 Theory

The reason for the technique comparison is to assess if a continuous monitoring probe, which is usually meant for long term monitoring, can be utilized in laboratory nitrate studies. The benefit of the probe is real-time data with extremely high temporal resolution. Other time effective methods of nitrate determination are ready to use cuvette tests. These however have several flaws, including waste that needs specialized disposal as well as the human error element. If the sample is not analyzed in situ, neglecting to adhere to proper handling and storage protocols might have a huge effect on the quality of the sample. For continuous monitoring, it is also paramount to follow a strict sampling plan to assure the consistency of samples. These factors mean that the accuracy and reliability of said tests is dependent on the user. The main difference between the measuring methods is that while the OPUS reads nitrate straight from the medium, the LCK 339 and the laboratory analysis uses reagents to produce an azo dye prior to identification.

2.1 Nitrate and the nitrogen cycle

Nitrate is an oxidized anion form of nitrogen atom with the chemical formula NO_3^-. It shares covalent bonds with 3 oxygen atoms, forming a triagonal molecule, as seen in Figure 1. The formal charge of the ion is -1. Nitrate is highly soluble in water and is one of the main forms of inorganic nitrogen in both freshwater and marine water bodies.

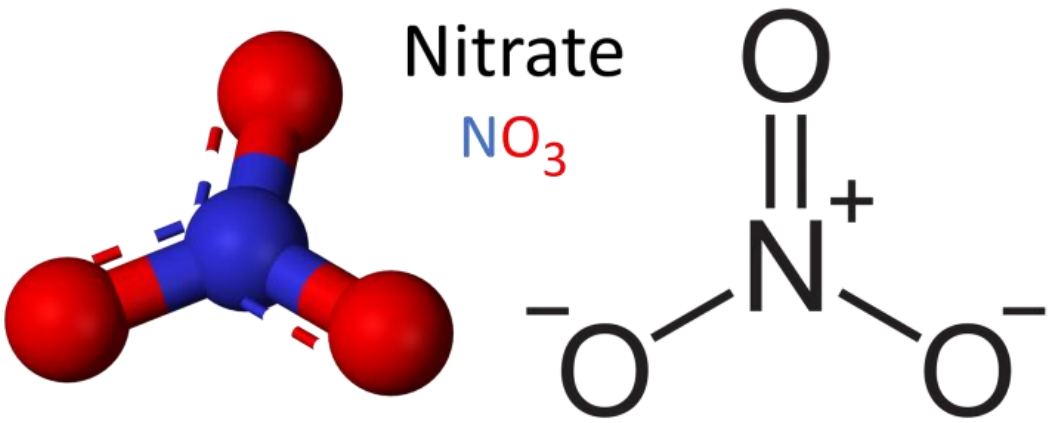


Figure 1 Molecular structure of the Nitrate ion

The influx of atmospheric gaseous nitrogen (N_2), into water bodies are mainly through influent, or surface runoff, and atmospheric fixation through either precipitation or nitrogen fixating surface algae. Within the water body, there is a constant flux between the different inorganic forms of nitrogen, the main forms being nitrate (NO_3^-), Nitrite (NO_2^-) and ammonia (NH_4^+), as shown in Figure 2. The main processes in this flux are nitrification and denitrification. Nitrification occurs when nitrification bacteria (Nitrosomonas) oxidize ammonia into nitrite followed by nitrite being oxidized further into nitrate by Nitrobacter. Denitrification is when nitrates and nitrites are reduced to gaseous nitrogen (N_2) by denitrification bacteria. The dissolved nitrogen can then be assimilated by

nitrogen fixating microorganism to form organic nitrogen compounds or ammonia. (Wetzel 1983)

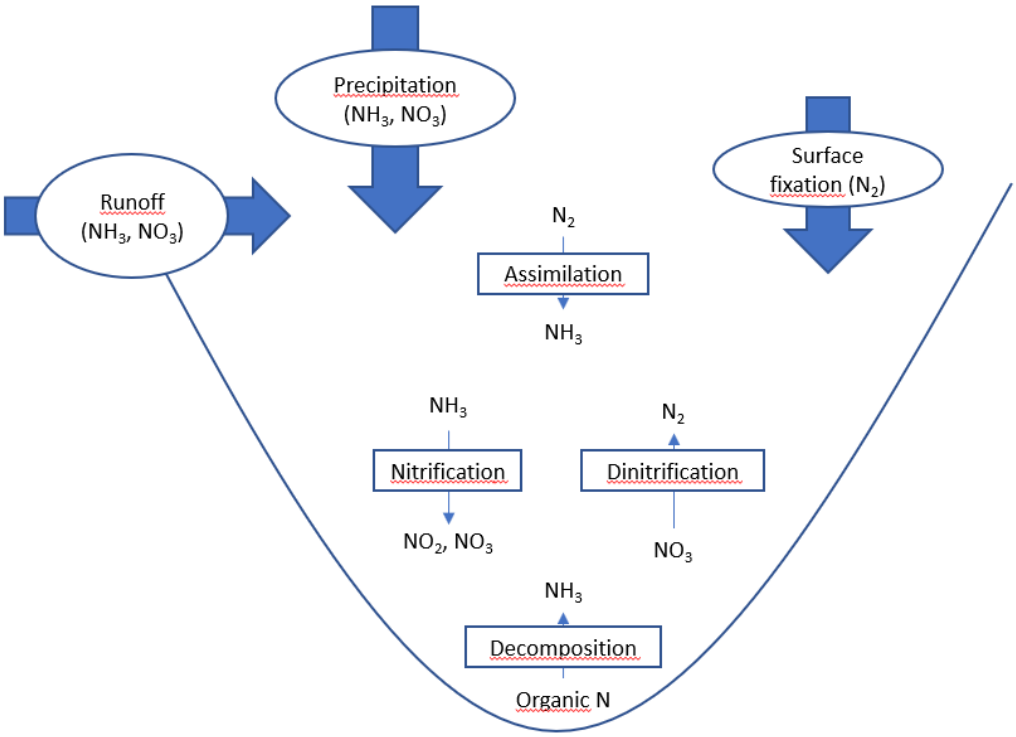


Figure 2 Simplified diagram of nitrogen flux in water bodies (Wetzel 1983)

Previous studies have shown that the anthropologic fixation of nitrogen and its utilization in agriculture has vastly altered the balance of the nitrogen cycle. Some studies have linked the rise in fresh water and ocean eutrophication to the commercialization of the Haber-Bosch process. The Haber-Bosch process is essential to the production of ammonia rich fertilizers. It fixates atmospheric nitrogen into ammonia and was develop in the early 20th century and awarded the inventors, German chemists Fritz Haber and Carl Bosch, the Nobel prize in 1910. (Galloway 2004; Gilbert 2014)

Nitrate is measured in either nitrate (NO_3^-) or nitrate nitrogen (NO_3-N). The former is the value of the entire molecular mass of the nitrate ion while the latter is the fraction of the ions molecular mass that is derived from the nitrogen

molecules only. As the molecular mass of nitrogen is 14,01 g/mol and the molecular mass of nitrate is 52,01 g/mol, the conversion rates between the two are as follows:

$$NO_3^- \rightarrow NO_3 - N = x \times 0{,}2259 \quad (1)$$

$$NO_3 - N \rightarrow NO_3^- = x \times 4{,}4268 \quad (2)$$

Prior research in nitrate determination shows that UV spectroscopy is a reliable and efficient method (Edwards 2001). Nitrate absorbs ultra-violet (UV) light at a wavelength of 200-205 nanometers (nm). Depending on the source of the sample, suspended solids, salts and other contaminates might have to be removed by filtering to achieve accurate results. There are also ion selective electrode instruments, but they are for freshwater only as other conductive ions in salt water interfere with the measurement.

The same model OPUS probe has been used in studies regarding nitrate in the Baltic Sea. It was attached to a conductivity-temperature-depth (CTD) probe to measure a vertical nitrate profile. CTD probes are sensors that measure said parameters whilst being lower to the bottom of the water body. By adding the OPUS, researchers can see the spatial nutrient gradient in the water column. (Meyer 2018)

If the sample is high in dissolved organic carbon, it might interfere with photospectrometric analyses. The effect of DOC that absorbs UV light at similar wavelengths as nitrate have been studied. As DOC also absorbs UV light at higher wavelengths, a two-wavelength approach was tested. It showed that by analyzing the same sample at two different wavelengths, one specifically for nitrate and another that DOC absorbed but nitrate did not, showed good results in overcoming carbon-based influence in the measurement values. (Edwards 2001) This approach is utilized as so-called turbidity correction. Previous studies have discussed that more cost and work intensive analyses methods

may prove more accurate, especially in determining lower levels of concentrations (Bartosova, 2012). However, ease of use and cost effectiveness may prove more important than the error margin when seeking high temporal resolution.

2.2 Origin by Ocean

The research in this thesis was performed for Origin by Ocean (Ocean Orchard Oy) to proof their research data and provide as a quality control for their measurements. Origin by Ocean is a company founded in 2019 by Mikael Westerlund and Mari Granström. At its core, Origin by Ocean aims to utilize marine biomass to create value materials and in doing so, removing excess nutrients from the Baltic Sea. One of these biomass feedstocks will be the brown algae *Fucus vesiculosus*, see Figure 3, commonly known as bladder wrack, as brown algae have been shown to effectively assimilate ammonia and nitrate (Wallenius 1984).

Figure 3 An adult *Fucus vesiculosus* specimen

As bladderwrack is a key species in the Baltic and already suffering from dwindling populations (Vahteri 2016), Origin by Ocean is intent on not utilizing wild biomass as their feedstock. Rather, Origin by Ocean aims to plant and grow macroalgae in the Baltic Sea. By doing so, the removed biomass and all the nutrients it has assimilated in its growth, will have a direct effect on the overall nutrient load of the Baltic Sea. It is therefore that the company is researching the intake quantity and rate of nitrate by *Fucus vesiculosus* in laboratory settings. Having this crucial data, they can then estimate the total amounts of nitrogen is removed from the Baltic with each harvest. The accuracy and reliability of this data will also play a crucial part in future research.

The main premise for this thesis is the ongoing measurement of nitrate flux in a variety of water mediums with emphasis on assimilation, or intake, by Fucus Veisculosus. The bulk of the research and experiments were performed at Origin by Oceans premises in Otaniemi, Espoo.

As *Fucus vesiculosus* are photosynthesizing brown algae, they need a photosynthetic radiation emitting (PAR) light source. The tanks were the *Fucus vesiculosus* are kept are therefore equipped with fluorescent lights that simulate a natural light and day cycle. Furthermore, to simulate a natural environment for the *Fucus vesiculosus*, each tank is fitted with a flow through pump that creates a current and aids in the mixing of the water.

For most of the research, the OPUS probe was submerged in the overflow tank to constantly measure the nitrate concentration. Occasionally, the sonde was also used for other purposes, for example, determining nitrate concentrations in sample and field measuring.

2.3 Spectrophotometric cuvette technique

The LCK 339 Nitrate cuvette test is a ready to use, analytical consumable tool for the determination of NO3 within the range of 0.23-1.,5 mg/l NO_3-N in an array of water types. The test is delivered with pre-loaded cuvettes and accompanying reagent. The cuvettes are filled with a set amount of 2.6-dimethylphenol. The reagent, which is added after the sample, contains sulfuric and phosphoric acids. Nitrate ions in the sample react with dimethylphenol to create 4-nitro-2.6-dimethylphenol. (Rudde 2012)

The procedure for the LCK 339 can be seen in Figure 4. The test is performed by adding 1 ml of sample into the cuvette. Then 0,2 ml of the provided reagent is added. The cuvette is then sealed and agitated to thoroughly mix the components. The solution is left to react for 15 minutes before inserted into a spectrophotometer.

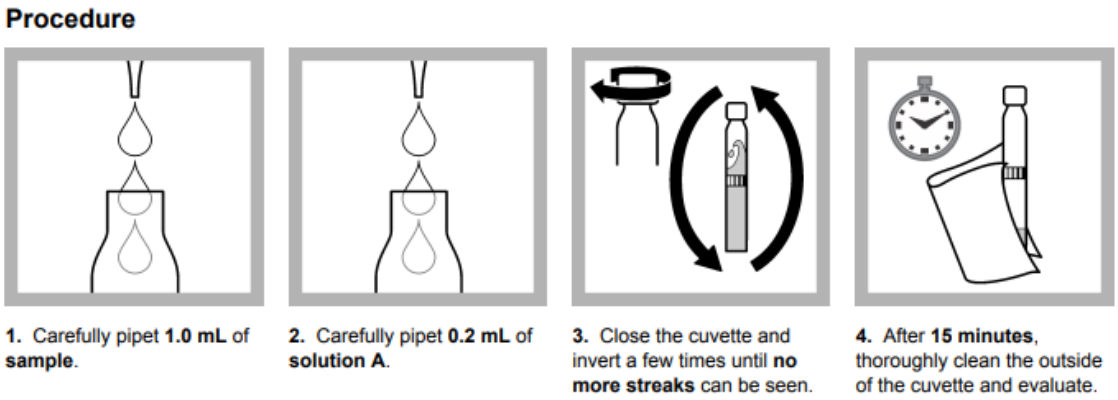


Figure 4 Illustration of the LCK 339 procedure

The cuvette is labeled with a bar-code that is automatically scanned by the spectrometer. The bar-code provides the device with details regarding the cuvette that is being analyzed and changes internal settings accordingly. The sample is analyzed, and the measurement is shown on the LCD screen. The result is showed in milligrams per liter (mg/l) and the value can be set to either nitrate nitrogen (NO3-N) or nitrate (NO3)

The method is a proprietary method under the European Patent Office, EP2500722B1, created by Heinz Rudde and currently assigned to Hach Lange GmbH.

The method of utilizing 2.6-dimethylphenol to measure nitrate was used prior to the invention but usually had limitations due to chlorides. This limitation would reduce the accuracy when measuring salt and brackish water (Rudde, 2012).

The technical data for the validation of the LCK 339 cuvette test states that calibrations produced a linear function. This function shows no evidence of outlier data points and is normally distributed. The detection limit of the method is 0,210 mg/l NO_3 or 0,047 mg/l NO_3-N, meaning that it is the lowest concentration that the method can determine that nitrate is present in the sample. The quantification limit is 0,629 mg/l NO_3 or 0,142 mg/l NO_3-N, meaning that this is the lowest concentration at which the method can provide reliable result of the concentration. The LCK 339 test range is set at 1-60 mg/l NO_3, for which the method standard deviation is 0,19mg/l NO_3 and 95% confidence interval is ± 0,45 mg/l NO_3. HACH LANGE GmbH performed said calibration within the guidelines set by international standard for calibration ISO 8466-1 Water Quality – Calibration and evaluation of analytical methods and estimation of performance characteristics as shown in the official Quality certificate for the methods (ISO 8466-1).

The LCK also has a recommended sample temperature of 20° C, which is not always convenient as samples taken might suffer from nutrient flux as a result from warming or cooling.

Furthermore, samples that are high in suspended solids or colored dissolved organic matter (CDOM), which often occurs in samples taken in estuaries, should be prepared prior to sampling in the cuvette as both interfere with the analysis. CDOM levels must be under 200 mg/l for the test to give reliable results. Other interfering elements that interfere are chromium and cobalt in

amounts over 5 mg/l and potassium, chloride and sodium at levels exceeding 500 mg/l, as can be seen in appendix 4.

2.4 DR3900^{TM} Spectrophotometer

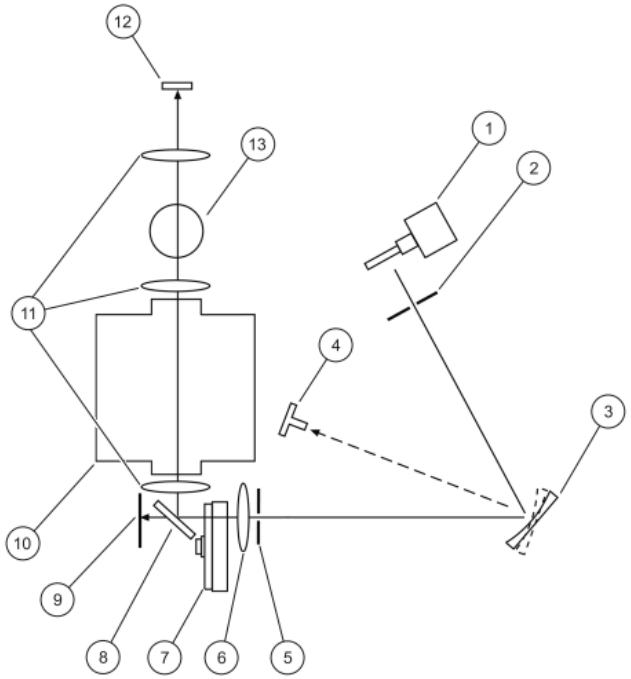
The DR3900^{TM} is a user-friendly, automated benchtop spectrophotometer developed and manufactured by HACH LANGE GmbH. It is specifically designed for the HACH LANGE GmbH cuvette test product range. The system is completely automated for ease of use and high accuracy. The instrument has an inboard barcode reader that identifies the cuvette to be analyzed and programs the settings, including specific wavelength spectrum, accordingly. Every sample is analyzed 10 times, where after the inboard software eliminates stray and outlier readings to nullify the probability of false results due to imperfections in the cuvette, i.e., dirt, fingerprints and scratches.

It uses a halogen lamp to expose the cuvette to specific wavelengths. On the opposite side of the cuvette, a digital reading element intercepts the excess light, as seen in figure 4 and the absorbance at each wavelength is calculated and analyzed, as shown in Figure 5. With the pre-programmed calibrations, results are then presented as concentrations of the specific compound on the LCD screen.

3.7 Beam path

Figure 7 shows the beam path of the DR 3900.

Figure 7 Beam path



1	Halogen lamp	**8**	Splitter mirror
2	Entrance slit	**9**	Reference element
3	Grating	**10**	Cell compartment (2) for rectangular cells
4	Grating angle indicator	**11**	Lens
5	Exit slit	**12**	Reading element
6	Lens	**13**	Cell compartment (1) for round cells
7	Filter wheel		

Figure 5 Diagram of the beam path of the DR3900, taken from the user manual (Hach Company 2020)

The wavelength range of the DR3900™ is 320-1100 nm with an accuracy of ±1,5 nm at 340-900 nm and a resolution of 1 nm. (Hach Company 2020)

2.5 OPUS Trios

The Trios OPUS is a spectral sensor for online measurement of both nitrogen and carbon compounds. It is a high-end miniature spectrophotometer with real-time Internet of Things (IoT) capabilities to deliver analysis data to the chosen platform. It utilizes spectral analysis by light absorption to determine specific ions and compounds in solution. Its applications areas as stated by the manufacturer

(TriOS Mees- und Datentechnik)) are environmental monitoring as well as industrial monitoring e.g., water treatment plants. The light pathway comes in several lengths, ranging from 0,3 mm to 50 mm. These affect the measuring range of the instrument and should be selected according to the expected concentration and turbidity of the monitored water. It also has inboard turbidity cancellation for high accuracy and reliability. The detector has a lower wavelength range, 200-360 nm due to the specific compounds and has a resolution of 0,8 nm.

The OPUS utilizes a Xenon flash lamp to emit light through the optical path. This light, or rather the remaining light is detected and analyzed by the inboard spectrometer, as seen in Figure 6 The absorbance of the measured medium is then compared to the absorbance of ultra-pure water. Depending on the specific compound to be determined, the absorbance at different wavelengths is calculated.

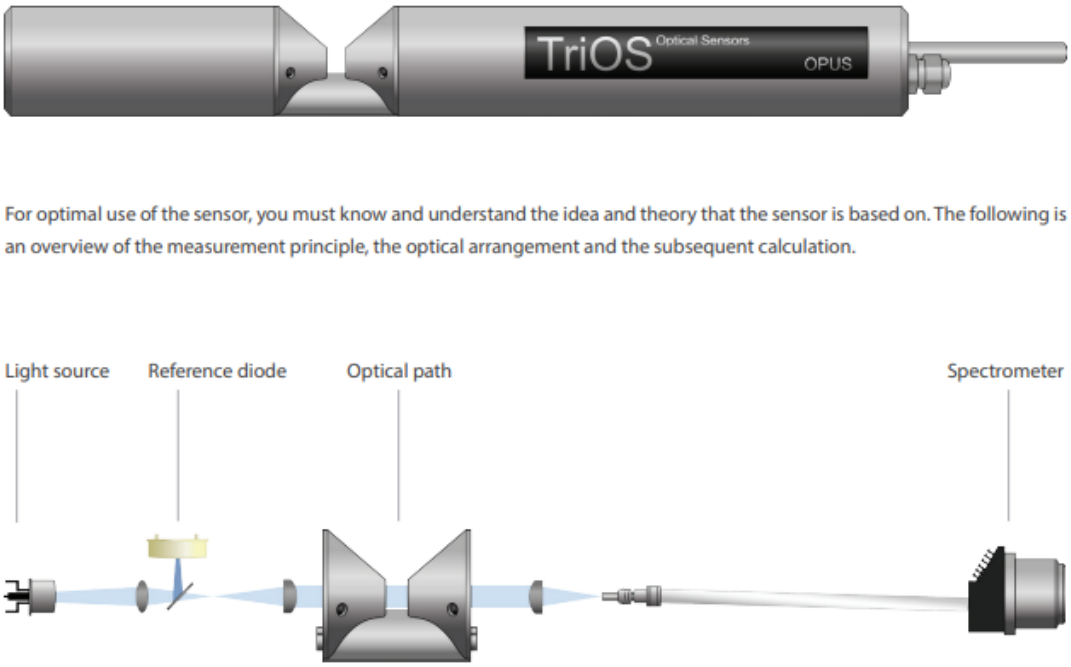


Figure 6. Diagram of operating principle of the OPUS sonde, taken from the OPUS user manual (TriOS 2017)

As stated by its manufacturer, it is usually used as a continuous monitoring apparatus, where small temporal fluctuations are mitigated. These fluctuations can be caused by microcurrents in the water, suspended air bubbles or large particles. These can have a strong effect on the transmitted data and can even cause the meter to spike, i.e., measuring peak values. However, the lens housing is coated by a nano-film to prevent fouling and reduce air catchment on the window. It is also possible to attach the sonde to a compressed air tank. This then allows the OPUS to deliver a jet of compressed air into the pathway prior to measurements to avoid obstructions of light.

2.6 Bland-Altman Method

When comparing analysis techniques, a standard practice is to use the simple linear correlation methods or statistical tests. Studies have shown that correlation is not necessarily agreement (Bland 1986). The Bland-Altman plot is a method comparison tool for visually representing the agreement of two different analysis techniques. Not only does it visualize the scatter of differences but also in relation to the actual value measured. This method was chosen so that the agreement at different concentrations could be estimated.

Originally developed for medical research, the Bland-Altman method evaluates the mean differences between two methods of measurement. The method was developed by English statisticians John Martin Bland and Douglas Graham Altman. In their research, Bland and Altman discuss the need to not only see the average agreement of measurements but also the agreement of induvial agreement. If one of the methods has a tendency to give both high and low deviations from the control methods in equal measure, the overall agreement is high even though individual deviations are strong, as shown in Figure 7 (Bland 1986; Bland 1999)

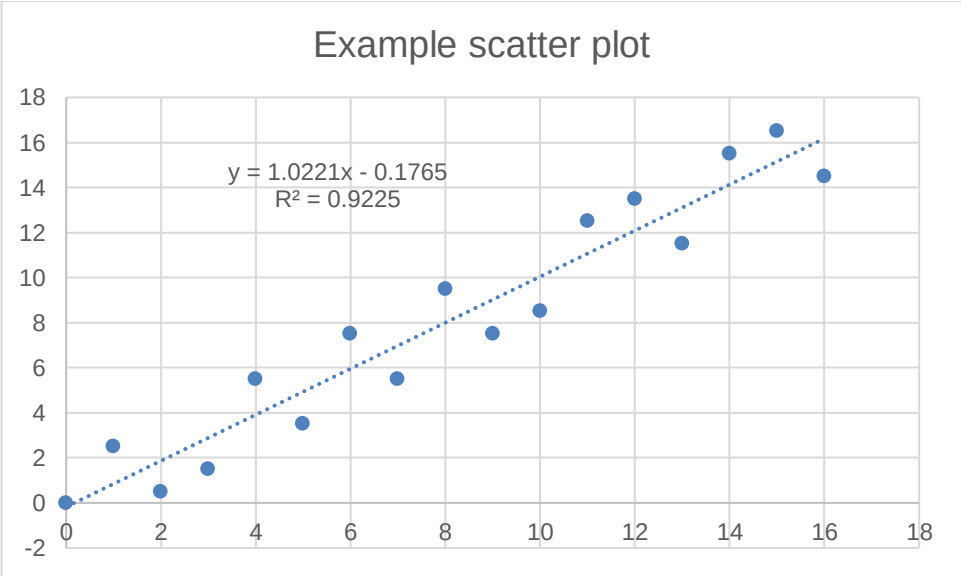


Figure 7 Scatter plot showing high correlation with persistent deviation

The method includes plotting the data with a bias and an upper and lower level of agreement (LOA). The data points are plotted with the mean of the paired values on the x-axis and the difference of the same values on the y-axis. Thus, for a given data pair (S) b, the coordinate would be plotted according to Equation (3).

$$S(x,y) = (\frac{a+b}{2}, a-b) \tag{3}$$

$$a\ is\ \ value\ of\ technique\ 1$$

$$b\ is\ \ value\ of\ technique\ 2$$

The bias is calculated by the mean difference of the individual measurements. The equation for the bias is calculated using Equation (4).

$$bias = \ \bar{x} = \frac{\sum_1^n((a_1-b_1)+\ldots(a_n-b_n))}{n} \tag{4}$$

$$a\ is\ \ value\ of\ technique\ 1$$

$$b\ is\ \ value\ of\ technique\ 2$$

The limits of agreement are calculated from the standard deviation of the difference of all samples. This standard deviation is then multiplied by 1,96 for a 95 % confidence interval.

$$limits\ of\ agreement = \ bias\ \mp 1{,}96 * \sqrt{\frac{\sum_1^n((a_1-b_1)-\bar{x})^2+\ldots((a_n-b_n)-\bar{x})^2}{n}} \quad (5)$$

$$a\ is\ \ value\ of\ technique\ 1$$

$$b\ is\ \ value\ of\ technique\ 2$$

The results are a scatter plot where the bias and LOA are depicted as a straight and broken lines. In figure 7 we see an example Bland Altman plot with the sample values randomly generated with a normal distribution between 0 and 1. The bias, or the mean difference is represented with a solid horizontal line in the middle of the chart. The levels of agreements are represented as broken horizontal lines. The scatter plot itself shows the difference between any set data pair. By reading the chart from left to right, one can determine if the scattering correlates with increase or decrease in value as the x-axis represents the mean of the paired sets.

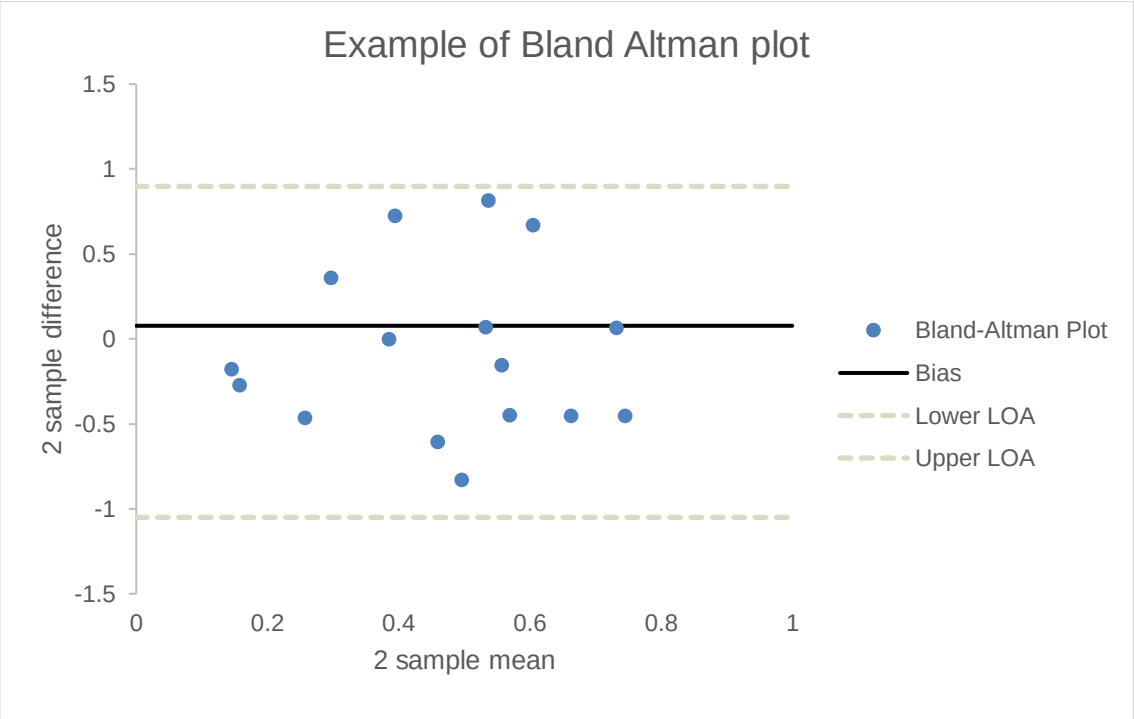


Figure 8 Example of Bland Altman plot with random values

As can be seen in Figure 8, there is no discrimination between upper and lower values on the x plane. There is also no outlier as all data points fall within the upper and lower limits of agreement. The Bland Altman plot does not show definitive answers to the agreement of the two methods but is a visual tool for assessment. (Bland 1986; Bland 1999)

2.7 Thesis boundary

The main purpose of this work is to monitor and assess the accuracy and reliability of the OPUS probe by technique comparison. The data received from the probe will be compared to discrete samples taken and analyzed by nitrate test cuvettes. This thesis will not analyze the actual results from the studies conducted by Origin by Ocean but rather analyze the validity of the nitrate measurement data. The boundary is illustrated in Figure 9.

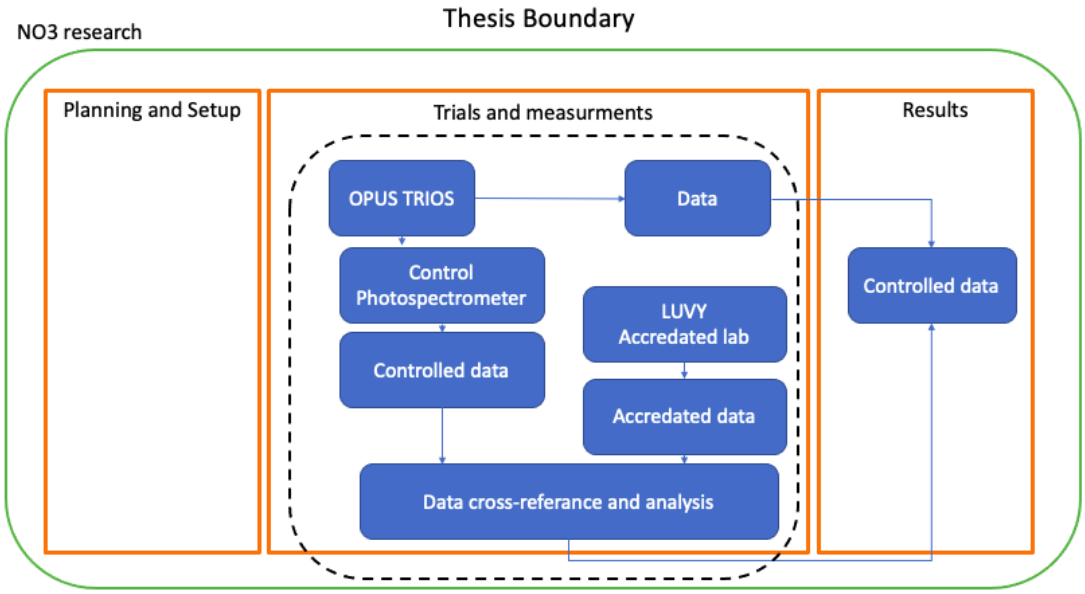


Figure 9 Boundary of thesis

The results from this thesis can be utilized by Origin by Ocean as a quality control plan for both current and future studies regarding nitrate.

3 Materials and Methods

The main instruments used in this thesis project were the OPUS probe, the LCK339 cuvette test and the DR39000 bench-top photospectrometer. The OPUS probe was under a leasing agreement from Luode Consulting OY, a market leader in water quality monitoring in Finland. The agreement included data processing and near-real time result via a web-based user interface. The raw data collected by the probe was sent via a connected logger to Luodes servers where it was processed and made visible as actual values on their website, as seen in Figure 10 This eliminated the need to manually calibrate the device and establish the correlation between absorbance and concentration.

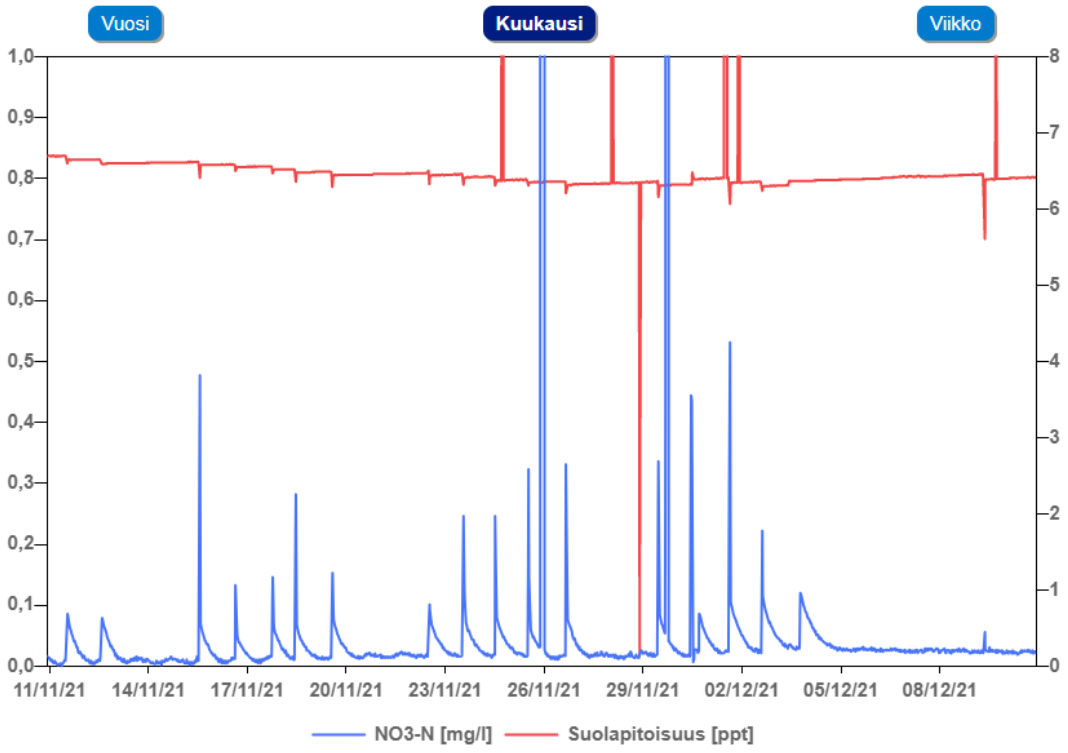


Figure 10 Screen shot of the online result page provided by Luode Consulting Oy

For the majority of the project, discrete samples from different sources were collected and time stamped to pair them with the corresponding OPUS measurement. Samples were prepared according to the LCK339 procedure without delay to minimize sample contamination or decay. These samples were collected either from the overflow tank of the ongoing experiment or from wastewater samples. These samples were often diluted to accommodate the OPUS measurement range. The sample was diluted in a cylindrical 1000 ml measuring flask whereafter the OPUS was placed vertically in the same flask.

3.1 Nutrient intake slopes

For this experiment, a separate tank system was assembled. The system was built from a single aquarium which was filled with 45 liters of tap water. 270 grams of Instant Ocean® artificial sea salt was added to replicate the natural

salinity in the water of origin, i.e., 6 practical salinity unit (psu). The tank was illuminated with two fluorescent light tubes that emitted 110 µE m-2s-1 of photosynthetically active radiation (PAR). The lights were fitted with a timer that automatically turned the lights on and off to simulate natural day and night conditions. The light/dark cycle was set at 16 hours of light and 8 hours of dark. The system also included a flow through pump to create a current within the aquarium. The pump was set at a flow rate of 200 liters / hour. Due to the lack of cooling, the temperature of the water fluctuated between 22 and 26° Celsius.

The OPUS sonde was submerged in the tank to continuously measure the nitrate concentration.

For the nutrient intake, 3 randomly selected mature *Fucus vesiculosus* individuals were used. The Fucus individuals were harvested from Porkkalanniemi, Kirkkonummi (59.979419, 24.393488) from a depth of 3 meters on the 20th of July 2021. After the harvest, the Fucus individuals were stored in dark and cold (4° Celsius) for 48 hours. These were then moved to the aquarium and acclimatized for a period of 24 hours.

For the additional nitrate, wastewater from a local aquarium (SEA LIFE Helsinki) was used. The wastewater had a nitrate concentration of 7 mg/l. This wastewater was added in pulses as to raise the nitrate concentration in the tank containing the *Fucus vesiculosus*.

The tap water naturally contained trace amounts of nitrate that was measured at an average value of 0.3 mg/l. After the Fucus individuals had acclimatized in the tank, this was reduced to a value of 0.126 mg/l.

Once the nitrate pulse was added, discrete samples were taken at regular interval and analyzed with the LCK 339 test kit. A total of 4 samples were taken during each run. The values measured from the samples were then referenced with the corresponding time stamped OPUS measurement and plotted in MS Excel.

The first LCK sample, T_0, was taken approximately 10 minutes after the pulse was added. This was to allow thorough mixing of the nitrate pulse in the tank. The following samples were taken with 2–4-hour intervals, with T_x referring to minutes elapsed since initial sample.

The nutrient runs were performed at different times of day to test if the time had any influence on the nitrate uptake of the *Fucus vesiculosus*.

3.2 Calibration curve

In defining the accuracy of any analytical method, a calibration curve needs to be established. A calibration curve is formed when a known value is measured with a given analytical method. The correlation between the known value and the measurement value is then analyzed. Depending on the type on analysis, this correlation can be for example linear, exponential or logarithmic. A linear calibration function was established in this research with 5 samples in conformity with international standard ISO 8466-1:1990 Water quality – Calibration and evaluation of analytical methods and estimation of performance characteristics. (ISO 8466-1 1990).

The aim of this experiment was to assess the accuracy of the analysis methods with known concentrations of NO_3-N. Also, using a purer solution with extremely low concentrations of interfering organic compounds would produce a more accurate comparison of the actual ability of the methods to determine nitrate. For this, a standard nitrate solution was used. The solution, (Certipur® Nitrate standard solution, 1198110500, Lot numb. HC02068511, Sigma-Aldrich) was certified reference material (CRM) with a nitrate concentration of 1000 mg/l. 5 different solutions were created by diluting the CRM. The solutions were created with a linear increase in concentration. These solutions were also used to create a calibration curve for both instruments.

For this experiment, the following solutions were created that are shown in Table 1.

Table 1 List of solutions prepared for calibration curve and their corresponding concentrations

Solution volume (ml)	CRM (ml)	CCRM (mg/l)	C NO_3 (mg/l)	C NO_3-N
250	0	1000	0,00	0,00
250	0,5	1000	2,00	0,452
250	1	1000	4,00	0,904
250	1,5	1000	6,00	1,356
250	2	1000	8,00	1,808

The calculations of the concentrations were made with the standard dilution formula number 6:

$$C_1V_1 = C_2V_2 \rightarrow \frac{C_1V_1}{V_2} = C_2 \quad (6)$$

C_1 and V_1 represents the initial volume and concentration of the dilutant and V_2 and C_2 refer to final volume and concentration of the dilution.

The samples were prepared by placing and empty 250 ml plastic bottle on a tared toploader scale (Kern 440). Using a micropipette (Thermo Scientific ™ Finnpipette™ F1), the CRM was added to the bottle. Then, the bottles were filled with de-ionized water until the scale read 250 g. This same procedure was repeated 5 times. The accuracy and repeatability of the pipette was tested prior to initiating this experiment. The pipette was tested by pipetting a set volume into a clean decanter on a tared analytical balance (KERN ADB 200-4) with a 0,0001 g accuracy. As the experiment required volumes of 1ml and 0,2 ml, these were the tests volumes and provided the following results that are shown in Table 2.

Table 2 Accuracy and reliability test of the Thermo ScientificTM FinnpipetteTM with mean and standard deviation values

	1 ml	0,2 ml
1	0.9957	0.1987
2	0.9968	0.1979
3	0.9982	0.1991
4	0.9969	0.2003
5	0.9983	0.1995
6	0.9944	0.1984
7	0.998	0.1986
8	0.9909	0.1991
9	0.9984	0.1979
10	0.9983	0.1986
Mean	0.99659	0.19881
Stan Dev	0.002399	0.0007295

The results of the pipette showed very high accuracy and repeatability with a mean of 0.997 and 0.199 for 1 ml and 0.2 ml respectively.

For the samples a set of 5 250 ml and 5 100 ml bottles were prepared. The bottles were unused HD-PE plastic bottles that were rinsed with de-ionized water to remove any residues left from the production. The samples were prepared straight into the 250 ml bottles. Once mixed, the bottles were shaken vigorously to ensure a homogenous sample. Next, 100 ml of each sample was transferred into the smaller bottles. These smaller bottles were immediately placed in a Styrofoam container with a frozen cold pack. The samples were then transported to Länsi-Uudenmaan vesi ja Ympäristö ry´s (LUVY´s) laboratory for delivery. The samples were handed over for analysis within 2 hours of initial mixing.

From each sample, 1 ml was extracted for testing with the LCK339 test cuvettes. The OPUS Trios was equipped with a calibration sleeve. The calibration sleeve greatly reduces the required amount of liquid for measurements by creating a watertight seal around the pathway, creating a cavity with the volume of approx. 80 ml. The solutions were added in order and left within the pathway for 20 minutes, i.e., two measurements. Once one sample had been measured, the pathway was emptied and rinsed with deionized water to remove residue from the prior sample.

Using linear regression, a calibration curve was established. For this curve, three different methods of measurements were used. Alongside the OPUS and the LCK cuvette tests, sample were transported to LUVY laboratory for external analysis. LUVY is a FINAS accredited laboratory that analyzes nitrate in water samples in accordance with the standard ISO 13395:1996 Water Quality. Determination of nitrite nitrogen and nitrate nitrogen and the sum of both by ISO 13395:1997 flow injection analysis (FIA) and spectrometric detection (ISO 1996.)

In the process, the sample is fed into a continuously flowing buffer solution. With metallic cadmium acting as a reagent, nitrate is reduced to nitrite. Both the nitrite originally present in the sample, as well as the nitrite formed during the reaction is then mixed with sulfanilamide to form diazonium salt. Finally, the newly formed salt reacts with N-1(1-Naphthyl)-ethylenediamine dihydrochloride with the result of a red azo dye, that can then be analyzed via spectrophotometry. This is also known as the Griess Reaction. (Sastry 2002; Pasquali 2006) This method gives the sum of both nitrate and nitrite. The original nitrite is determined by analyzing the reduced cadmium and the nitrate is the given by the difference between total nitrite and original nitrite.

4 Results

During the test period that lasted from the 26th of May to the 25th of November, a total of 33 paired data points were recorded. The datapoints were recorded with time stamps and other meta data. The entire spreadsheet can be seen in appendix 1. Several samples were discarded due to obvious errors that would have needlessly lower the accuracy and reliability of this research. These errors included paired samples taken from different sources, flawed methods of discrete sampling and obstructions in the OPUS pathway. Some might argue that these types of errors are inherent in analysis research and should be included in the overall results, but the author chose not to do so. The reasoning behind this decision is that most of the errors was due to the inexperience of the person performing them.

4.1 Comparing data between the OPUS and LCK

After analyzing all the 33 paired data point, a dataset was achieved. The methods show a high agreement with some outliers. However, the average deviation between the methods was 0.272 mg/l NO_3-N, which is high regarding the values measured.

By plotting the data in a scatter plot with the OPUS values on the x-axis and the LCK on the why axis, we can then analyze for linear correlation, seen in figure 11.

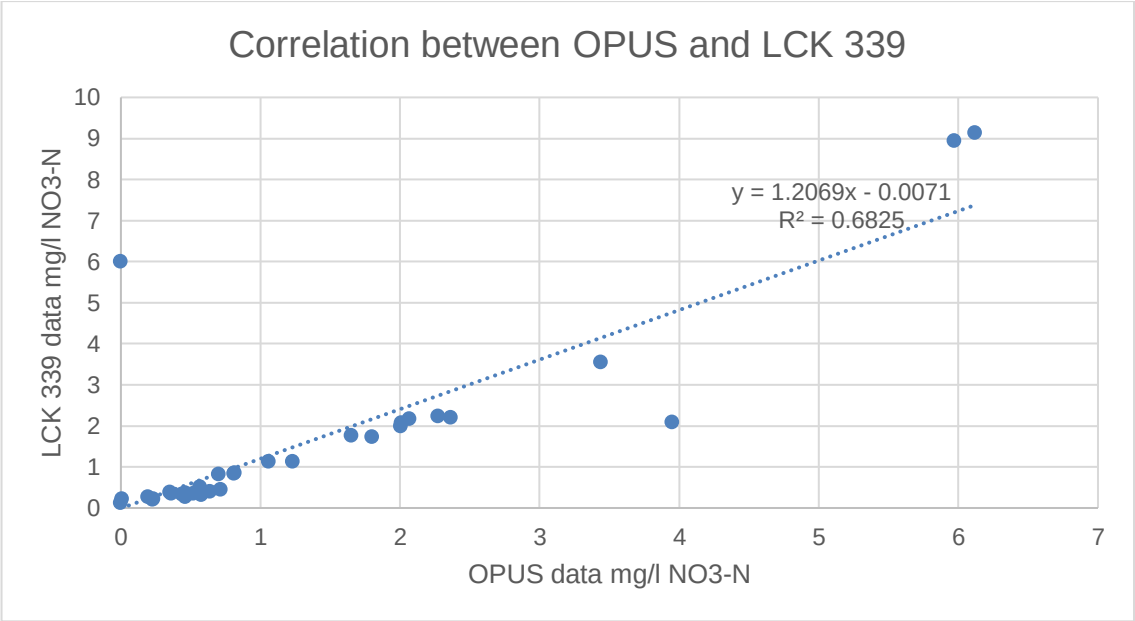


Figure 11 Scatter plot with linear correlation curve between OPUS data and LCK data with R2 value

The closer the R^2 value is to 1, the higher the correlation. This method of comparison gave a R^2 value of 0,682. Ask earlier discussed, even though the OPUS upper range, as stated by the manufacturer, is 2 mg/l NO_3-N, it may still give values above this limit. The reliability of these values is, however, not assured by the manufacturer; therefore, removing them allows comparison of the methods within their given specifications and confidence intervals. This shows a scatter plot with a higher linear correlation, as seen in Figure 12.

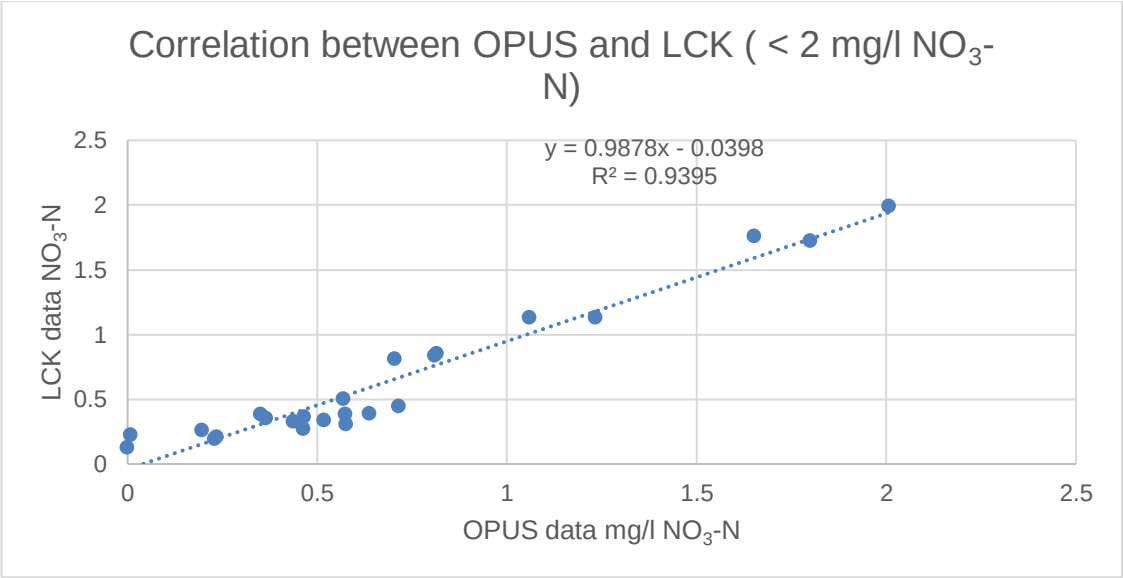


Figure 12 Scatter plot with linear correlation between OPUS data and LCK data with values below 2 mg/l NO3-N

By excluding the data points above the OPUS range limit, we see a high linear correlation coefficient of 0.9878 and a R^2 value of 0.9395. There is no clear difference in method deviation at higher and lower concentrations.

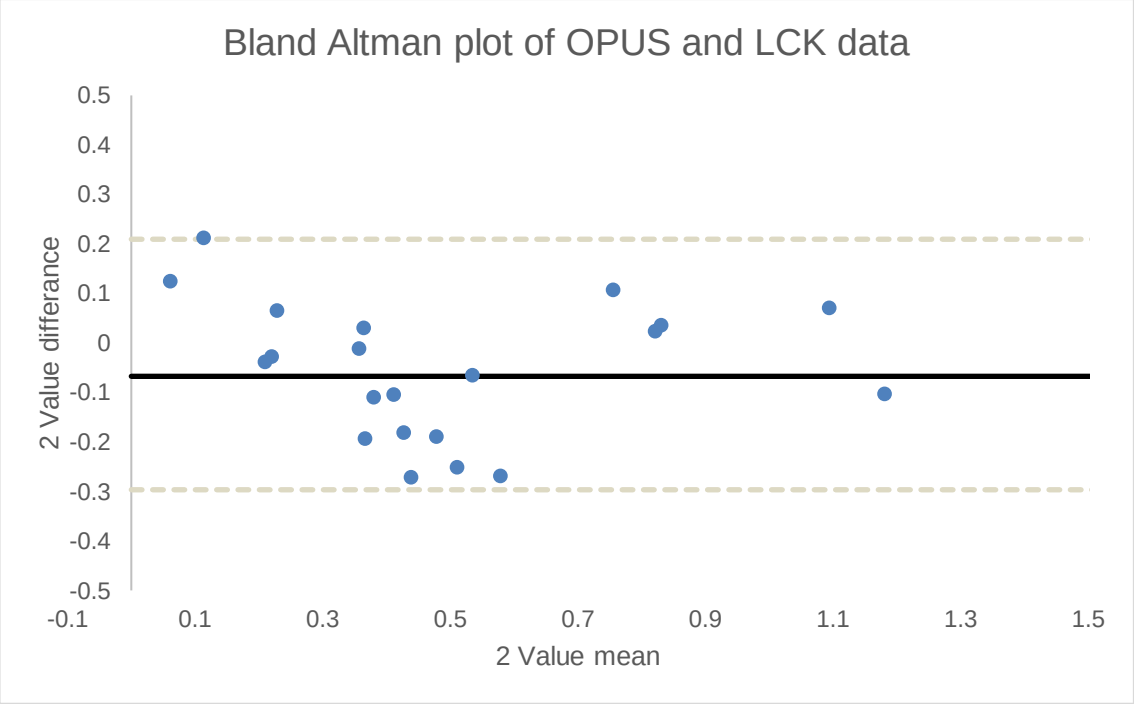


Figure 13 The Bland Altman plot shows consistent agreement with only one outlier at 0.11 mg/l NO3-N

When analyzing the data following the Bland Altman method, a clear graph was achieved, as seen in Figure 13. The plot shows that the majority of values fall in the lower range of concentrations. The values that exceeded methods range was once again excluded. There are still some values bordering on the limits of agreement. It is also difficult to state if the level of agreement varies from low and high values as the higher end of values has clearly fewer data points. It is also important to consider the values of the measurements.

4.2 Nutrient intake slopes

The agreement between the OPUS and LCK 339 data within the nutrient experiments was poor. Even though the deviation was within 0.5 mg/l range, with the highest deviation being 0.386 mg/l NO_3-N, the LCK data points showed very low correlation to the data provided by the OPUS. Due to the high temporal resolution of the OPUS sonde, any drastic changes in nitrate concentrations can be excluded. The decrease of nitrate was highly similar in every run.

However, the LCK data showed a high fluctuation rate. Every run had at least one inconsistent measurement with the LCK.

The first nutrient run was performed between 13:30 and 22:30, lasting a total of 9 hours. This nutrient run showed the best slope correlation between the two methods with very small deviations between the data points. The LCK measurements had a R^2 value for the LCK of 0,6358. The first LCK measurement at T_0 shows a deviation of -0,202 mg/l NO_3-N from the OPUS. The measurement is inaccurate due to the fact that both concentrations were known, and no large nitrogen flux could have occurred within the limited time between mixing and sampling. The following LCK measurements at T_{70}, T_{300} and T_{540} all correlate with the OPUS with deviations of -0,005, 0,029 and -0,039 mg/l NO_3-N respectively as seen in Figure 14. Furthermore, the end concentration approached the lower level of the LCK measuring range and data is therefore unreliable.

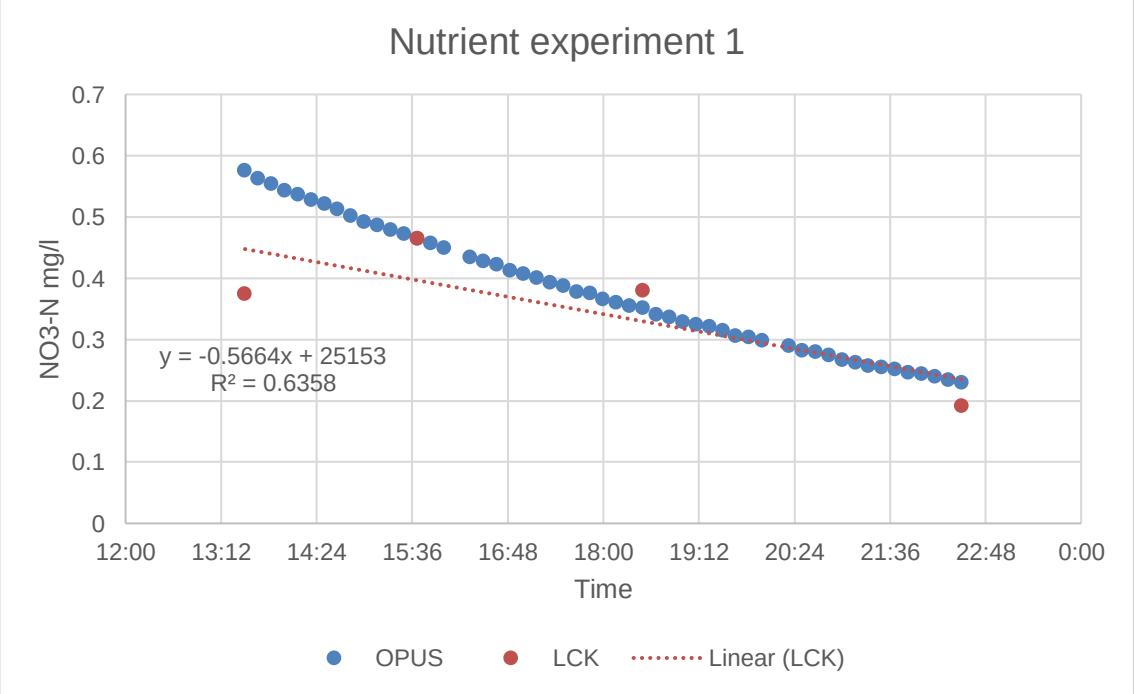


Figure 14 Scatter plot of nutrient run 1 with LCK trendline, equation and R squared value

The second nutrient run showed the highest deviations between the measuring methods with an average deviation of -0,165 mg/l NO_3-N. However, the last pair of datapoints showed a complete agreement with a 2-decimal accuracy. If the last data pair is disregarded, the slope of the LCK measurements are almost identical to the OPUS measurements, although with an average 0,22 lower value. The second nutrient run was also the only run which showed the increases in nitrate concentrations by the OPUS, although very small, as shown in Figure 15.

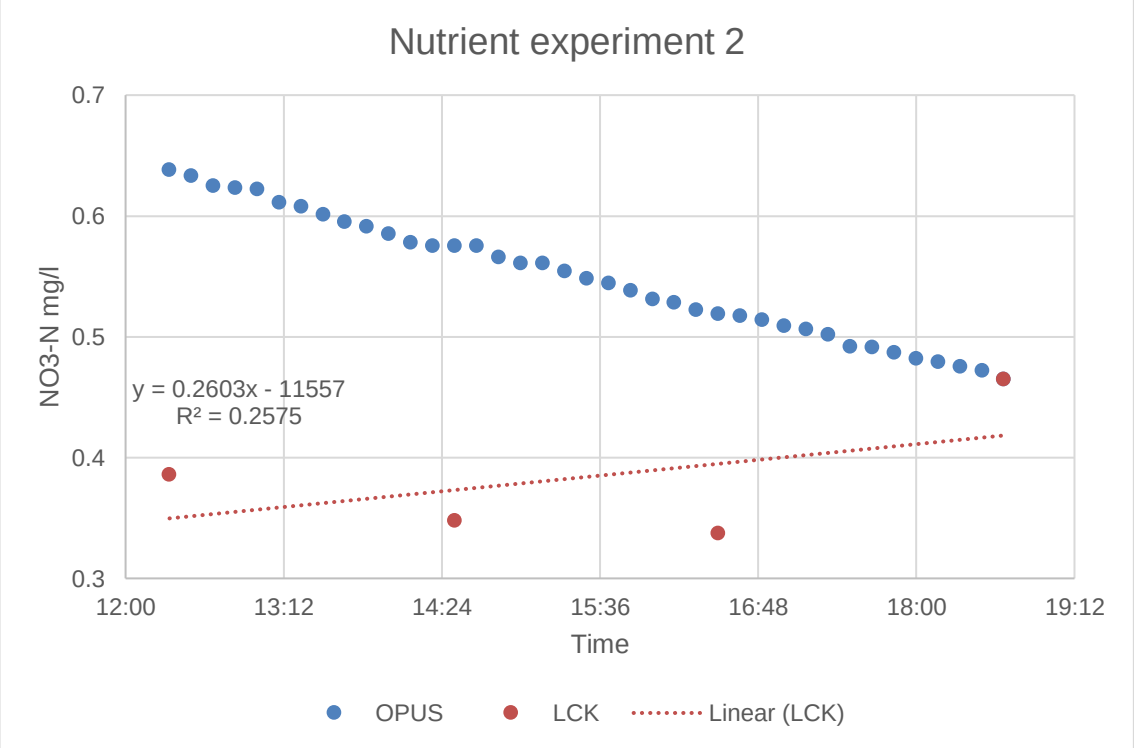


Figure 15 Scatter plot of nutrient run 2 with LCK trendline equation and R2 values

The third nutrient run was initiated at 18:00 and terminated at 01:00. The LCK data had an average deviation of -0.131 mg/l NO_3-N. This run had the single highest deviation between the methods of all the runs, -0.27 mg/l. Otherwise, the LCK data correlated well with the OPUS, producing a linear slope fairly similar to that of the OPUS, as seen in Figure 16.

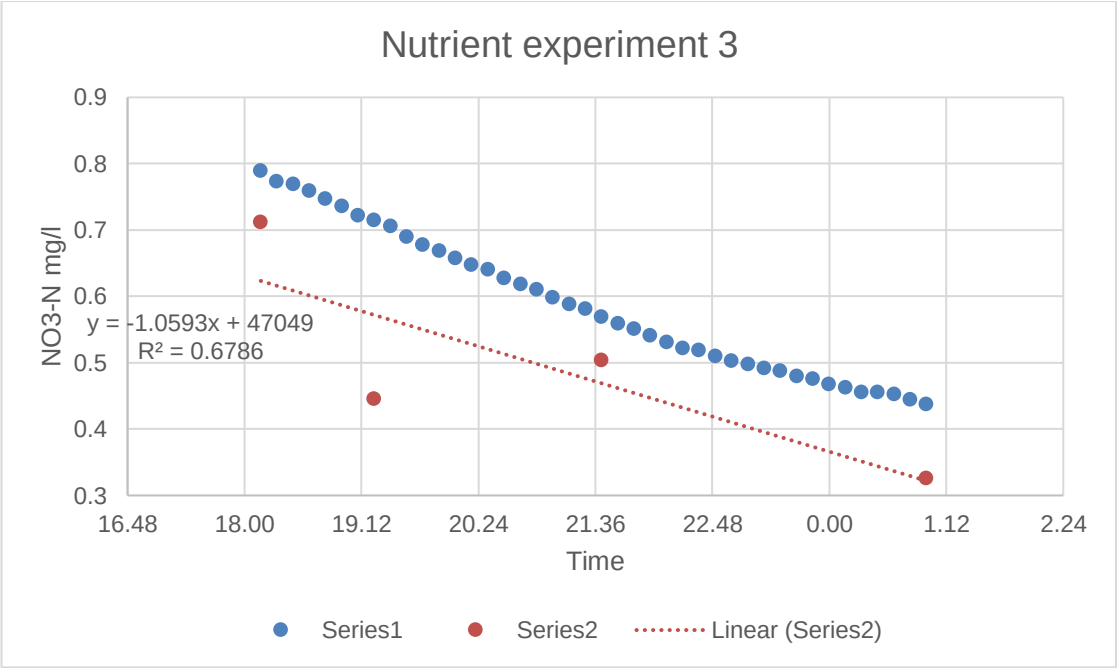


Figure 16 Scatter plot of nutrient run 3 with LCK data trendline and R2 values

These plots do not however take into consideration the error margin of both methods. As both the first and third nutrient run had fairly similar slopes with a few exceptions, the error margins need to be taken into account when comparing the two data sets. When comparing the data from the first run and allowing for the OPUS error margin, we see that only LCK measurements agree with the OPUS, as seen in Figure 17.

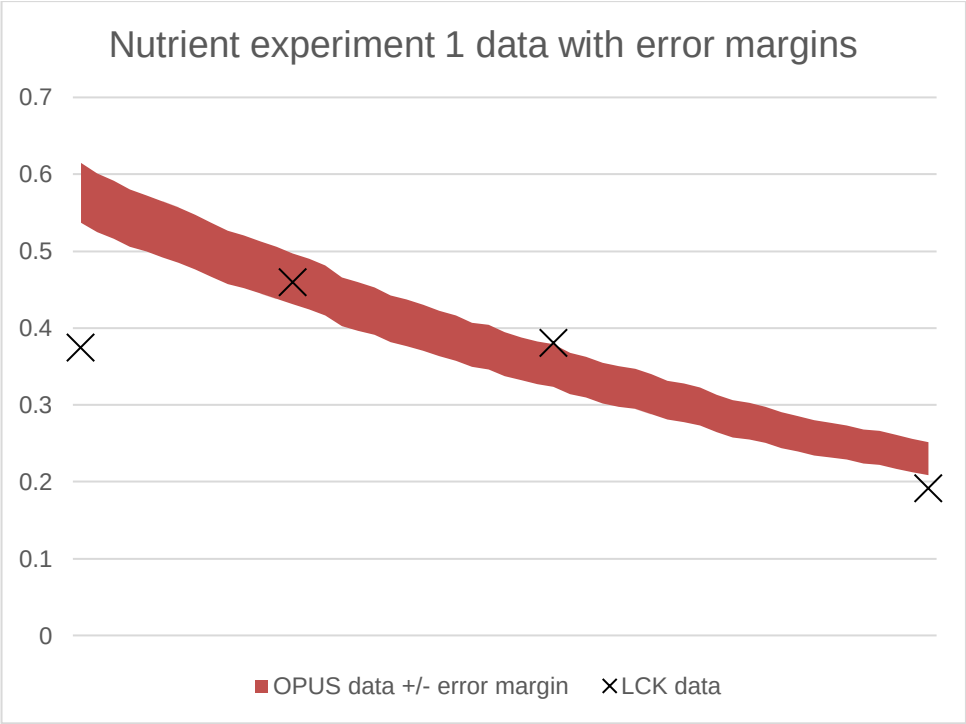


Figure 17 Nutrient run # 1 with OPUS error margin

As the first nutrient run had the lowest average deviation between the two methods, it also had the highest agreement level. However, when analyzing the third nutrient run, and considering both error margins, we see a fairly high level of agreement is present, as seen in Figure 18.

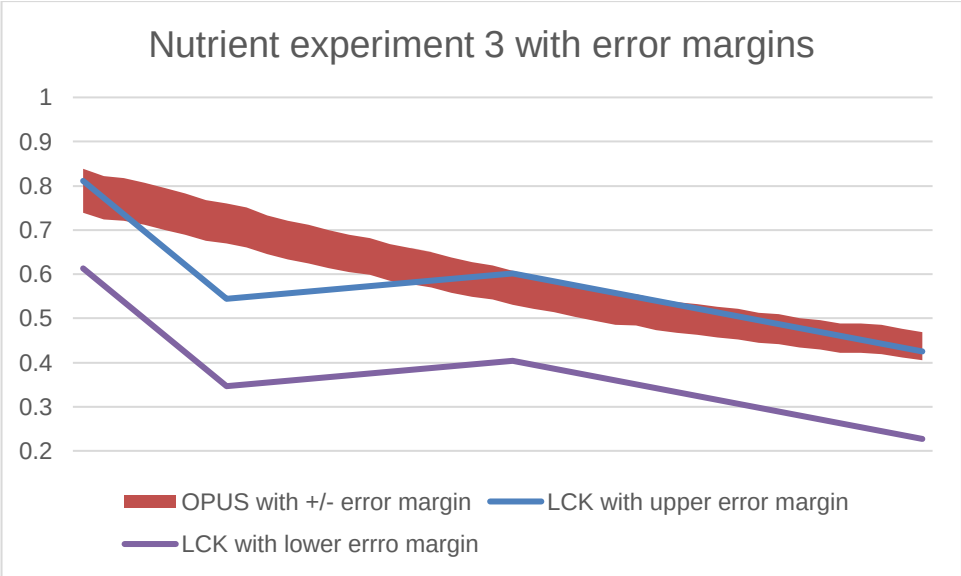


Figure 18 Nutrient run # 3 with both error margins

If the second LCK measure would be disregarded, the two methods would have an extremely high level of agreement. The fact that all the three runs had one single LCK measurement that did not correlate with the rest of the data might result from error in sampling or the preparation of the cuvette test.

4.3 Calibration curve

Due to an error in the plumbing of the lab, a faucet from the regular water main was labeled as de-ionized water. This faucet was used to dilute the nitrate standard solution, which led to different concentrations in the samples than originally calculated. However, the control sample was analyzed first, and the error was discovered. Then it was only a matter of nullifying the base nitrate concentration of the water from the main line when calculating the accuracy of the analysis methods. The nitrate concentration of the water used to dilute the samples was 0.36 mg/l NO_3-N. The determination was made from the mean of the control samples measurements by the three methods. This error still introduced the possibility of DOC interference. The DOC levels of the water were later analyzed and proven to be insignificant but not negligible. The actual

concentrations of the samples as well as the measured values can be seen in table 3.

Table 3 Table of values for calibration samples each method

Sample	Actual value (mg/l)	OPUS (mg/l)	LCK (mg/l)	LUVY (mg/l)
0	0.36	0.37	0.35	0.37
1	0.82	0.81	0.82	0.82
2	1.27	1.24	1.12	1.7
3	1.72	1.65	1.75	1.8
4	2.17	2.35	2.19	2.2

As can be seen in Table 3, the figures are in very high agreement, except for Sample 3 analyzed by LUVYLab. When plotted, LUVYLab has the lowest performance with regards to correlation coefficient even though the margin is very small. However, the error margin of their data is ±10%. It can also be observed that the agreement deteriorates as the measured value increases. The standard deviation between the methods for each sample in increasing order is: 0,009, 0,004, 0,25, 0,06 and 0,08. With the actual measuring area being so small, it cannot be confirmed if this trend continues with even higher values.

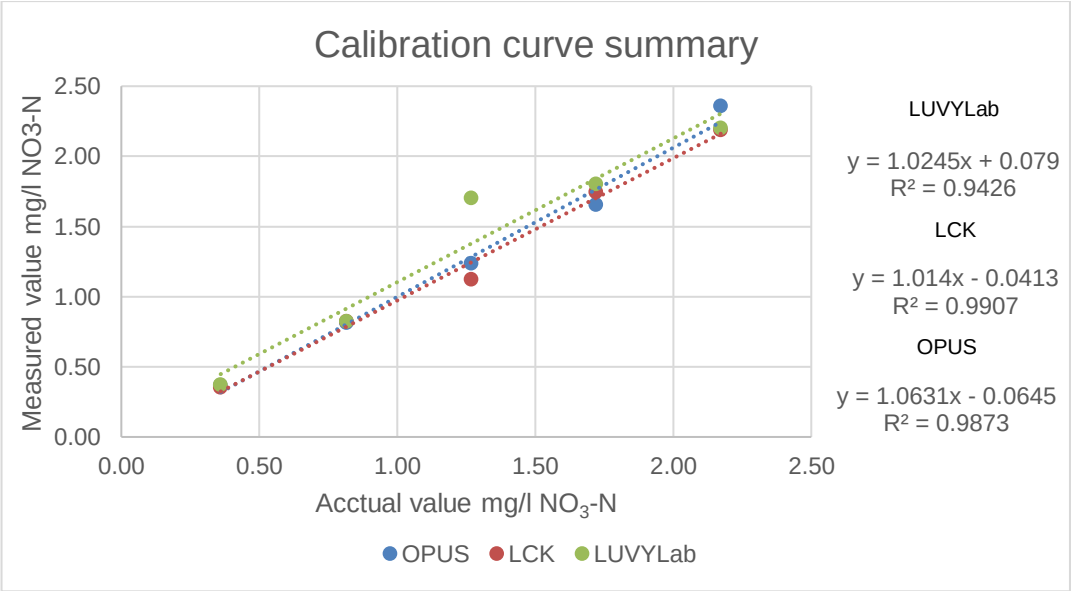


Figure 4 Summary of calibration curve data

It can be seen from the summary of the calibration curve data in figure 19 that every method had a very high correlation coefficient as well as a R^2 value of over 0,94 with the LCK having 0,990. Due to the error with the dilution, there is a degree of uncertainty with these results.

A study in the efficiency of an OPUS *in situ* was performed by Műnevver *et al*. and published in 2021 where a set of 5 OPUS probes were deployed in the Atlantic and results were compared to traditional wet-chemical analysis methods. The conclusion of the study shows an excellent agreement between both techniques. (Műnevver 2021) The actual *ex situ* method is not elaborated on in the publication, but the execution of the study was similar to this thesis with the exception that Műnevver et al. conducted their study in the field. The publication emphasizes on the outstanding functionality of the OPUS in a variety of field conditions like a depth resistance of 6000 meters. By comparing the findings of these studies, the usefulness of the OPUS probe and similar continuous monitoring probes in water quality monitoring cannot be understated.

5 Discussion

As discussed previously, the amount of data points could have been greater. These results are in no way definitive as method comparisons should have sample size of over 100 as pointed out in Bland´s and Altman´s body of work. In statistical analyzes, the accuracy and reliability usually increases with samples size. However, as the reason for this study was not to introduce a novel method of analysis, rather confirming the suitability of a certain method for a certain type of study.

The overall comparison showed a high level of agreement between the techniques. Especially when removing values above the measuring range of the OPUS. As the OPUS is designed for a wide range of water qualities, ranging from wild waters to municipal wastewater, the turbidity compensation maxes out if the water has a high level of interfering particles. But when it measures a sample with virtually no other compounds than nitrate, as can be seen the overall data, values way above 2 mg/l NO_3-N can be measured. As the manufacturer states that the upper range for the given confidence interval is 2 mg/l NO_3-N, there is no assurance that these values are valid.

For the nutrient runs, several inconsistencies were found. After allowing for the inherent error margin and confidence interval of the two methods, a high level of agreement can be stated. However, the inconsistent data points from the LCK measurements lower the overall performance of the method and therefor also its reliability in this setting. When measuring concentrations below 1 mg/l NO_3-N, the low error margin of the methods still has a meaningful effect on the overall results of the experiment. For these experiments, there is a huge benefit from high temporal resolution when it comes to determining concentration changes. It would be possible to perform LCK tests with smaller intervals, but it would be impractical, time consuming and not financially sound. The LCK test is also only as accurate and reliable as the person performing it and the tools utilized. Even though all the measurements taken with the LCK was performed with the same tools and by the same person, the percentage of faulty or

unreliable test was 25 %. As the OPUS analyses the water as it is with automated precision, the human element as well as the inaccuracy with the reaction chemicals are removed.

Surprisingly, the calibration curve showed that the strongest outlier data point was measured by the accredited laboratory. As they had the highest error margin of the three methods, the sample was still almost lacking in interfering material. This shows that measurement techniques and their results are always questionable and validates the need for studies of this type. This also challenges the reliability of other studies, like environmental monitoring, that usually due to lack of resources rely of singe discrete samples with extremely low spatial and temporal resolution. If a 1 km^2 lake can be monitored with a single discrete sample once a month, how accurate is the data?

Within the framework of this thesis and the questions it addressed, the data shows that the OPUS is the optimal method for analysis of this nature, strongly due to the consistency and the high temporal resolution. As the studies performed by Origin by Ocean is more dependent on the temporal flux rather than the absolute values, perfect accuracy is not the main objective.

As a follow up for this thesis, studies in higher concentrations should be performed. This would however need a probe with a shorter path length to increase its measuring range. More samples could also be taken where the concentration is in the upper end of the probes range.

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Raw sample data

Spread sheet of raw sample data including meta data

Sample	Date	Time	OPUS	LCK	LUVY	OPUS low	OPUS high	LCK 339 low	LCK 339 high	Raw dev	Data Mean	Location	Sample description
1	26.5.2021	8:50	0,815	0,849 x		0,764	0,866	0,750	0,948	0,034	0,832	Otaniemi	SeaLife #1, wastewater 10% dilution
2	2.7.2021	12:50	0,235	0,206 x		0,213	0,257	0,107	0,305	-0,028375	0,221	Otaniemi	Seawater, fucus habitat, static
3	19.7.2021	11:10	0,197	0,261 x		0,177	0,217	0,162	0,360	0,064	0,229	Otaniemi	Seawater, fucus habitat, static
4	21.7.2021	8:50	0,009	0,22	0,008	-0,001	0,019	0,121	0,319	0,211	0,115	Otaniemi	Kustavi, mesocosmos sample
5	21.7.2021	16:00	0,704	0,81 x		0,659	0,749	0,711	0,909	0,106	0,757	Otaniemi	SeaLife #2, wastewater 10% dilution
6	29.7.2021	12:20	1,06	1,13 x		0,997	1,123	1,031	1,229	0,07	1,095	Matinkylä	SeaLife #3, wastewater 10% dilution (tap water base NO3 0,35l)
7	29.7.2021	13:30	0,576	0,304 x		0,537	0,615	0,205	0,403	-0,272	0,440	Matinkylä	O, fucus habitat, 1st SL nitrate run h0, (bad discrete sample)
8	29.7.2021	15:40	0,464	0,27 x		0,431	0,497	0,171	0,369	-0,194	0,367	Matinkylä	O, fucus habitat, 1st SL nitrate run h2
9	29.7.2021	18:30	0,351	0,38 x		0,323	0,379	0,281	0,479	0,029	0,366	Matinkylä	O, fucus habitat, 1st SL nitrate run h5
10	29.7.2021	22:30	0,23	0,191 x		0,209	0,252	0,092	0,290	-0,039	0,211	Matinkylä	O, fucus habitat, 1st SL nitrate run h9
11	31.7.2021	12:20	0,638	0,386 x		0,596	0,680	0,287	0,485	-0,252	0,512	Matinkylä	O, fucus habitat, 2st SL nitrate run h0
12	31.7.2021	14:30	0,575	0,384 x		0,536	0,614	0,285	0,483	-0,191	0,480	Matinkylä	O, fucus habitat, 2st SL nitrate run h2
13	31.7.2021	16:30	0,519	0,337 x		0,483	0,555	0,238	0,436	-0,182	0,428	Matinkylä	O, fucus habitat, 2st SL nitrate run h4
14	31.7.2021	18:40	0,465	0,36 x		0,432	0,498	0,261	0,459	-0,105	0,413	Matinkylä	O, fucus habitat, 2st SL nitrate run h6
15	6.8.2021	17:40	2,008	1,99 x		1,888	2,118	1,891	2,089	-0,018	1,999	Matinkylä	Uusikaupunki, wastewater 20% dilution (tap water base NO3 0,37l)
16	6.8.2021	19:20	0,735	0,445 x		0,669	0,761	0,346	0,544	-0,27	0,580	Matinkylä	O, fucus habitat, 1st UK nitrate run h 1
17	6.8.2021	21:40	0,569	0,503 x		0,531	0,607	0,404	0,602	-0,066	0,536	Matinkylä	O, fucus habitat, 1st UK nitrate run h 3
18	6.8.2021	1:00	0,437	0,326 x		0,405	0,469	0,227	0,425	-0,111	0,382	Matinkylä	O, fucus habitat, 1st UK nitrate run h 7
19	3.8.2021	10:40	1,8	1,72 x		1,700	1,900	1,621	1,819	-0,08	1,760	Otaniemi	SeaLife #4, wastewater25% dilution (tap water base unknown)
20	9.9.2021	14:20	0	0,124 x		-0,010	0,010	0,025	0,223	0,124	0,062	Otaniemi	Hanko sea water
21	15.9.201	11:30	6,119	9,13 x		5,803	6,435	9,031	9,229	3,011	7,625	Otaniemi	FFax sample, batch 1, room temperature 3 days, 25% dilution (Di-water) 30 min reaction time
22	15.9.2021	13:30	5,97	8,94 x		5,662	6,279	8,841	9,039	2,97	7,455	Otaniemi	FFax sample, batch 1, cold, 25% dilution (Di-water)
23	21.9.2021	10:10	3,44	3,54 x		3,268	3,622	3,441	3,639	0,1	3,490	Otaniemi	FFax sample. Batch 1, cold, 10% dilution (Di-water)
24	21.9.2021	12:30	0	6 x		-0,010	0,010	5,901	6,099	6	3,000	Otaniemi	FFax sample. Batch 2, cold, 50% dilution (Di-water) OPUS over range
25	21.9.2021	14:30	3,95	2,09 x		3,743	4,158	1,991	2,189	-1,86	3,020	Otaniemi	FFax sample. Batch 1, boiled, 10% dilution (Di-water) after fucus
26	27.9.2021	10:20	2,276	2,235 x		2,152	2,400	2,136	2,334	-0,041	2,256	Otaniemi	FFax sample. Batch 1, boiled, 4% dilution (Di-water) microscale
27	27.9.2021	10:40	2,01	2,07 x		1,900	2,121	1,971	2,169	0,06	2,040	Otaniemi	FFax sample. Batch 1, boiled, 4% dilution (Di-water) measuring flask
28	27.9.2021	11:30	2,07	2,16 x		1,957	2,184	2,061	2,259	0,09	2,115	Otaniemi	FFax sample. Batch 2, boiled, 4% dilution (Di-water) measuring flask
29	25.11.2021	21:50	0,365	0,352	0,37	0,337	0,393	0,263	0,451	-0,013	0,359	Otaniemi	CIMR dilution 1000 mg/l (0.0%)
30	26.11.2021	22:10	0,811	0,834	0,82	0,760	0,862	0,735	0,933	0,023	0,823	Otaniemi	CIMR dilution 1000 mg/l (0.2%)
31	26.11.2021	22:30	1,234	1,13	1,7	1,162	1,306	1,031	1,229	-0,104	1,182	Otaniemi	CIMR dilution 1000 mg/l (0.4%)
32	25.11.2021	22:50	1,652	1,76	1,8	1,559	1,745	1,661	1,859	0,108	1,706	Otaniemi	CIMR dilution 1000 mg/l (0.6%)
33	25.11.2021	23:10	2,364	2,19	2,2	2,236	2,492	2,091	2,289	-0,174	2,277	Otaniemi	CIMR dilution 1000 mg/l (0.8%)

Results from LUVY Lab Oy

Result summary for 5 samples delivered to Länsi-Uudenmaan Vesi- ja Ympräistö Oy

LUVYLab Oy Ab
PL 51
08101 LOHJA

TESTAUSSELOSTE
¤Yleinen
30.11.2021

21-4106
#1

1 (2)

Ocean Orchard Oy
Tekniikantie 2
02150 ESPOO

FINAS
Finnish Accreditation Service
T147 (EN ISO/IEC 17025)

Tilausnro 130120 (X/S), saapunut 25.11.2021, näytteet otettu 25.11.2021
Näytteenottaja: Tilaaja

NÄYTTEET

Lab.nro	Näytteen kuvaus
9718	OBO # 0
9719	OBO # 1
9720	OBO # 2
9721	OBO # 3
9722	OBO # 4

MÄÄRITYSTULOKSET / NÄYTTEET

Määritys	Yksikkö	9718	9719	9720	9721
*Nitraatti- ja nitriittitypen summa(SFA)	µg/l	370	820	1700	1800
*Nitriittityppi	µg/l	<2	<2	<2	<2
*Nitraattityppi (SFA)	µg/l	370	820	1700	1800

Määritys	Yksikkö	9722
*Nitraatti- ja nitriittitypen summa(SFA)	µg/l	2200
*Nitriittityppi	µg/l	<2
*Nitraattityppi (SFA)	µg/l	2200

Merkintöjen selityksiä: P = määritys kesken, E = ei tehty, ~ = noin, < = pienempi kuin, « = pienempi tai yhtäsuuri kuin, > = suurempi kuin, » = suurempi tai yhtäsuuri kuin.
*=akkreditoitu menetelmä; V=vaatimus S=suositus T=tavoitetaso; Määrityksen edessä 1), 2), 3) ja/tai 7) = alihankinta

Milla Holopainen
Vastaava laborantti

Tässä testausselosteessa esitetyt testaustulokset pätevät ainoastaan testatuille näytteille.Akkreditointi ei koske lausuntoa. Testausselosteen saa kopioida vain kokonaan. Menetelmä-, mittausepävarmuus- ja määrityspäivätiedot liitteenä/toimitetaan pyydettäessä.

Katuosoite	Postiosoite	Puhelin	Sähköposti	Alv.rek.
Länsi-Louhenkatu 31	PL 51			2940757-6
08100 LOHJA	08101 LOHJA	*019 323895	laboratorio@luvylab.fi	

LUVYLab Oy Ab
PL 51
08101 LOHJA

TESTAUSSELOSTE 21-4106 2 (2)
¤Yleinen #1
30.11.2021

MENETELMÄTIEDOT

Määritys	Menetelmän nimi ja tutkimuslaitos (suluissa)
*Nitraatti- ja nitriittitypen summa(SFA)	ISO 13395:1996, SFA-tekniikka (TL64)
*Nitriittityppi	SFS 3029:1976 (TL64)
*Nitraattityppi (SFA)	ISO 13395:1996, SFA-tekniikka (TL64)

TUTKIMUSLAITOSTIEDOT

Tunnus	Tutkimuslaitoksen nimi
TL64	LUVYLab Oy Ab (FINAS T147)(EN ISO/IEC 17025: 2017)

MITTAUSEPÄVARMUUSTIEDOT

Määritys	Näyte	Tuloksen epävarmuus	Määrityspvm.
*Nitraatti- ja nitriittitypen summa(SFA)	2021/9718	±10%	26.11.2021
	2021/9719	±10%	26.11.2021
	2021/9720	±10%	26.11.2021
	2021/9721	±10%	26.11.2021
	2021/9722	±10%	26.11.2021
*Nitriittityppi	2021/9718	Määritysrajan alitus	25.11.2021
	2021/9719	Määritysrajan alitus	25.11.2021
	2021/9720	Määritysrajan alitus	25.11.2021
	2021/9721	Määritysrajan alitus	25.11.2021
	2021/9722	Määritysrajan alitus	25.11.2021
*Nitraattityppi (SFA)	2021/9718	±10%	29.11.2021
	2021/9719	±10%	29.11.2021
	2021/9720	±10%	29.11.2021
	2021/9721	±10%	29.11.2021
	2021/9722	±10%	29.11.2021

LCK 339 Quality certificate

Quality certificate provided by Hach Lange GmbH with analytical parameters

Quality certificate
Technical data for Validation
of LCK339 (1-60 mg/l Nitrate)

Quality certificate

Technical data for cuvette test LCK339
(Results as NO_3)

Sensitivity	0.0182 Abs./(mg/l)
Ordinate intersect	0.048 Abs.
Residual standard deviation	0.0035 Abs.
Method variation coefficient	0.57 %
Method standard deviation	0.19 mg/l
Confidence intervall (95%)	± 0.45 mg/l

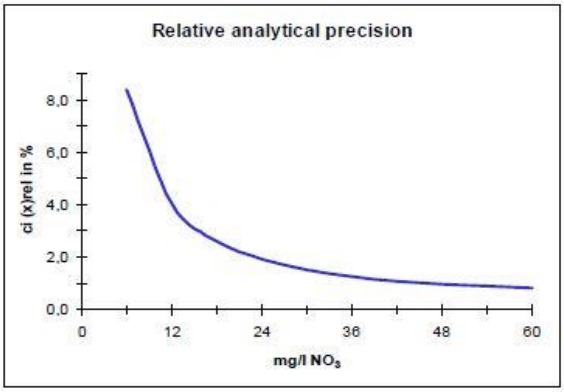
The technical data for cuvette test LCK339 were determined in conformity with ISO 8466-1 and DIN 38402 A51 „Calibration of analysis methods".

The series of the smallest and largest calibration standards exhibit normal distribution and are outlier- and trend-free.
The calibration gives a linear function.

Technical data in conformity with DIN 32645

Detection limit	0.210 mg/l
Quantitation limit	0.629 mg/l

The detection and the quantitation limits were determined in conformity with DIN 32645.



Result	Confidence intervall
12.0 mg/l	± 0.48 mg/l
24.0 mg/l	± 0.46 mg/l
36.0 mg/l	± 0.46 mg/l
48.0 mg/l	± 0.47 mg/l
60.0 mg/l	± 0.50 mg/l

HACH LANGE GmbH
Quality Management

Dr. Ralf Kloos

HACH LANGE
UNITED FOR WATER QUALITY

HACH LANGE GmbH
Willstätterstraße 11
D-40549 Düsseldorf
Tel.+49 (0)211-52 88-320
Fax+49 (0)211-52 88-143
Kundenservice@hach-lange.de
www.hach-lange.de

Working procedure: LCK 339 Nitrate

Brochure of sampling procedure and interference parameters

LCK 339 Nitrate

DOC312.53.94016

0.23–13.50 mg/L NO_3-N or 1–60 mg/L NO_3 **LCK 339**

Scope and application: For wastewater (beware of interferences), drinking water, raw water, surface water, soils, substrates and nutrient solutions.

Test preparation

Test storage

Storage temperature: 15–25 °C (59–77 °F)

pH/Temperature

The pH of the water sample must be between pH 3–10.

The temperature of the water sample and reagents must be between 20–24 °C (68–75 °F).

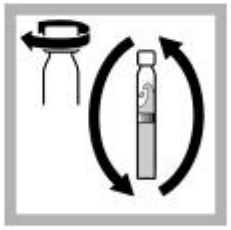
Before starting

In case of not working at the correct recommended temperature an incorrect result may be obtained.
Not more than **3 hours** should elapse between sampling and analysis. **Store in a cool place!**
Review safety information and expiration date on the package.
Review the Safety Data Sheets (MSDS/SDS) for the chemicals that are used. Use the recommended personal protective equipment.
Dispose of reacted solutions according to local, state and federal regulations. Refer to the Safety Data Sheets for disposal information for unused reagents. Refer to the environmental, health and safety staff for your facility and/or local regulatory agencies for further disposal information.

Procedure

1. Carefully pipet **1.0 mL** of **sample**.

2. Carefully pipet **0.2 mL** of **solution A**.



3. Close the cuvette and invert a few times until **no more streaks** can be seen.

4. After **15 minutes**, thoroughly clean the outside of the cuvette and evaluate.

5. Insert the cuvette into the cell holder.
DR 1900: Go to LCK/TNTplus methods.
Select the test, push **READ**.

Interferences

The ions listed in the table have been individually checked against the given concentrations and do not cause interference. The cumulative effects and the influence of other ions have not been determined.

High loads of oxidizable organic substances (COD) cause the reagent to change color and to give high-bias results. The test can only be used for waste water analyses if the COD is less than 200 mg/L.

The measurement results must be subjected to plausibility checks (dilute and/or spike the sample).

Removal of Interferences

Nitrite concentrations of more than 2.0 mg/L interfere (high-bias results) and can be removed by the addition of a spatula-tip full of amidosulphonic acid. The chloride can be precipitated out as silver chloride by adding silver sulphate. High calcium concentrations cause turbidity. This interferes with the determination but can be prevented by adding a spatula-tip full of EDTA to the sample.

Interference level	Interfering substance
500 mg/L	K^+, Na^+, Cl^-
100 mg/L	Ag^+
50 mg/L	Pb^{2+}, Zn^{2+}, Ni^{2+}, Fe^{3+}, Cd^{2+}, Sn^{2+}, Ca^{2+}, Cu^{2+}
10 mg/L	Co^{2+}, Fe^{2+}
5 mg/L	Cr^{6+}

Summary of method

Nitrate ions in solutions containing sulphuric and phosphoric acids react with 2.6-dimethylphenol to form 4-nitro-2.6-dimethylphenol.

HACH LANGE GMBH
Willstätterstraße 11
D-40549 Düsseldorf
Tel. +49 (0) 2 11 52 88-0
Fax +49 (0) 2 11 52 88-143
info-de@hach.com
www.hach.com

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