Program Design and Performance Evaluation of a New Seawater Nitrate and Nitrite Analyzer

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ABSTRACT:

The Chemical Sensor lab's new prototype nitrate and nitrite analyzer currently uses an older, adapted LabView program to analyze seawater samples. Our goal was to design a program specific to the analyzer to streamline the automated analyzing process and evaluate the analyzer's performance. This was accomplished by translating the older iteration of the program into a user-friendly, polished LabView program that meets the requirements for needed inputs and outputs. From there, we tested the new analyzer by running nitrite and nitrate standards to verify the method's precision. We also analyzed ocean samples that were collected at different depths offshore Monterey, California as a case study. The nutrient profiles were then compared with data from previous cruises that occupied the same stations in 2019 and 2020 to validate the method. The profiles were mostly akin to the older profiles, with variations in nitrate concentrations in deeper water. The analyzer's precision estimate was 1.42%.

INTRODUCTION:

Nitrate and nitrite are important chemical compounds to measure in oceanography as they are necessary nutrients and often limiting nutrients for all oceanic primary producers (Webb, 2021). These tiny photosynthetic organisms play an integral role in the nitrogen cycle as well as

marine food chains. Nitrite concentrations are always lower than nitrate concentrations because they are chemically unstable compounds that have an affinity to bond with oxygen to become nitrate (Zafiriou and True, 1979). Nitrate consumption often leaves the surface of the ocean with low concentrations, as these producers use these nitrogenous compounds to photosynthesize and build their bodies (Webb, 2021). When these producers die and sink to deeper depths, heterotrophic organisms remineralize the organic matter back to nitrate, enriching deep water (Burkhardt *et al.*, 2014). Oceanic regions often have lower concentrations of nitrate and nitrite when compared to coastal regions as factors such as agricultural runoff and freshwater input contribute to higher concentrations.

To measure nitrate and nitrite concentrations in seawater, a colorimetric method can be employed by mixing the seawater sample with reagents, first sulphanilamide to create a diazonium ion, then N-(1-napthyl)-ethylenediamine (NED) to create a pink azo dye that binds to nitrite (Aminot and Chaussepied, 1983; Grasshoff *et al.*, 1983; Sakamoto *et al.*, 1990). The developed color is proportional to the concentration and has a peak absorbance at 540 nm (Wierzbicka, 2020). In order to know these concentrations in samples, nitrite standards are made with low nutrient sea water and then used to create a regression model of concentration versus absorbance. Absorbances from seawater samples can be plugged into the regression equation to find concentrations of nitrite. Since in this colorimetric method sulphanilamide and NED only react with nitrite, a second replicate of the sample passes through a cadmium column that reduces all nitrate into nitrite. The sample is then reacted with the two coloring reagents that develop absorbance for the reduced nitrate plus nitrite that is natural in the sample, in a step that "tricks" the spectrophotometer. Therefore, the nitrate absorbance is equal to the difference between the original nitrite measurement and the measurement of the nitrite and reduced nitrate.

An alternative method to finding nitrate and nitrite concentrations from seawater is using the In Situ Ultraviolet Spectrophotometer (ISUS), which uses ultraviolet light instead of colorimetry to determine nitrite and nitrate concentrations (Johnson and Coletti, 2002). In the Chemical Sensors lab at MBARI, the nitrate and nitrite analyzer was recently developed to analyze nitrate concentrations in the low nutrient seawater that is used to calibrate the ISUS sensors from the GO-BGC and SOCCOM projects (Matsumoto *et al.*, 2022). The goal is to make sure that the low nutrient seawater used for the calibration is actually low in nitrate and nitrite. The analyzer is a cheaper and simpler version of an auto-analyzer to measure nitrate and nitrite. It can be replicated in any laboratory relatively easy.

A large part of this project is creating a LabView program designed uniquely for this new nitrate and nitrite analyzer (Figure 1), which is based on Sakamoto's original design (Sakamoto *et al.*, 1996). The previous LabView program used for this analyzer was an adaptation of a pH program, with many elements that were not needed for the instrument, and some that needed to be added too. Modifications would allow for a simpler and more efficient process when running the analyzer. To check and make sure that this instrument and applied chemical methodology are precise and accurate, we went on a cruise to collect ocean samples in the Monterey Bay region to analyze and compare the data from previously collected data in the same area. We hypothesize that the relative standard deviation of all the cast study samples is equal to or less than 2%.

METHODS:

LABVIEW

To begin working on LabView, I spent two weeks on National Instrument's LabView Core 1 course to be proficient enough to properly redesign the program. The old LabView design

for the nitrate and nitrite analyzer was adapted from a pH spectrophotometry system, which includes many functions that are not needed for the analyzer's function (Figure 2). To achieve an updated version that is unique for the analyzer, most of the Front Panel interface was removed or rearranged. The Block Diagram was also rearranged to make reading the program and debugging easier after the Front Panel's features were cemented. The final product gives the user an easier understanding of the program as well as a simpler path to running samples (Figure 3).

COLORIMETRIC METHOD

The method that the analyzer employs is colorimetric, in other words, using the visible spectra to determine absorbance of a target wavelength output through certain compounds. In this specific employment, the analyzer combines the seawater sample with sulphanilamide to create diazonium ions. From there, it injects N- (1-naphthyl)-ethylenediamine (NED) to create a pink azo dye (Aminot and Chaussepied, 1983). Using the spectrophotometer detector, a full spectra of visible light wavelength intensities is output. The pink azo dye has a peak intensity at 540 nm, so the corresponding intensity value is the raw intensity for the nitrate or nitrite in the sample. Using the Beer Lambert Law,

$$A = log_{10} \frac{I_o}{I}$$

where A is absorbance, I_o is incident light, and I is transmitted light, we could change raw intensities into absorbances. This output absorbance is then adjusted to account for the variation from the true absorbance by subtracting the value at 730 nm from 540 nm as the absorbance at

730 nm should always be 0, yet the spectrophotometer is slightly off by differing amounts each time.

NITRATE REDUCTION

This analyzer only reads nitrite absorbance in the spectrophotometer, so to calculate nitrate, all of the nitrate must be reduced via the cadmium column to nitrite and then read, meaning the first spectra is only nitrite and the second taken spectra in the analyzer's method is nitrite and nitrate, and the difference in concentration between the two values is the nitrate concentration. Column efficiency is taken into account as the cadmium coil does not reduce 100% of nitrate from a sample into nitrite. To calculate column efficiency, a nitrate standard and a nitrite standard of the same concentration were run. The column efficiency is shown by this relationship,

$$CE = \frac{NO_{3_{abs}}}{NO_{2_{abs}}}$$

where CE is the column efficiency, NO_3 abs is nitrate absorbance, and NO_2 abs is nitrite absorbance. The final adjusted concentration of nitrate is determined by adding the product of the nitrate sample concentration and the column efficiency to the original sample's nitrate concentration.

REGRESSION MODEL

To visualize the analyzer's precision, we used standards of 0μM to 5μM and ran them five times each to find its average and standard deviation along with the regression model with its R^2 value (Figure 4).

Then, to accurately record values for the sample's nitrate and nitrite concentrations, standards were made by adding stock solution into low nutrient seawater. A battery of standards of different concentrations were run daily to create a regression model and equation by which absorbances can be translated to concentrations (Figure 5). First, a blank of just low nutrient sea water is run. Its absorbance is subtracted from every standard's absorbance. Then, standards of differing nitrite concentrations were prepared using low nutrient sea water. Concentrations of standards differ depending on the estimated concentration. Each sample concentration must fall within the regression model, or else the equation will be inadequate to accurately calculate the concentration. Samples that are deemed too concentrated to be accurately analyzed (abs > 0.95) were diluted by 50% to allow for measurements within the absorbance values of zero to 0.99. The relative standard deviations of the absorbance averages of each sample for nitrate and nitrite is calculated to determine precision for the case study.

CASE STUDY: COLLECTING SAMPLES IN A RESEARCH CRUISE

The samples used to evaluate the performance of the analyzer were collected using a rosette that sampled multiple depths aboard the R/V Western Flyer on the cruise YODA2022 in the Monterey Bay area between 6/21/2022 and 6/25/2022 (Figure 6). One sample from each Niskin was collected and frozen until analysis onshore. There were 72 total samples, 60 of which were analyzed in duplicate. The samples from station 90 were not filled all the way, so that cast's samples were only analyzed once.

ANALYSIS

Samples were concomitantly collected from the same Niskins to be analyzed using MBARI's auto-analyzer in order to evaluate the analyzer's performance. However, the employee that performs the analysis went on medical leave and was unable to provide the dataset on time. Alternatively, we compared our results with data collected from cruises that collected nitrate and nitrite at the same stations during 2019 and 2020. Further, temperature, salinity, and oxygen levels from the same YODA2022 casts are also plotted to identify any trends that might affect nitrate and nitrite concentrations.

RESULTS:

The first phase of this project, redesigning the LabView program, was successful in its goal of being unique to the nitrate and nitrite analyzer (Figure 3). The new LabView program is much more streamlined for the purpose of running the nitrate and nitrite analyzer. The main buttons and indicators are arranged in a logical way as to make the analyzing process simple and easy. For example, the run sample, recharge, and rinse buttons are front and center as these are the three main modes the analyzer uses. The columns for samples are arranged to be easily input with the option of a corresponding note, and the graph of the output absorbance spectra makes it quick to identify any potential problems in the collected data.

The newly developed LabView program was then used to find the precision of the analyzer using standards. The standard deviations were very low, with the regression model's R^2 value at an outstanding 0.9999 (Figure 4). The analyzer was then used on the samples that were

successfully collected from the cruise YODA2022 to create nitrite and nitrate profiles for all six stations (Figures 7, 8). The nitrate profiles show the more coastal casts starting around 10 μM (Figure 7B) and plateauing around 30 to 35 μM NO_3 in deeper water (Figure 7A). The stations more in the open ocean have starting nitrate values of 0 μM which increase only after 50 meters or so. The deeper concentrations are all similar to each other. Similarly, the nitrite concentrations from the coastal waters start higher than the oceanic concentrations, yet most concentrations converge at around 0.1 μM NO_2 in deeper waters.

When comparing prior cruise data to the current data (Figures 9, 10), nitrate and nitrite concentrations appear to be similar in most profiles. However, there are some cases for nitrate specifically that show the 2022 data is significantly less than the 2019 and 2020 data for various depths (Figure 9 C, D, E). As for the nitrite profiles, they are all similar to the older data besides one (Figure 10A), which shows a large spike in nitrite concentration from around 50 to 100 meters.

The relative standard deviation of the nitrite sample absorbance was 2.16%, and for nitrate it was 0.68% (Figure 11).

DISCUSSION:

Comparing the current data to the data collected in 2019 and 2020, most nitrate and nitrite profiles look very similar to each other. However, there are some discrepancies where the nitrate concentrations fall much lower than expected (Figure 9 C, D, E). There are many variables that could affect this, including variations in the subtropical gyre movement and upwelling, changes in temperature, salinity, and oxygen, or imperfections in the analyzer when a sample reaches higher concentrations of nitrate. Differences in upwelling could account for some

of this change, as the Monterey Bay region receives high nutrient waters periodically (Sakamoto *et al.*, 1996), and this data could have been collected during a quieter season. However, when these profiles are compared to temperature, salinity, and oxygen profiles from the same locations (Figure 12), there does not seem to be a proportional trend for any of these measurements at the same depths. Chlorophyll a is used to estimate biomass of phytoplankton (Pennington *et al.*, 2015), so often nitrate concentrations are inversely proportional to chlorophyll a abundance due to phytoplankton's affinity of taking up the nitrite and nitrate around them. Figure 13 (Ward, 2005) shows the change of chlorophyll a and nitrate over time in Monterey Bay. This could be an explanation for small variation in the upper 200 meters, however phytoplankton need to be close to the surface to photosynthesize and thus cannot account for deeper water fluctuations of nitrate.

A significant reason why some of these readings could be off besides environmental conditions is that the analyzer might not be as effective at analyzing samples with higher concentrations of nitrite, or that there was error in sample dilution. While samples were diluted by 50% for the deeper casts (above 25 μM NO_3), it is possible that during the dilutions, there was contamination of the samples, leading its calculated concentration values to be below what it really is. Further, this analyzer is primarily used for very low concentrations of nitrite, so the presence of high concentrations of nitrite may not be the most dependable. If this same case study was run again, I recommend starting dilutions much earlier (and more than 50% if necessary) to keep the analyzed sample's nitrite concentrations to what it usually analyzes. Since the analyzer is used to make sure low nutrient sea water has low concentrations of nutrients, it is plausible that its accuracy is greatly diminished with such saturated samples.

As for the nitrite concentrations, the only graph that looks out of place when comparing the 2022 data to the 2019 and 2020 data is at C1 (Figure 10A). This station is still within the

Bay, and thus very close to land. The major spike in nitrite at around 60 meters could be an indication of higher rates of agricultural runoff or variations in freshwater input to the Bay, and overall smaller amounts of biological activity then previous years.

The relative standard deviations of the sample absorbances (Figure 11) are ideal for a high precision analyzer. Sakamoto's precision estimate for the 1996 analyzer was 2% (Sakamoto *et al.*, 1996), so the goal was to match that or be even lower. The overall sample relative standard deviation of absorbances is 1.42%, which matches the hypothesis that it would be equal or less than 2%.

This project sheds light on the accuracy and precision of MBARI's nitrate and nitrite analyzer. Further samples will need to be run to determine the precise upper and lower bounds of concentrations that the analyzer can accurately analyze before dilutions are necessary. However, its main purpose of analyzing low nutrient sea water can be replicated with high precision using the new program unique to the analyzer. The Chemical Sensors lab intends to make the imprints for the analyzer as well as the LabView software public to allow any lab access to a much cheaper alternative way of analyzing nitrate and nitrite compared to auto-analyzers. To achieve this goal, I recommend that a Matlab program is made for the analyzer to add variety to usable software for this method. As for the hardware, testing the process with different spectrophotometers and fiber optic light sources will give variety and more access to those trying to replicate the analyzer.

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Zafiriou, O.C. & True, M.B., 1979. Nitrite photolysis in seawater by sunlight. *Marine Chemistry*, 8(1), pp. 9-32.

TABLES AND FIGURES:

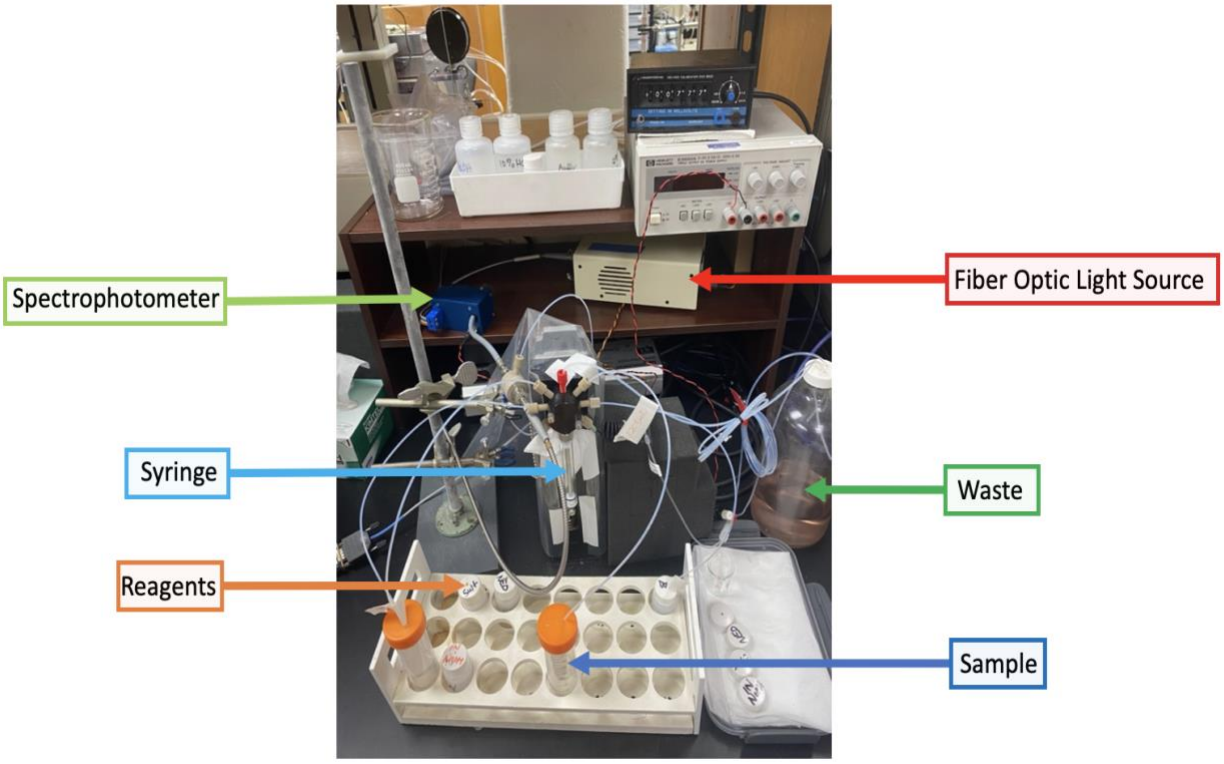

Figure 1. MBARI's nitrate and nitrite analyzer

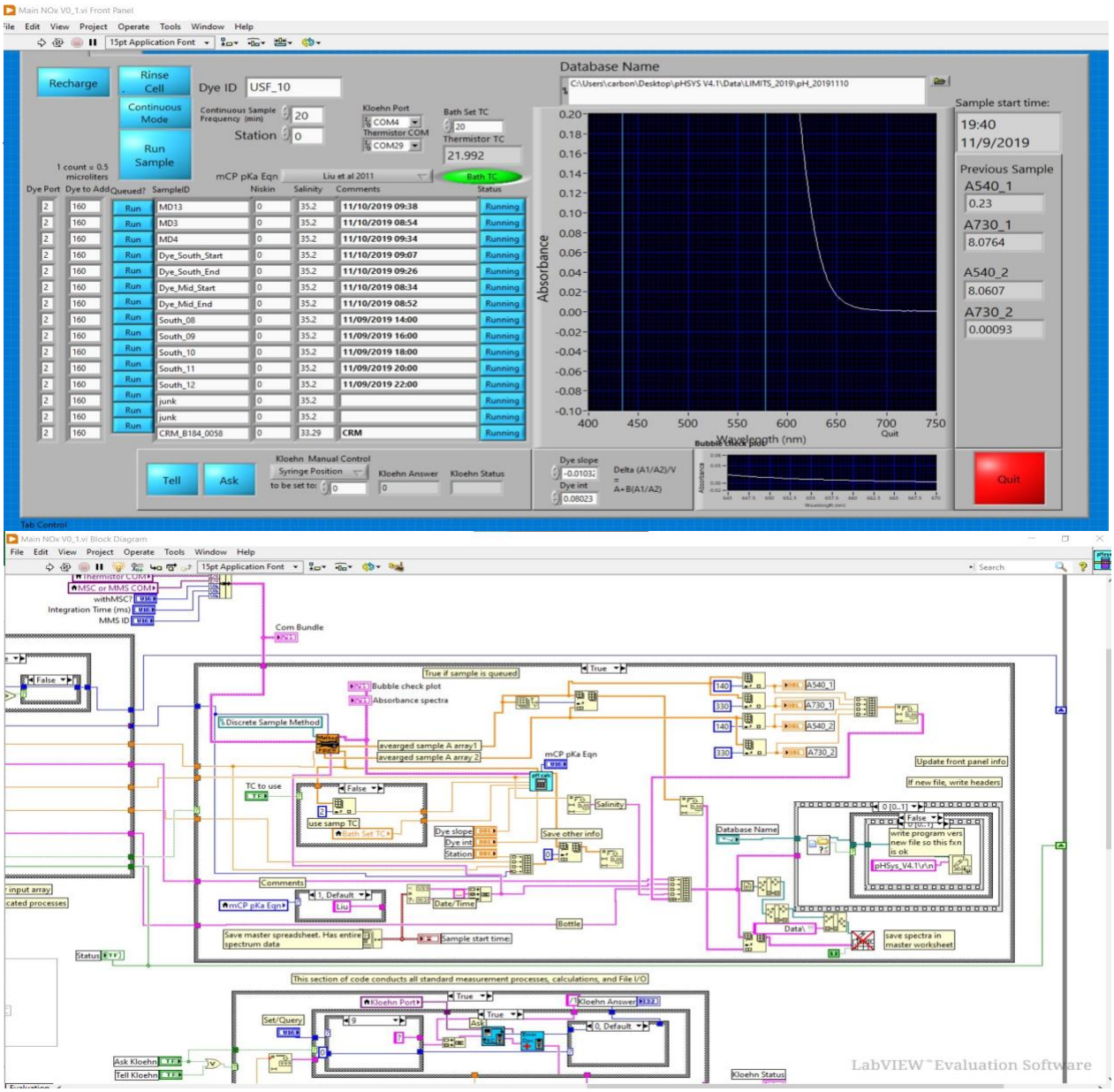

Figure 2. Old LabView Front Panel and Block Diagram adopted from a pH spectrophotometry system

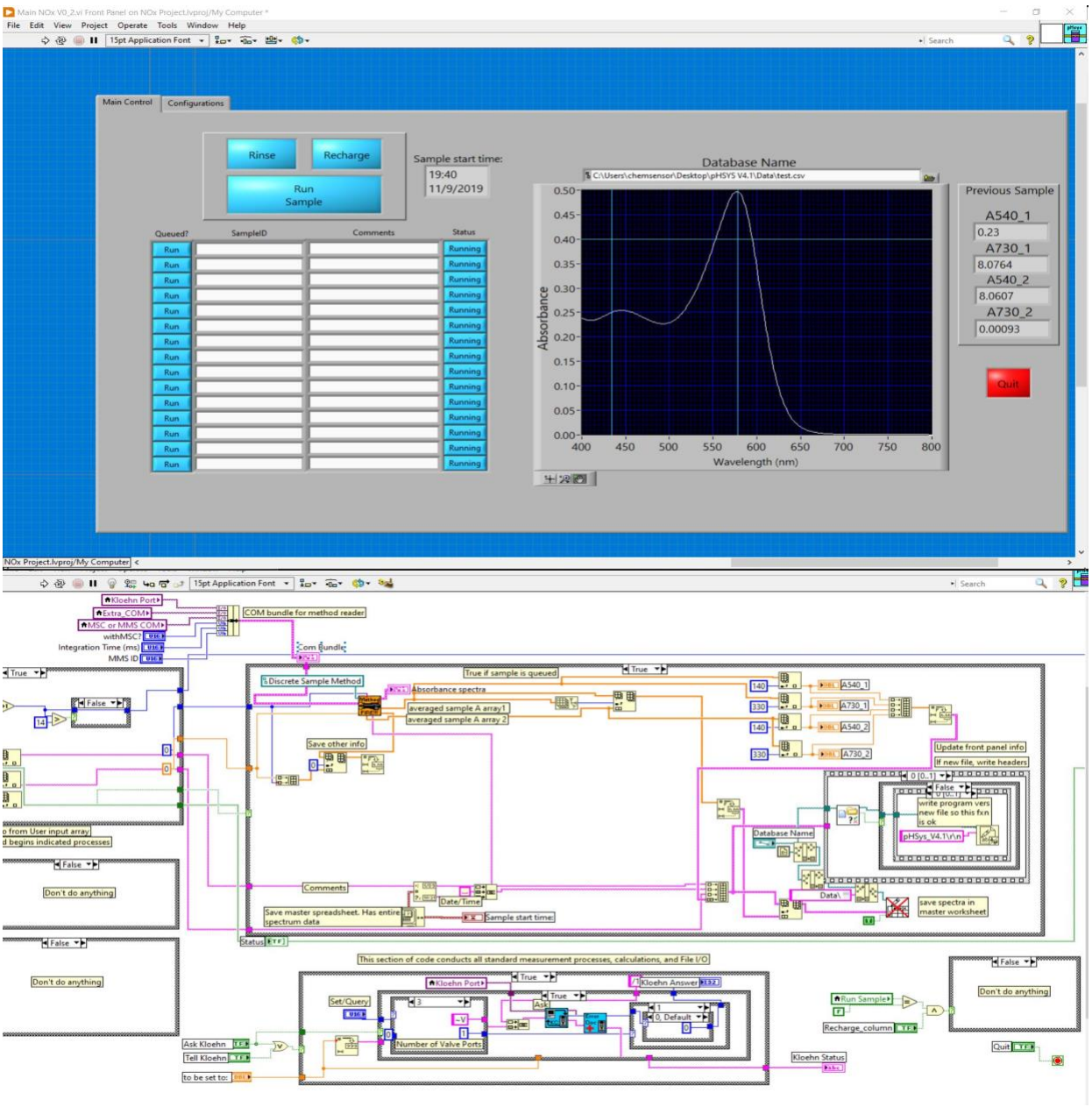

Figure 3. New LabView design for the nitrate and nitrite analyzer

Nitrite Standards		
	Averages	Standard Deviations
0uM	0.0039	0.0005
1uM	0.0436	0.0002
2uM	0.0853	0.0002
3uM	0.1244	0.001
4uM	0.1637	0.002
5uM	0.2022	0.003

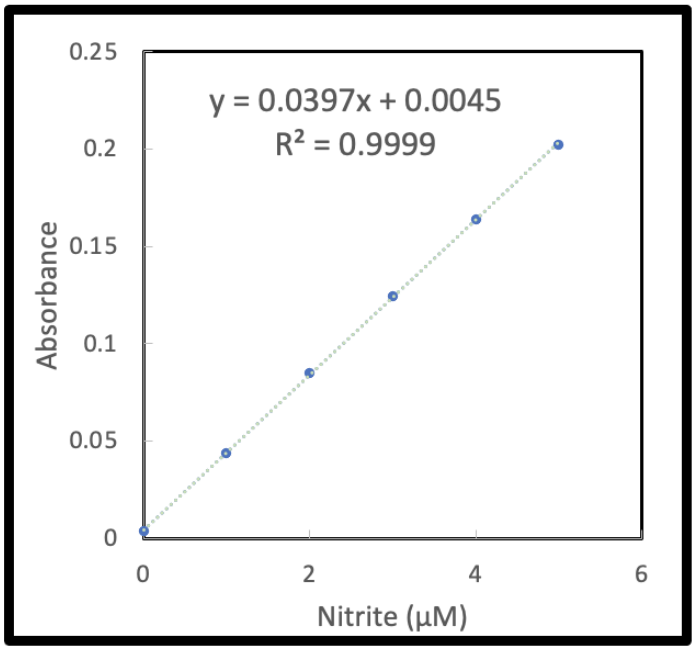

Figure 4. Regression curve created by standards. The table indicates the average absorption of each standard, with the standard deviation to show precision.

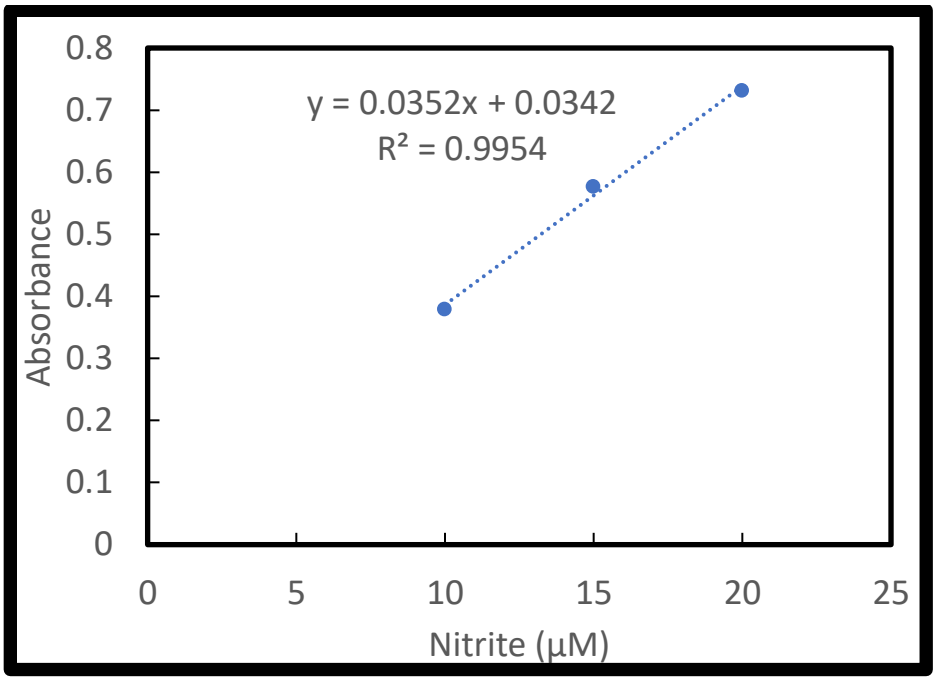

Figure 5. Example of regression model with higher standard concentrations.

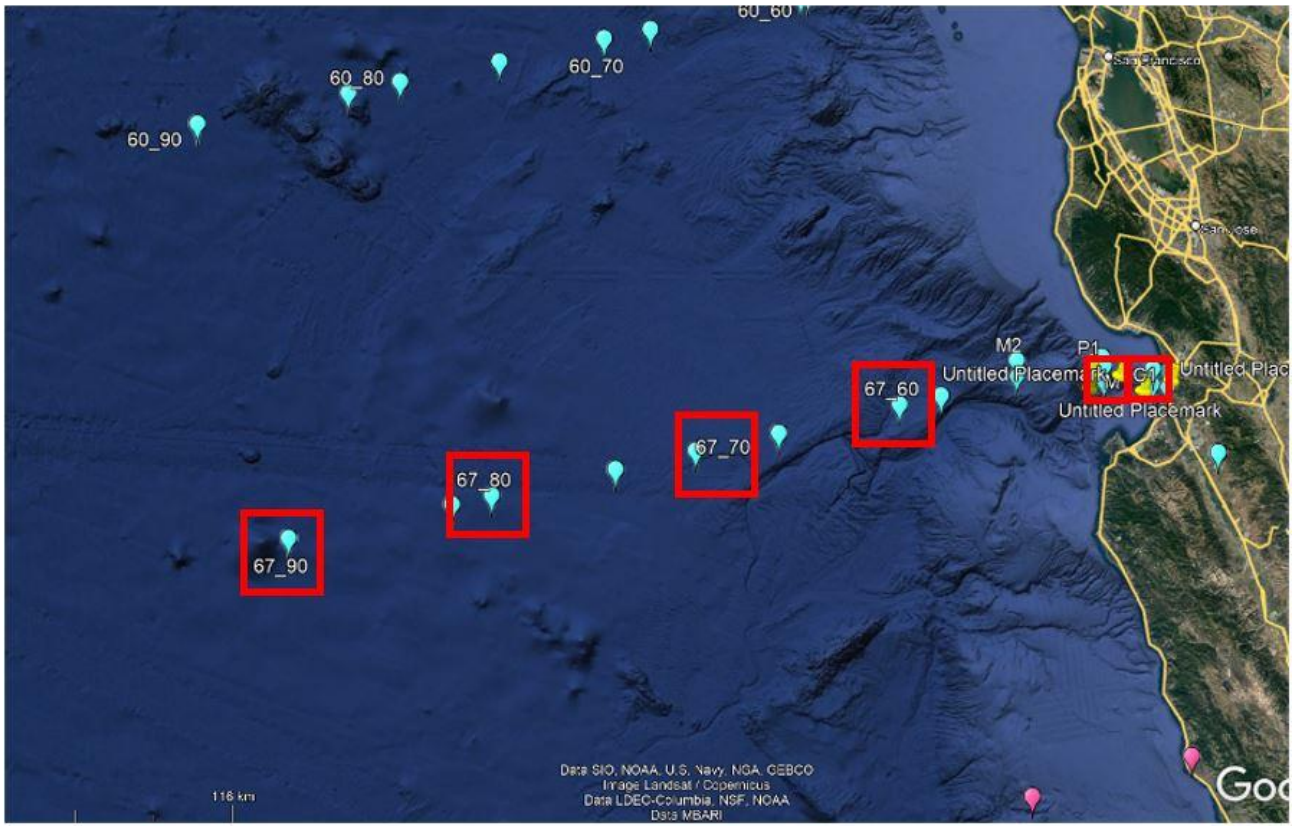

Figure 6. Map of stations where samples were collected off of the California coast aboard R/V Western Flyer on the cruise YODA2022. Samples were collected in the stations highlighted inside the red squares.

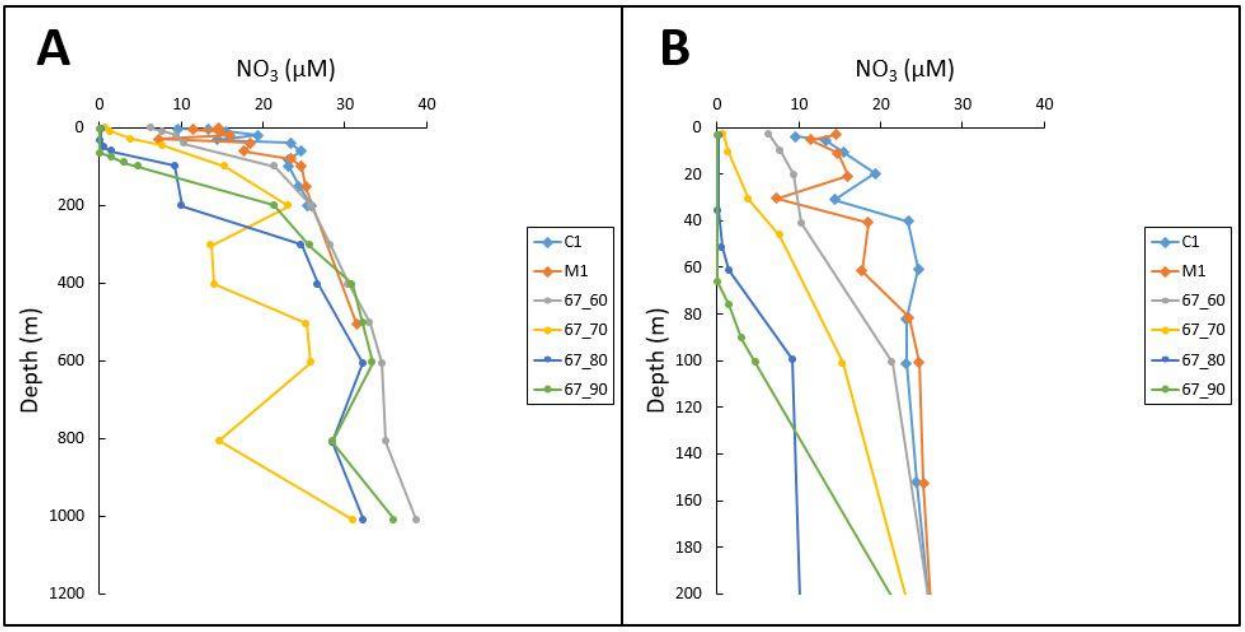

Figure 7. Nitrate Profiles of all six sampled stations on YODA2022. (A) Full profile (B) Top 200 meters.

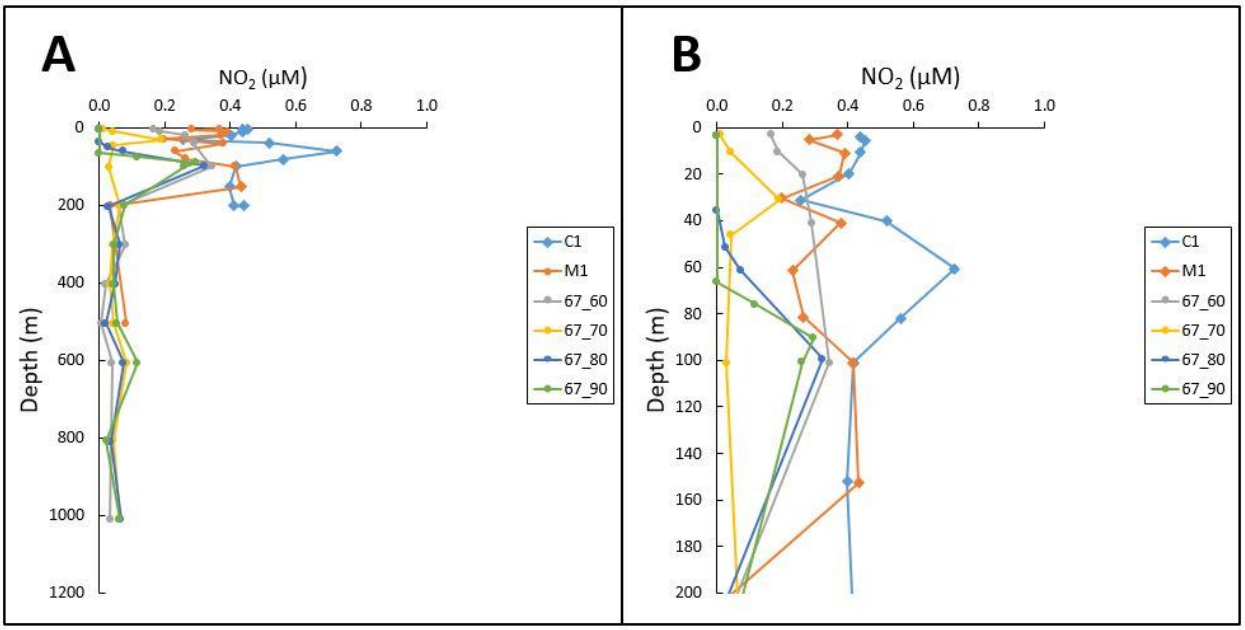

Figure 8. Nitrite profiles of all six sampled stations on YODA2022. (A) Full profile (B) Top 200 meters.

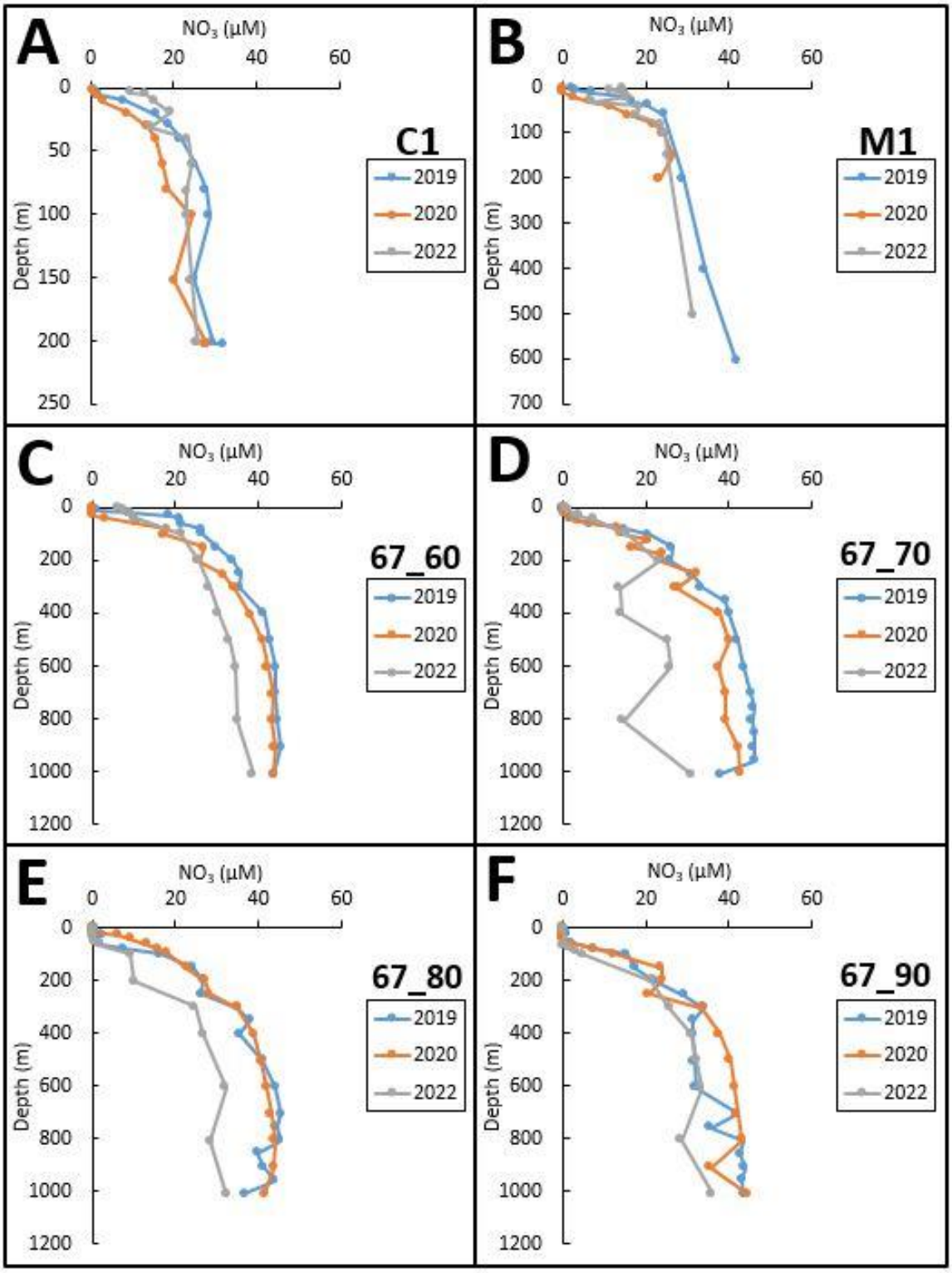

Figure 9. 2019, 2020, 2022 nitrate data from each sampling station.

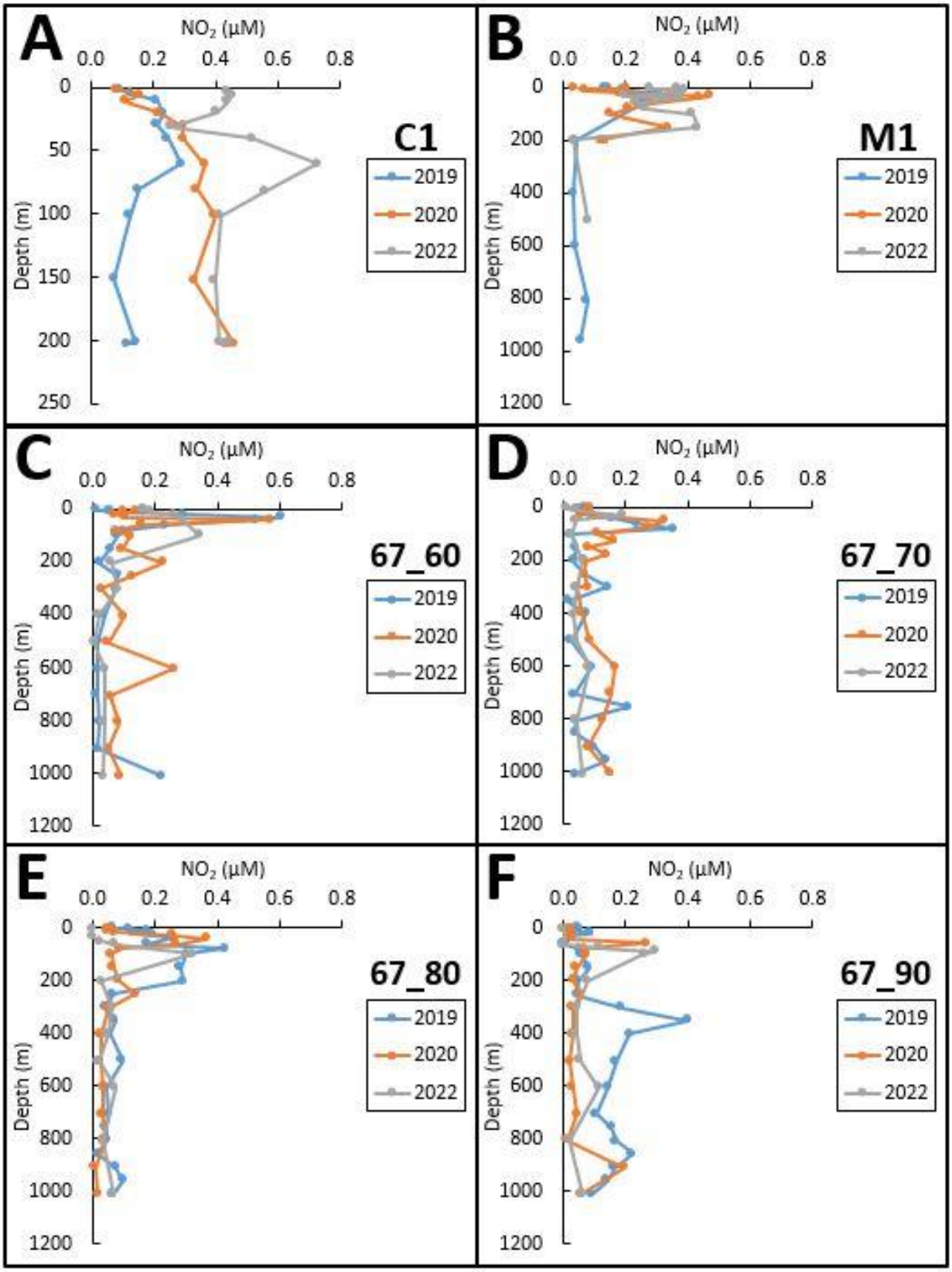

Figure 10. 2019, 2020, 2022 nitrite data from each sampling station.

Sample Deviations		
	NO2	NO3
Standard Deviations	0.0002	0.0033
Relative Standard Deviations	2.16%	0.68%

Figure 11. Mean standard deviations and relative standard deviations of absorbances of all collected samples.

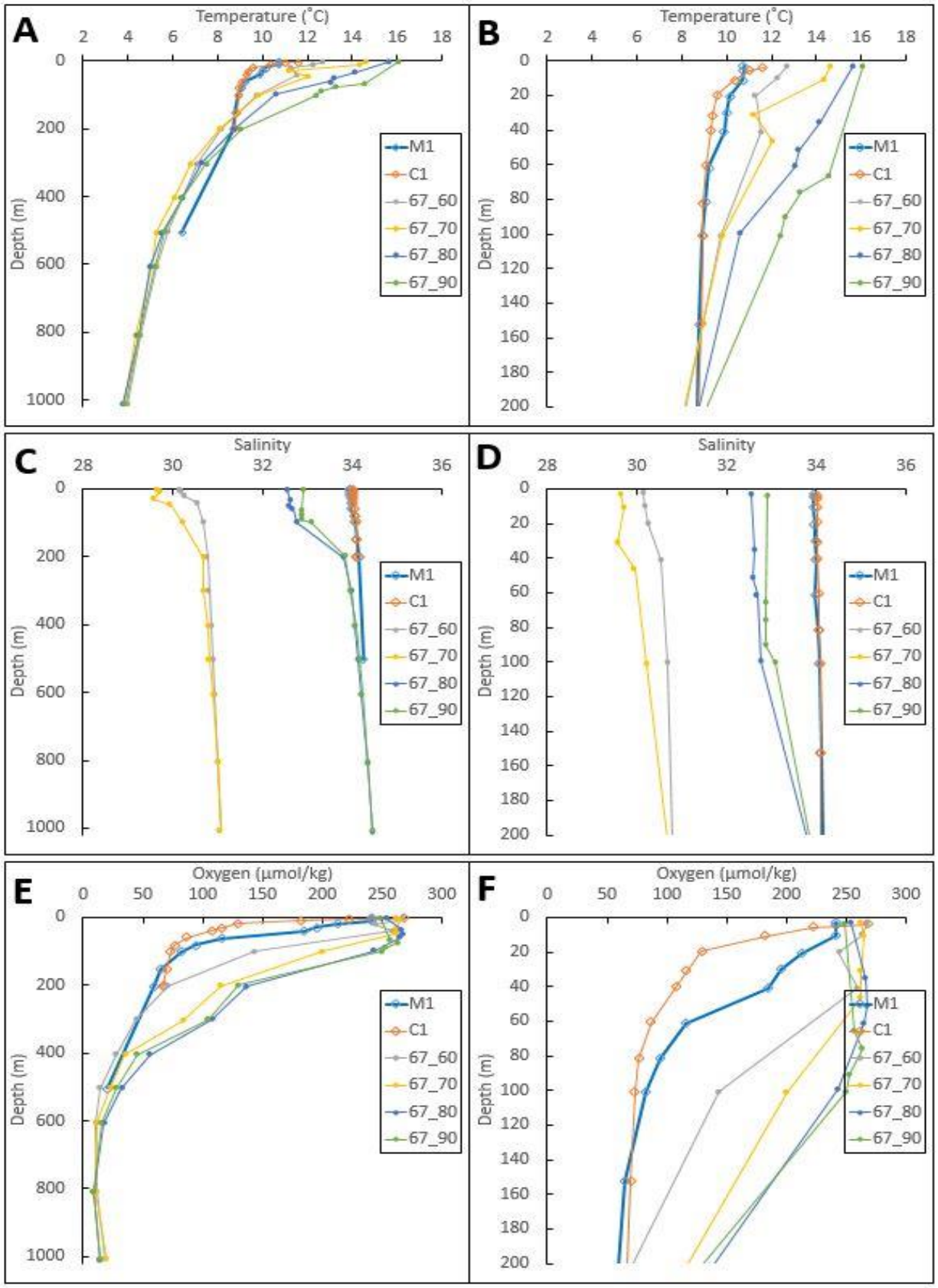

Figure 12. Temperature, Salinity, Oxygen for all sampling stations on YODA2022. (B),(D), and (F) are the top 200 meters.

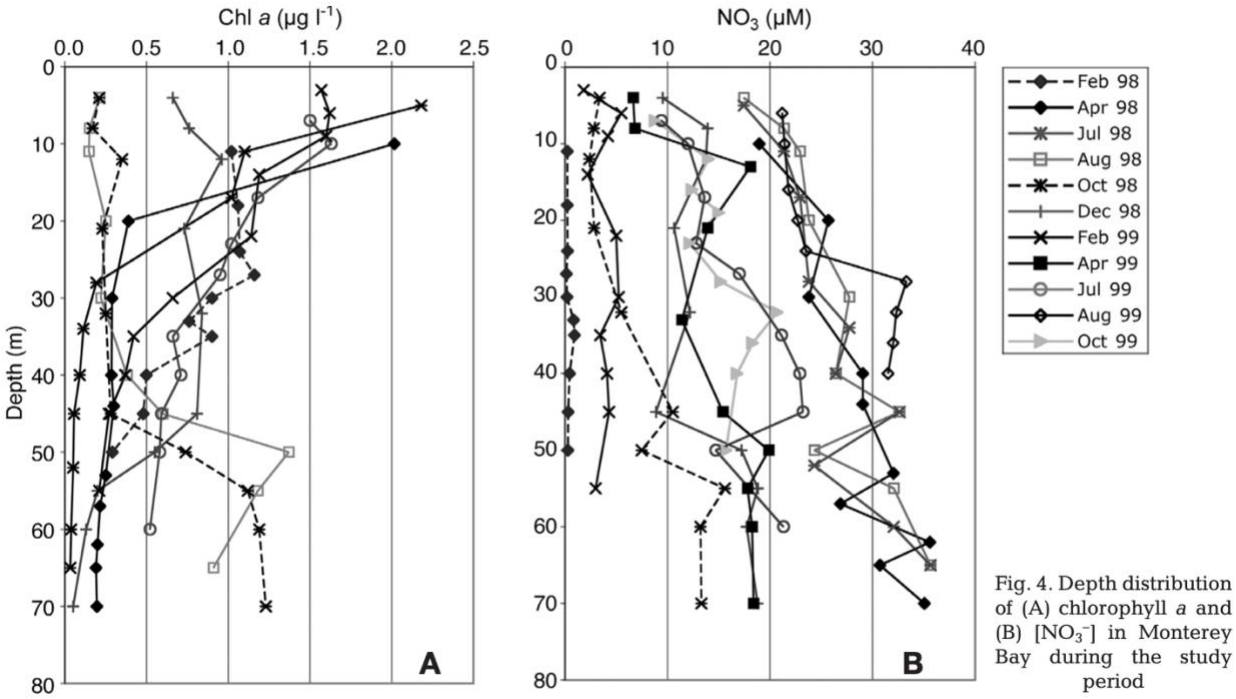

Fig. 4. Depth distribution of (A) chlorophyll *a* and (B) [NO_3^-] in Monterey Bay during the study period

Figure 13. Graph of chlorophyll a and nitrate in Monterey Bay. (Ward B.B., 2005).