# Saanich Inlet field and laboratory methods related to experimental rate data acquired aboard the R/V John Strickland during two cruises in August, 2021 and June, 2022

Website: https://www.bco-dmo.org/dataset/962576

**Data Type**: Other Field Results

Version: 1

Version Date: 2025-10-01

#### **Project**

» <u>Collaborative Research: Nitrous oxide reduction in oxygen minimum zones: an understudied but critical loss</u> term in ocean greenhouse gas cycling (oxygen minimum zones)

Contributors	Affiliation	Role
Grundle, Damian	Bermuda Institute of Ocean Sciences (BIOS)	Principal Investigator
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Bristow, Laura	University of Southern Denmark	Scientist
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#### Abstract

These data include oceanographic, chemical, and biogeochemical rate measurements relating to oxygen, nitrogen, and sulfur species from Saanich Inlet, British Columbia, Canada, spanning lower oxycline and anoxic depths in the basin. Data and samples were collected aboard the R/V John Strickland during two cruises in August, 2021 and June, 2022. Data from 2021 capture the dynamics of an oxygen renewal event of the deep anoxic basin, which occurred between the second and third sampling days. Data from 2022 were collected during stable, stratified conditions with complete anoxia in the deep basin throughout the sampling period. Rate measurements are from 15N-labeled tracer experiments tracking the production and consumption of nitrous oxide (N2O), a potent greenhouse gas that is produced under hypoxic and anoxic conditions. These data evaluate the extent to which consumption of N2O in the deep basin balances out is production in overlying waters and were collected by Dr. Sheryl Murdock (Arizona State University) and Dr. Laura Bristow (University of Gothenburg).

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#### Coverage

**Location**: Seasonally anoxic basin on Vancouver Island, Canada (48.6 N, 123.5 W). Maximum depth 225 meters.

**Spatial Extent**: N:48.6275 **E**:-123.4988 **S**:48.59 **W**:-123.51

Temporal Extent: 2021-08-01 - 2022-06-22

#### Methods & Sampling

#### Sample collection

For all rate experiments, water was sampled through gas-tight Viton tubing into vials or glass bottles filling from the bottom with three times overflow. Vials or bottles were closed bubble-free with deoxygenated butyl rubber stoppers and kept cool/dark until return to the laboratory. Collection vessels included 20-mL glass vials (2021) or 250-mL glass serum bottles (2022) for  $N_2O$  production experiments, 500-mL glass bottles for  $N_2O$  consumption, anaerobic ammonia oxidation, denitrification, dissimilatory nitrite reduction to ammonium, and nitrite oxidation experiments, and 250-mL glass serum bottles for dark carbon fixation experiments.

#### **Experimental set-up**

**Laboratory set up for N<sub>2</sub>O production:** In 2021, a 2-mL N<sub>2</sub> headspace was introduced in each 20-mL vial before it underwent 2 cycles composed of 30 seconds of vigorous shaking followed by 30 seconds of flushing with N<sub>2</sub>. Each vial was then amended with one of three  $^{15}$ N-labeled tracer solutions ( $^{15}$ NH<sub>4</sub>+/ $^{14}$ NO<sub>2</sub>-,  $^{15}$ NO<sub>2</sub>-/ $^{14}$ NH<sub>4</sub>+,  $^{15}$ NO<sub>3</sub>-/ $^{14}$ NH<sub>4</sub>) to quantify rates of N<sub>2</sub>O production from NH<sub>4</sub>+ oxidation and NO<sub>3</sub>- and NO<sub>2</sub>- reduction. Each tracer solution was added to six vials for a 2  $\mu$ mol·L-1 increase in the concentration of both the  $^{15}$ N and  $^{14}$ N substrates. In 2022, a helium headspace was added to 250-mL bottles followed immediately by purging of the water for 30 minutes with helium. Each bottle was then amended with one of the three tracer solutions and the liquid dispensed into replicate 12-mL exetainer vials for incubation. Each vial was injected with 1 $\mu$ M unlabeled  $^{14}$ N<sub>2</sub>O to stem the loss of  $^{15}$ N-labeled N<sub>2</sub>O to other processes (2022 only). In both years, vials were incubated in the dark at in situ temperature and were terminated at 0 and 12 hours (2021) or 0, 9, and 18 hours (2022) by injection of saturated mercuric chloride.

**Laboratory set up for N\_2O consumption:** A headspace was introduced followed immediately by purging of the water for 30 minutes with helium. Water was dispensed with > 2 volumes overflow into 12-mL exetainer vials that were immediately closed with deoxygenated chlorobutyl rubber septa. Each vial received a 2-mL helium headspace before undergoing 2 cycles composed of 30 seconds of vigorous shaking followed by 30 seconds of flushing with helium. Each vial was then injected with  $^{15}$ N $_2$ O (1  $\mu$ M) and incubated in the dark at in situ temperature, and were terminated at four time points, 0, 6, 12 and 24 hours, by injection of saturated mercuric chloride.

Laboratory set up for anaerobic ammonia oxidation, denitrification, dissimilatory nitrite reduction to ammonium, and nitrite oxidation: A headspace was introduced and  $^{15}\text{NO}_2$  added (5  $\mu$ M) followed immediately by purging of the water for 30 minutes with helium. Water was dispensed with > 2 volumes overflow into 12-mL exetainer vials that were immediately closed with deoxygenated chlorobutyl rubber septa. Each vial received a 2-mL helium headspace before undergoing 2 cycles composed of 30 seconds of vigorous shaking followed by 30 seconds of flushing with helium. Vials were then incubated in the dark at in situ temperature, and were terminated at four time points, 0, 6, 12 and 24 hours, by injection of saturated mercuric chloride.

**Laboratory set up for dark carbon fixation:** <sup>13</sup>C-DIC was introduced into each serum bottle and a 20-mL helium headspace inserted, before undergoing 2 cycles composed of 30 seconds of vigorous shaking followed by 30 seconds of flushing with helium. A sample of the headspace was placed into a 3 mL glass vial, and preserved with mercuric chloride, headspace free for labeling percentage determination (averaged 20 %). Serum bottles were then incubated in the dark at in situ temperature, and duplicate bottles were terminated at two time points 0 and 24 hours by filtration onto pre-combusted glass fiber filters (GF-75). Filters were subsequently oven dried and stored at room temperature until analysis.

**N2O production:** In 2021, rates of 15N2O production were calculated using equations in Ji et al (2020) and the bulk d15N-N2O measured by CRDS in each experimental vial. The 15N enrichment of each substrate pool (F) was calculated from stock tracer solution concentrations, confirmed using an Astoria Nutrient Autoanalyzer following the methodology of Barwell-Clarke and Whitney 1996, and a tracer addition volume of 0.1 mL. For 2022 experiments, the production of 15N15N and 15N15N18O were measured on a coupled gas chromatograph-isotope ratio mass spectrometer (GC-IRMS) and corrected for the labelling in the initial substrate pool (Bourbonnais et al 2021, Dalsgaard et al 2012). The 15N enrichment of each substrate pool (F) was determined by the concentrations of substrate before and after tracer addition as in (Dalsgaard et al 2012).

**N2O consumption:** The production of 15N15N was analyzed on a gas chromatography isotope ratio mass spectrometer (Dalsgaard et al 2012).

**Anaerobic ammonia oxidation and Denitrification:** The production of 14N15N and 15N15N was analyzed on a GC-IRMS and corrected for the labelling in the initial substrate pool (Dalsgaard et al 2012).

**Dissimilatory nitrite reduction to ammonium:** After conversion of 15NH4 to N2 with alkaline hypobromite (Warembourg 1993) the production of 14N15N and 15N15N was analyzed on a GC-IRMS and corrected for the labelling in the initial substrate pool (Dalsgaard et al 2012).

**Nitrite oxidation:** After conversion of 15NO3 to N2 with cadmium/sulfamic acid (Bristow et al 2016), the production of 14N15N and 15N15N was analyzed on a GC-IRMS and corrected for the labelling in the initial substrate pool (Dalsgaard et al 2012).

**Dark carbon fixation:** After acidification, the 13C enrichment was analysed by an elemental analyzer - isotope ratio mass spectrometer.

#### **BCO-DMO Processing Description**

- \* converted nd to blanks (no data, i.e., experiments were not run)
- \* converted dates and times to ISO formats

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#### **Data Files**

File

**962576\_v1\_rate.csv**(Comma Separated Values (.csv), 9.74 KB)
MD5:7145a44a2b74f703a922d2c86cebc706

Primary data file for dataset ID 962576, version 1

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## **Supplemental Files**

#### File

#### Cruise casts and sampling

filename: SaanichN2O\_event\_log.csv

(Comma Separated Values (.csv), 12.58 KB) MD5:685b8050053156e638486938e30bb492

List of all field sampling events and sample types collected during each event over two cruises in August 2021 and June 2022 (cruises JS202206 & JS202108). Column descriptions:

Column Name, Column Description, Units of measurement

Event, "Unique event identity - format ""FieldDay\_year\_event#\_instrument""",

Field Day, Sampling day designation from SI01-SI11,

Sampling\_Date, Calendar day - format YYYYMMDD,

Cast\_Start\_Time, Pacific daylight time (UTC minus 7 hours) at start of event,

Tidal\_height, Tidal height measured at Patricia Bay. Data from Fisheries and Oceans Canada (https://www.tides.gc.ca/en/stations/07277/), meters Station, "Two sampling stations & S3, S4",

Latitude, Station latitude (decimal degrees), decimal degrees

Longitude, Station longitude (decimal degrees), decimal degrees

Cast, Year\_cast#,

Instrument, "Instrument being lowered (CTD=CTD rosette, RNAS=RNA sampler)",

Sample\_Depth, "Depth where bottles were closed, or RNA sampler filtered", meters

Depth Code, Sampling depth categories described in cruise report,

Interface, "Oxic/anoxic interface depth, where oxygen reached the detection limit of the SBE43 sensor", meters

Event\_Type, "Sampling CTD cast, oceanographic profile CTD cast, or RNA sampler test",

Sample\_Nutrients, Sampled from rosette for nutrients concentrations (nutrient analyzer),

Sample\_Nitrite, Sampled from rosette for nitrite concentrations (colorimetric method),

Sample Ammonium, Sampled from rosette for ammonium concentrations (fluorometric method),

Sample\_Oxygen, Sampled from rosette for dissolved oxygen concentrations (Winkler titration),

Sample Nitrous Oxide, Sampled from rosette for nitrous oxide concentrations (cavity ring-down spectrometry),

Sample\_Sulfide, Sampled from rosette for sulfide concentrations (colorimetric method),

Sample\_DNA, "Sampled from rosette for DNA analysis (amplicon sequencing, metagenomics)",

Sample\_RNA, Sampled from rosette for RNA analysis (metatranscriptomics),

Sample N2Oprod Rates, Sampled from rosette for 15N-tracer incubations (nitrous oxide production pathways),

Sample N2Ocons Rates, Sampled from rosette for 15N-tracer incubations (nitrous oxide consumption),

Sample\_Anammox\_Rates, Sampled from rosette for 15N-tracer incubations (anaerobic ammonia oxidation),

Sample\_Denit\_Rates, Sampled from rosette for 15N-tracer incubations (denitrification),

Sample\_DNRA\_rates, Sampled from rosette for 15N-tracer incubations (dissimilatory nitrite reduction to ammonium),

Sample\_NitOx\_Rates, Sampled from rosette for 15N-tracer incubations (nitrite oxidation),

Sample\_darkCfix\_rates,Sampled from rosette for 13C-tracer incubations (dark carbon fixation),

Comments, Additional information,

#### Depth\_Code\_descriptions.csv

(Comma Separated Values (.csv), 932 bytes) MD5:4db07cfd06314d3509e96560347001fc

Description of the sampling depth categories

#### SaanichN2O\_rate\_data\_kinetics.csv

(Comma Separated Values (.csv), 3.59 KB) MD5:3c7880c7854525e9f0075eacd82fb266

Kinetics data on geochemical rates. Column descriptions:

Column Name, Column Description, Units of measurement

Event, "Unique event identity - format ""FieldDay year event# instrument""", unitless

Station, "Two sampling stations D S3, S4", unitless

Latitude, Latitude of sampling site, decimal degrees

Longitude, Longitude of sampling site, decimal degrees

Sample\_Depth, "Depth where bottles were closed, or RNA sampler filtered", meters

Depth\_Code,Sampling depth categories described in cruise report,unitless

Manipulated\_Conc,"Range of additions of either N2O, oxygen, or sulfide in N2O consumption kinetics experiments",nanomoles per liter

Manipulated\_Conc\_SD,"Standard deviation of manipulated concentrations of N2O, oxygen, or sulfide in N2O consumption kinetics experiments",nanomoles per liter

N2Ocons\_Rates, "N2O conumption rate under manipulated N2O, oxygen, or sulfide conditions", nanomoles per liter per day N2Ocons SE, Standard error of manipulated N2O consumption rates, nanomoles per liter per day

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#### **Related Publications**

Bourbonnais, A., Frey, C., Sun, X., Bristow, L. A., Jayakumar, A., Ostrom, N. E., Casciotti, K. L., & Ward, B. B. (2021). Protocols for Assessing Transformation Rates of Nitrous Oxide in the Water Column. Frontiers in Marine Science, 8. https://doi.org/10.3389/fmars.2021.611937

Methods

Bristow, L. A., Dalsgaard, T., Tiano, L., Mills, D. B., Bertagnolli, A. D., Wright, J. J., Hallam, S. J., Ulloa, O.,

Canfield, D. E., Revsbech, N. P., & Thamdrup, B. (2016). Ammonium and nitrite oxidation at nanomolar oxygen concentrations in oxygen minimum zone waters. Proceedings of the National Academy of Sciences, 113(38), 10601–10606. https://doi.org/10.1073/pnas.1600359113

Methods

Dalsgaard, T., Thamdrup, B., Farías, L., & Revsbech, N. P. (2012). Anammox and denitrification in the oxygen minimum zone of the eastern South Pacific. Limnology and Oceanography, 57(5), 1331–1346. Portico. https://doi.org/10.4319/lo.2012.57.5.1331

Methods

Ji, Q., Jameson, B. D., Juniper, S. K., & Grundle, D. S. (2020). Temporal and Vertical Oxygen Gradients Modulate Nitrous Oxide Production in a Seasonally Anoxic Fjord: Saanich Inlet, British Columbia. Journal of Geophysical Research: Biogeosciences, 125(9). Portico. https://doi.org/10.1029/2020jg005631 <a href="https://doi.org/10.1029/2020JG005631">https://doi.org/10.1029/2020JG005631</a> Methods

Murdock, Sheryl (2025). Saanich Inlet nitrous oxide project cruise reports. http://hdl.handle.net/<u>1834/43479</u> Related Research

Warembourg FR (1993). Nitrogen fixation in soil and plant systems. Nitrogen isotope techniques 654: 157-180. *Methods* 

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#### **Parameters**

Parameter	Description	Units
Event	Unique event identity - format "FieldDay_year_event#_instrument"	unitless
Station	Two sampling stations Đ S3, S4	unitless
Sampling_Date	Calendar day	unitless
Cast_Start_Time	Pacific daylight time (UTC minus 7 hours) at start of event	unitless
Tidal_height	Tidal height measured at Patricia Bay. Data from Fisheries and Oceans Canada (https://www.tides.gc.ca/en/stations/07277/)	meters (m)
Latitude	Latitude of station location	decimal degrees
Longitude	Longitude of station location	decimal degrees
Sample_Depth	Depth where bottles were closed, or RNA sampler filtered	meters
Depth_Code	Sampling depth categories described in cruise report	unitless
NH4_N2Oprod_Rates	N2O production rate from ammonium (rate at which N2O is produced during the oxidation of ammonium to nitrite). "ns" indicates experiments were run but rates were not significant (p=0.05).	nanomoles per liter per day

NH4_N2Oprod_SE	Standard error of N2O production rate from ammonium. "ns" indicates experiments were run but rates were not significant (p=0.05).	nanomoles per liter per day
NO2_N2Oprod_Rates	N2O production rate from nitrite (rate at which N2O is produced from the reduction of nitrite). "ns" indicates experiments were run but rates were not significant (p=0.05).	nanomoles per liter per day
NO2_N2Oprod_SE	Standard error of N2O production rate from nitrite. "ns" indicates experiments were run but rates were not significant (p=0.05).	nanomoles per liter per day
NO3_N2Oprod_Rates	N2O production rate from nitrate (rate at which N2O is produced from the reduction of nitrate). "ns" indicates experiments were run but rates were not significant (p=0.05).	nanomoles per liter per day
NO3_N2Oprod_SE	Standard error of N2O production rate from nitrate. "ns" indicates experiments were run but rates were not significant (p=0.05).	nanomoles per liter per day
N2Ocons_Rates	N2O consumption rate (rate at which N2O is reduced to nitrogen gas). "ns" indicates experiments were run but rates were not significant (p=0.05).	nanomoles per liter per day
N2Ocons_SE	Standard error of N2O consumption rate. "ns" indicates experiments were run but rates were not significant (p=0.05).	nanomoles per liter per day
Anammox_Rates	Anaerobic ammonium oxidation rate (rate at which ammonium is converted into dinitrogen gas). "ns" indicates experiments were run but rates were not significant (p=0.05).	nanomoles per liter per day
Anammox_SE	Standard error of anaerobic ammonium oxiddation rate. "ns" indicates experiments were run but rates were not significant (p=0.05).	nanomoles per liter per day
Denit_Rates	Denitirification rate (rate at which nitrate and/or nitrite are reduced to dinitrogen gas). "ns" indicates experiments were run but rates were not significant (p=0.05).	nanomoles per liter per day
Denit_SE	Standard error of denitrification rate. "ns" indicates experiments were run but rates were not significant (p=0.05).	nanomoles per liter per day
DNRA_rates	Dissimiliatory nitrite reduction to ammonium rate (rate at which nitrite is converted to ammonium). "ns" indicates experiments were run but rates were not significant (p=0.05).	nanomoles per liter per day
DNRA_SE	Standard error of dissimiliatory nitrite reduction to ammonium rate. "ns" indicates experiments were run but rates were not significant (p=0.05).	nanomoles per liter per day

NitOx_Rates	Nitrite oxidation rates (rate at which nitrite is oxidized to nitrate). "ns" indicates experiments were run but rates were not significant (p=0.05).	nanomoles per liter per day
NitOx_SE	Standard error of nitrite oxidation rates. "ns" indicates experiments were run but rates were not significant ( $p$ =0.05).	nanomoles per liter per day
DarkCfix_rates	Dark carbon fixation rates (rate at which inorganic carbon is incorporated into cellular biomass). "ns" indicates experiments were run but rates were not significant ( $p=0.05$ ).	nanomoles per liter per day
DardCfix_SE	Standard error of dark carbon fixation rates. "ns" indicates experiments were run but rates were not significant (p=0.05).	nanomoles per liter per day
Notes	Notes	units

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# Instruments

Dataset- specific Instrument Name	Picarro Cavity Ring-Down Spectrometer
Generic Instrument Name	Cavity enhanced absorption spectrometers
Dataset- specific Description	Picarro Cavity Ring-Down Spectrometer: Used for measurement of nitrous oxide concentrations, both natural and in 15N-tracer experiments from 2021.
Generic Instrument Description	Instruments that illuminate a sample inside an optical cavity, typically using laser light, and measure the concentration or amount of a species in gas phase by absorption spectroscopy. Techniques include cavity ring-down spectroscopy (CRDS) and integrated cavity output spectroscopy (ICOS).

Dataset- specific Instrument Name	Trilogy Fluorometer - Turner Designs
Generic Instrument Name	Fluorometer
Dataset- specific Description	Trilogy Fluorometer (Turner Designs): Ammonium concentration measurements
	A fluorometer or fluorimeter is a device used to measure parameters of fluorescence: its intensity and wavelength distribution of emission spectrum after excitation by a certain spectrum of light. The instrument is designed to measure the amount of stimulated electromagnetic radiation produced by pulses of electromagnetic radiation emitted into a water sample or in situ.

Dataset- specific Instrument Name	Delta V Plus Isotope Ratio mass spectrometer
Generic Instrument Name	Isotope-ratio Mass Spectrometer
Dataset- specific Description	Delta V Plus Isotope Ratio mass spectrometer: Used for measurement of 15N-dinitrogen and -nitrous oxide from 15N-tracer experiments.
Generic Instrument Description	The Isotope-ratio Mass Spectrometer is a particular type of mass spectrometer used to measure the relative abundance of isotopes in a given sample (e.g. VG Prism II Isotope Ratio Mass-Spectrometer).

Dataset- specific Instrument Name	Small rosette containing 12 x 2.5L Niskin bottles
Generic Instrument Name	Niskin bottle
Dataset- specific Description	12-bottle sampling rosette: Small rosette containing 12 $\times$ 2.5L Niskin bottles was used for all sample collections.
	A Niskin bottle (a next generation water sampler based on the Nansen bottle) is a cylindrical, non-metallic water collection device with stoppers at both ends. The bottles can be attached individually on a hydrowire or deployed in 12, 24, or 36 bottle Rosette systems mounted on a frame and combined with a CTD. Niskin bottles are used to collect discrete water samples for a range of measurements including pigments, nutrients, plankton, etc.

Dataset- specific Instrument Name	Astoria Nutrient Analyzer
Generic Instrument Name	Nutrient Autoanalyzer
Dataset- specific Description	Astoria Nutrient Analyzer: Nitrate and nitrite concentration measurements
	Nutrient Autoanalyzer is a generic term used when specific type, make and model were not specified. In general, a Nutrient Autoanalyzer is an automated flow-thru system for doing nutrient analysis (nitrate, ammonium, orthophosphate, and silicate) on seawater samples.

Dataset-specific Instrument Name	High-sensitivity oxygen sensor
Generic Instrument Name	Oxygen Sensor
	High-sensitivity oxygen sensor: More accurate oxygen measurements from nanomolar concentrations
	An electronic device that measures the proportion of oxygen (O2) in the gas or liquid being analyzed

Dataset- specific Instrument Name	Seabird SBE 19plus
Generic Instrument Name	Sea-Bird SBE 19plus V2 SEACAT CTD
Dataset- specific Description	Seabird SBE 19plus CTD: Oceanographic data collection
	Self-contained self-powered CTD profiler. Measures conductivity, temperature and pressure (Digiquartz sensor) in both profiling (samples at 4 scans/sec) and moored (sample rates of once every 5 seconds to once every 9 hours) mode. Available in plastic or titanium housing with depth ranges of 600m and 7000m respectively. Miniature submersible pump provides water to the conductivity cell. Compared to the previous 19plus, the V2 incorporates an electronics upgrade and additional features, with six differentially amplified A/D input channels, one RS-232 data input channel, and 64 MB FLASH memory.

Dataset-specific Instrument Name	Seabird SBE 43 oxygen sensor
Generic Instrument Name	Sea-Bird SBE 43 Dissolved Oxygen Sensor
Dataset-specific Description	Seabird SBE 43 oxygen sensor: Measuring real-time, in-situ oxygen concentrations across the hypoxic/anoxic boundary (low micromolar detection limit)
Generic Instrument Description	The Sea-Bird SBE 43 dissolved oxygen sensor is a redesign of the Clark polarographic membrane type of dissolved oxygen sensors. more information from Sea-Bird Electronics

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# Deployments

# JS202206

Website	https://www.bco-dmo.org/deployment/954643	
Platform	R/V John Strickland	
Report	http://hdl.handle.net/1834/43479	
Start Date	2022-06-13	
End Date	2022-06-22	
Description	During R/V John Strickland cruise JS202206, sampling was conducted in a seasonally anoxic basin on Vancouver Island (British Columbia, Canada)	

# JS202108

Website	https://www.bco-dmo.org/deployment/954560
Platform	R/V John Strickland
Report	http://hdl.handle.net/1834/43479
Start Date	2021-08-01
End Date	2021-08-13
Description	During R/V Strickland cruise JS202108, sampling was conducted in a seasonally anoxic basin on Vancouver Island (British Columbia, Canada)

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### **Project Information**

Collaborative Research: Nitrous oxide reduction in oxygen minimum zones: an understudied but critical loss term in ocean greenhouse gas cycling (oxygen minimum zones)

Coverage: Saanich Inlet — a seasonally anoxic fjord on Vancouver Island, British Columbia, Canada

#### **NSF Award Abstract:**

Nitrous oxide (N2O) is a gas produced by microbes in both aquatic and terrestrial environments, and, like other greenhouse gases, it contributes to global warming. Furthermore, N2O can destroy ozone, a gas responsible for protecting the earth from dangerous ultraviolet radiation. In the ocean, N2O production is largely controlled by the amount of available dissolved oxygen, with more N2O being produced under low oxygen concentrations; however, when no oxygen is available, a scenario referred to as anoxia, microbes in the ocean switch from producing N2O to consuming N2O. In recent years, it has become evident that zones of low oxygen are expanding in some areas of the oceans, and this has raised concern that more N2O will be produced. If this occurs, more N2O will be emitted to the atmosphere, and will lead to further global warming and ozone destruction. Because of this, research has largely focused on understanding how much N2O is produced in the ocean under low oxygen conditions. If, however, anoxic zones also increase in size, this could act to balance out, at least to some degree, the predicted increase in N2O production caused by the expansion of zones where oxygen is present but in low concentrations. This study aims to simultaneously measure N2O production and consumption, in both low oxygen and anoxic zones and identify the microbes responsible for N2O production and consumption. Our results will: 1) lead to a much better understanding of how N2O consumption in anoxic zones could help to balance out an increase in N2O production if low oxygen zones in the ocean continue to expand, 2) help to inform models aimed at predicting oceanic N2O production and emissions to the atmosphere under future ocean conditions, and 3) allow us to better understand the microbes involved in N2O production and consumption. Our study will support a postdoc and undergraduate students who will work at the interface of marine chemistry and community genomics. The PIs plan to specifically consider applications from underrepresented minorities and students at institutions with limited opportunities. The PIs also plan a number of other educational/outreach programs ranging from teachertraining workshops, teacher internships, and academic and public lecture series.

The oceanic production of the potent greenhouse and ozone destroying gas nitrous oxide (N2O) increases as dissolved oxygen (DO) concentrations transition from oxic to hypoxic. Marine DO concentrations have decreased globally with climate change and oceanic hypoxic zones have expanded and predicted to continue expanding. This increase is cause for concern that N2O production in the ocean will increase in the future which would lead to higher emissions to the atmosphere. As a result, much research has focused on quantifying the oxygen thresholds that correspond to large increases in N2O production. In contrast, relatively few studies have aimed to quantify the capacity for net N2O consumption, resulting from microbial N2O reduction to N2 under anoxic conditions, to buffer against predicted N2O production increases if anoxic zones expand in conjunction with hypoxic zones. To this end, this study aims to simultaneously quantify N2O production and consumption from oxic-hypoxic-anoxic water column zones, in order to determine the potential for N2O consumption to counteract predicted increases in N2O production. Our field work be conducted in Saanich Inlet, a British Columbian fjord which is an ideal natural laboratory for our study, as it is characterized by a well-established oxycline and anoxic zone. Specifically, we aim to 1) measure bulk N2O

concentrations, and, using 15N tracer techniques, quantify N2O production and consumption rates as DO concentrations decrease from oxic to anoxic conditions, 2) quantify the magnitude by which N2O consumption in the anoxic zone balances increased N2O production in the overlying hypoxic region, and 3) definitively link observed N2O production and consumption rates to the microorganisms mediating this process, focusing specifically on distinguishing N2O consumption via denitrifier (NO3- to N2) versus non-denitrifier (N2O to N2 only) taxa. Ultimately, our results will provide quantitative information on N2O consumption rates over fluctuating ocean conditions, thereby helping constrain models of oxygen effects on net N2O production and ocean-to-atmosphere greenhouse gas fluxes. Furthermore, this work will identify the taxonomic breadth of microbes capable of N2O reduction and their linkage to actual N2O reduction rates, thereby providing a quantitative understanding of whether or not the detection of specific bio-signatures is predictive of marine N2O dynamics.

This award reflects NSF's statutory mission and has been deemed worthy of support through evaluation using the Foundation's intellectual merit and broader impacts review criteria.

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## **Funding**

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-2023430
NSF Division of Ocean Sciences (NSF OCE)	OCE-2022991

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