Natural abundance N2O isotopomers measured from seawater samples collected in the Eastern Tropical North Pacific during R/V Sally Ride (SR1805) cruise from March to April 2018

Website: https://www.bco-dmo.org/dataset/832995
Data Type: Cruise Results
Version: 1
Version Date: 2020-08-02

Project
» Collaborative Research: Mechanisms and Controls of Nitrous Oxide Production in the Eastern Tropical North Pacific Ocean (N2O in ETNP)

Contributors	Affiliation	Role
Casciotti, Karen L.	Stanford University	Principal Investigator
Baya, Pascale A.	Stanford University	Scientist
Kelly, Colette LaMonica	Stanford University	Scientist
Travis, Nicole M.	Stanford University	Scientist
Gerlach, Dana Stuart	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager

Abstract
Seawater samples for dissolved nitrate, nitrite, and nitrous oxide were collected from CTD casts during R/V Sally Ride 1805 cruise (March/April 2018) in the Eastern Tropical North Pacific oxygen minimum zone.

Table of Contents

- Coverage
- Dataset Description
  - Methods & Sampling
  - Data Processing Description
- Data Files
- Related Publications
- Parameters
- Instruments
- Deployments
- Project Information
- Funding

Coverage

Spatial Extent: **N**:17.683 **E**:-102.35 **S**:10 **W**:-112.9998
Temporal Extent: 2018-03-13 - 2018-04-04

Methods & Sampling

Seawater samples for dissolved nitrate, nitrite, and nitrous oxide isotope analysis were collected from either a 30-liter, 12-bottle rosette or a 12-liter, 24-bottle rosette. Samples for nitrate and nitrite isotopic analysis were collected, unfiltered, into 500 mL Nalgene polypropylene bottles following three rinses of the bottle, caps, and threads of at least 10% of the bottle volume. After collection from the rosette, samples for nitrate isotopic analysis were syringe-filtered with a 60 mL syringe through a 0.22 µM pore size Sterivex filter into 60-mL high density polyethylene bottles, then frozen at –20ºC

For **nitrite isotopic analysis**, samples were preserved within two hours of collection for δ15N-NO2- and δ18O-NO2- using the azide method (McIlvin and Altabet, 2005), along with nitrite isotope reference materials (Casciotti et al., 2007) in different amounts. Briefly, seawater samples were added to 20 mL vials in volumes

targeted to achieve 10 nmol nitrite, based on shipboard colorimetric nitrite analysis (Grasshoff et al., 1999), then capped with a gray butyl septum (National Scientific) and sealed with an aluminum crimp seal. Where [NO2–] > 0.25 µM (limit of detection for these analyses, Figure S1 in Kelly et al, 2020) but < 2 µM, the maximum volume allowable for analysis (10 mL) seawater was subsampled, regardless of actual nitrite concentration. Reference materials (Table S1, Kelly et al., 2020) were diluted into nitrite-free seawater and prepared in 5 nmol and 10 nmol amounts to bracket low-nitrite samples. Vials were purged with N2 gas for 15 minutes to remove background N2O, then treated with a sparged sodium azide/acetic acid solution to chemically convert dissolved nitrite into N2O. The reaction was halted after 30 minutes with the addition of 6 M sodium hydroxide solution (McIlvin and Altabet, 2005).

For **nitrous oxide isotopic analysis**, samples were collected into 160 mL glass serum bottles (Wheaton), following standard gas-sampling procedures: gas-tight tubing (Tygon) was used to overflow each serum bottle with sample three times, after which a ~1 mL air headspace was introduced, and the bottle was capped with a gray butyl septum (National Scientific). Given the trace amount of N2O in the atmosphere (NOAA Global Monitoring Laboratory) and complete flushing of the bottle during analysis, the effect of this headspace and N2O partitioning between the gas and liquid phase falls within the analytical uncertainty for N2O concentration measurements. Samples for N2O isotopic analysis were promptly preserved by adding 100 µL mercuric chloride (HgCl2) to each 160 mL bottle, then sealed with an aluminum crimp seal and stored at lab temperature (20-22ºC).

Data Processing Description

Sample processing:
Preserved samples were analyzed for nitrate, nitrite, and nitrous oxide isotopes via mass spectrometry using a Thermo Finnigan DELTA V in continuous flow mode. For nitrate isotopic analysis, filtered and frozen samples were analyzed for NOx concentration with a discrete analyzer system (Westco), which uses a cadmium column to convert NO3- to NO2- and colorimetric methods to quantify the concentration of NO2-. Following nitrate concentration analysis, filtered and frozen samples were prepared for nitrate isotopic analysis using the denitrifier method (Sigman et al., 2001; Casciotti et al., 2002), with updates from McIlvin and Casciotti (2011). Samples with any detectable nitrite ([NO2-] < 0.076 µM) were treated with sulfamic acid to convert the nitrite present to sulfuric acid + N2 gas (Granger and Sigman, 2009), then prepared similarly with the denitrifier method. Three reference materials with a range of δ15N and δ18O values as well as a process blank were prepared alongside each run of samples (USGS 32, USGS 34, and USGS 35; Table S1 from Kelly et al., 2020). USGS reference materials were also treated with sulfamic acid for sulfamic-treated sample runs.

Samples prepared with the denitrifier method were analyzed via a purge-and-trap system coupled to a Thermo Finnigan DELTA V isotope ratio mass spectrometer in continuous flow (McIlvin and Casciotti, 2011). Likewise, samples preserved shipboard with the azide method (McIlvin and Altabet, 2005) were analyzed for the δ15N and δ18O of nitrite on the same IRMS system, following the injection of 100 uL antifoam emulsion (Sigma-Aldrich) into each vial. Samples with nitrate < 0.1 µM or nitrite < 0.25 µM were excluded from the nitrate or nitrite δ15N and δ18O datasets, respectively, due to the large error associated with these low concentrations.

For nitrous oxide isotope analysis, each 160 mL bottle was analyzed on a custom-built purge-and-trap system coupled to a Finnigan DELTA V isotope ratio mass spectrometer (McIlvin and Casciotti, 2010) against a tank of pure N2O calibrated by S. Toyoda (Tokyo Tech). Molecular masses 44, 45, and 46 were measured for sample and reference gases, as well as fragment ion masses 30 and 31. These values, along with a set of coefficients to account for “scrambling” at the ion source, were used to solve for the δ15N2O, δ15N2Oβ, and δ18O-N2O of each sample. See Kelly et al. (2020) for a full description of the scrambling calculation. N2O concentrations were obtained from the N2O peak area, known instrument sensitivity (conversion of mass 44 peak area to nmols N2O), and sample weights pre- and post-analysis (McIlvin and Casciotti, 2010).

Data processing:
Samples prepared via the denitrifier method were corrected for drift and any offset/blank as described in McIlvin and Casciotti (2011). For samples prepared for nitrite isotopic analysis via the azide method, first, a correction was applied to account for instrument drift over the course of the run, using the run numbers and raw, measured δ15N and δ18O of the high-concentration reference materials. Subsequently, a size correction was calculated from two different sizes of standard curves (5 nmol and 10 nmol) and their respective slopes and intercepts; Finally, a size-corrected standard curve was used to calculate the actual isotope ratios from the drift-corrected values.

The isotope ratios of N2O and that of the fragment ion NO — namely, 45/44, 46/44, and 31/30 — were first corrected via comparison to direct injections of a common N2O reference gas. Following this correction, a linearity relation was applied to these isotope ratios, in order to correct for variations in measured isotope ratios due to peak size. To obtain from these three isotope ratios the individual isotopomers of N2O, it was necessary to correct for “scrambling” at the ion source, or the phenomenon by which the NO fragment ion actually contains the outer, beta N atom from the linear N2O molecule, instead of the inner, alpha N atom (Toyoda and Yoshida, 1999). Finally, a two-point scale decompression (Mohn et al., 2014) was applied to correct for a consistent offset between measured and inter-calibrated standard values. See Kelly et al. (2020) for a full discussion of the N2O isotopomer data corrections.

Problem Report:
Issues were occasionally encountered during analysis of nitrous oxide stable isotopes (clogged lines, etc.). Samples for which issues occurred are flagged in the data accordingly: no flag=good, 3=questionable, 4=bad.

BCO-DMO processing:
- Timestamp data from all cruises were joined to isotopomer dataset
- Timestamp converted to ISO DateTime UTC format in additional column
- Parameter names adjusted to comply with database requirements
- Added a conventional header with dataset name, PI name, version date
- Units added to parameter description metadata section
- Missing data identifier of 'nd' (no data) used

[table of contents | back to top]

Data Files

File	
nitrous_oxide_isotopomers.csv	(Comma Separated Values (.csv), 81.46 KB) MD5:6d5031fdc4fea15ccffb05a4e87a0f8b
Primary data file for dataset ID 832995	

[table of contents | back to top]

Related Publications

Casciotti, K. L., Böhlke, J. K., McIlvin, M. R., Mroczkowski, S. J., & Hannon, J. E. (2007). Oxygen Isotopes in Nitrite: Analysis, Calibration, and Equilibration. Analytical Chemistry, 79(6), 2427–2436. doi:10.1021/ac061598h
Methods

Casciotti, K. L., Sigman, D. M., Hastings, M. G., Böhlke, J. K., & Hilkert, A. (2002). Measurement of the Oxygen Isotopic Composition of Nitrate in Seawater and Freshwater Using the Denitrifier Method. Analytical Chemistry, 74(19), 4905–4912. doi:10.1021/ac020113w
Methods

Granger, J., & Sigman, D. M. (2009). Removal of nitrite with sulfamic acid for nitrate N and O isotope analysis with the denitrifier method. Rapid Communications in Mass Spectrometry, 23(23), 3753–3762. doi:10.1002/rcm.4307
Methods

Grasshoff, K., Kremling, K., & Ehrhardt, M. (Eds.). (1999). Methods of Seawater Analysis. doi:10.1002/9783527613984
Methods

Kelly, C. L., Travis, N. M., Baya, P. A., & Casciotti, K. L. (2020). Quantifying nitrous oxide cycling regimes in the eastern tropical North Pacific Ocean with isotopomer analysis. Global Biogeochemical Cycles. doi:10.1029/2020gb006637 https://doi.org/10.1029/2020GB006637
Results

,
Methods

McIlvin, M. R., & Altabet, M. A. (2005). Chemical Conversion of Nitrate and Nitrite to Nitrous Oxide for Nitrogen and Oxygen Isotopic Analysis in Freshwater and Seawater. Analytical Chemistry, 77(17), 5589–5595. doi:10.1021/ac050528s
Methods

McIlvin, M. R., & Casciotti, K. L. (2010). Fully automated system for stable isotopic analyses of dissolved nitrous oxide at natural abundance levels. Limnology and Oceanography: Methods, 8(2), 54–66. doi:10.4319/lom.2010.8.54
Methods

McIlvin, M. R., & Casciotti, K. L. (2011). Technical Updates to the Bacterial Method for Nitrate Isotopic Analyses. Analytical Chemistry, 83(5), 1850–1856. doi:10.1021/ac1028984
Methods

Mohn, J., Wolf, B., Toyoda, S., Lin, C.-T., Liang, M.-C., Brüggemann, N., … Yoshida, N. (2014). Interlaboratory assessment of nitrous oxide isotopomer analysis by isotope ratio mass spectrometry and laser spectroscopy: current status and perspectives. Rapid Communications in Mass Spectrometry, 28(18), 1995–2007. doi:10.1002/rcm.6982
Methods

Sigman, D. M., Casciotti, K. L., Andreani, M., Barford, C., Galanter, M., & Böhlke, J. K. (2001). A Bacterial Method for the Nitrogen Isotopic Analysis of Nitrate in Seawater and Freshwater. Analytical Chemistry, 73(17), 4145–4153. doi:10.1021/ac010088e
Methods

Toyoda, S., & Yoshida, N. (1999). Determination of Nitrogen Isotopomers of Nitrous Oxide on a Modified Isotope Ratio Mass Spectrometer. Analytical Chemistry, 71(20), 4711–4718. doi:10.1021/ac9904563
Methods

Weiss, R. F., & Price, B. A. (1980). Nitrous oxide solubility in water and seawater. Marine Chemistry, 8(4), 347–359. doi:10.1016/0304-4203(80)90024-9
Methods

[table of contents | back to top]

Parameters

Parameter	Description	Units
ISO_DateTime_UTC	Date and time (UTC) formatted to ISO8601 standard (YYYY-MM-DDThh:mm:ssZ)	unitless
Station	Station ID	unitless
Cast	Sequential CTD cast number	unitless
Target_Depth	Target sampling depth	meters
Niskin	Bottle number on CTD rosette	unitless
Latitude	Latitude at start of CTD deployment; South is negative	decimal degrees
Longitude	Longitude at start of CTD deployment; West is negative	decimal degrees
CTD_Pressure	Pressure	decibars
CTD_Depth	Depth	meters
Sigma_theta	Potential density	kilogram per cubic meter (kg/m^3)
Salinity	Salinity	practical salinity units (psu)

PAR	Photosynthetically active radiation	micro Einsteins per squared meter (µE/m^2)
Seabird_Oxygen	Oxygen from Seabird oxygen sensor	micromoles per liter (µmol/L)
Temperature	Water temperature	degrees Celsius
Nitrite	Concentration of nitrite	micromoles per liter (µmol/L)
Nitrate_Sample_ID	Sample identifier for nitrate isotopes	unitless
Sulfamic_treated	Whether nitrate isotope sample was sulfamic-treated: 1=yes, 0=no	unitless
NO3_rep1	Concentration of nitrate, first replicate analysis of same sample	micromoles per liter (µmol/L)
NO3_rep2	Concentration of nitrate, second replicate analysis of the same sample	micromoles per liter (µmol/L)
NO3_rep3	Concentration of nitrate, third replicate analysis of the same sample	micromoles per liter (µmol/L)
NO3_mean	Concentration of nitrate, average of replicate analyses	micromoles per liter (µmol/L)
NO3_std	Concentration of nitrate, standard deviation of replicate analyses	micromoles per liter (µmol/L)
d18O_NO3_meas1	delta18O of nitrate, first replicate analysis of the same sample	per mil vs. VSMOW
d18O_NO3_meas2	delta18O of nitrate, second replicate analysis of the same sample	per mil vs. VSMOW
d18O_NO3_meas3	delta18O of nitrate, third replicate analysis of the same sample	per mil vs. VSMOW
d18O_NO3_avg	delta18O of nitrate, average of replicate analyses	per mil vs. VSMOW
d18O_NO3_stdev	delta18O of nitrate, standard deviation of replicate analyses	per mil vs. VSMOW
d15N_NO3_meas1	delta15N of nitrate, first replicate analysis of the same sample	per mil vs. atmospheric N2
d15N_NO3_meas2	delta15N of nitrate, second replicate analysis of the same sample	per mil vs. atmospheric N2
d15N_NO3_meas3	delta15N of nitrate, third replicate analysis of the same sample	per mil vs. atmospheric N2
d15N_NO3_avg	delta15N of nitrate, average of replicate analyses	per mil vs. atmospheric N2
d15N_NO3_stdev	delta15N of nitrate, standard deviation of replicate analyses	per mil vs. atmospheric N2
d18O_NO2_rep1	delta18O of nitrite, first distinct replicate sample	per mil vs. VSMOW
d18O_NO2_rep2	delta18O of nitrite, second distinct replicate sample	per mil vs. VSMOW
d18O_NO2_avg	delta18O of nitrite, average of replicates	per mil vs. VSMOW
d15N_NO2_rep1	delta15N of nitrite, first distinct replicate sample	per mil vs. atmospheric N2

d15N_NO2_rep2	delta15N of nitrite, second distinct replicate sample	per mil vs. atmospheric N2
d15N_NO2_avg	delta15N of nitrite, average of replicates	per mil vs. atmospheric N2
N2O_sat	Saturation concentration of nitrous oxide, calculated from Weiss and Price (1980)	nanomoles per liter (nmol/L)
N2O_rep1	Concentration of nitrous oxide, first distinct replicate sample	nanomoles per liter (nmol/L)
N2O_rep2	Concentration of nitrous oxide, second distinct replicate sample	nanomoles per liter (nmol/L)
N2O_mean	Concentration of nitrous oxide, average of replicate samples	nanomoles per liter (nmol/L)
N2O_std	Concentration of nitrous oxide, standard deviation of replicate samples	nanomoles per liter (nmol/L)
N2O_supersaturation_rep1	Super-saturation of nitrous oxide, equal to actual nitrous oxide concentration minus saturation concentration, for first replicate concentration measurement	nanomoles per liter (nmol/L)
N2O_supersaturation_rep2	Super-saturation of nitrous oxide, equal to actual nitrous oxide concentration minus saturation concentration, for second replicate concentration measurement (n=2)	nanomoles per liter (nmol/L)
N2O_supersaturation_mean	Mean super-saturation of nitrous oxide, equal to actual nitrous oxide concentration minus saturation concentration	nanomoles per liter (nmol/L)
N2O_supersaturation_std	Standard deviation of super-saturation of nitrous oxide, equal to actual nitrous oxide concentration minus saturation concentration	nanomoles per liter (nmol/L)
d18O_N2O_rep1	delta18O of nitrous oxide, first distinct replicate sample	per mil vs. VSMOW
d18O_N2O_rep2	delta18O of nitrous oxide, second distinct replicate sample	per mil vs. VSMOW
d18O_N2O_mean	delta18O of nitrous oxide, average of replicate samples	per mil vs. VSMOW
d18O_N2O_std	delta18O of nitrous oxide, standard deviation of replicate samples	per mil vs. VSMOW
d15N_alpha_N2O_rep1	delta15N-alpha of nitrous oxide, first distinct replicate sample	per mil vs. atmospheric N2
d15N_alpha_N2O_rep2	delta15N-alpha of nitrous oxide, second distinct replicate sample	per mil vs. atmospheric N2
d15N_alpha_N2O_mean	delta15N-alpha of nitrous oxide, average of replicates	per mil vs. atmospheric N2
d15N_alpha_N2O_std	delta15N-alpha of nitrous oxide, standard deviation of replicates	per mil vs. atmospheric N2
d15N_beta_N2O_rep1	delta15N-beta of nitrous oxide, first distinct replicate sample	per mil vs. atmospheric N2
d15N_beta_N2O_rep2	delta15N-beta of nitrous oxide, second distinct replicate sample	per mil vs. atmospheric N2
d15N_beta_N2O_mean	delta15N-beta of nitrous oxide, average of replicate samples	per mil vs. atmospheric N2
d15N_beta_N2O_std	delta15N-beta of nitrous oxide, standard deviation of replicate samples	per mil vs. atmospheric N2

d15N_N2Obulk_rep1	delta15N-bulk of nitrous oxide, first distinct replicate sample	per mil vs. atmospheric N2
d15N_N2Obulk_rep2	delta15N-bulk of nitrous oxide, second distinct replicate sample	per mil vs. atmospheric N2
d15N_N2Obulk_mean	delta15N-bulk of nitrous oxide, average of replicate samples	per mil vs. atmospheric N2
d15N_N2Obulk_std	delta15N-bulk of nitrous oxide, standard deviation of replicate samples	per mil vs. atmospheric N2
SP_1	Site preference of nitrous oxide, first replicate sample	per mil vs. atmospheric N2
SP_2	Site preference of nitrous oxide, second replicate sample	per mil vs. atmospheric N2
SP_mean	Site preference of nitrous oxide, average of replicates	per mil vs. atmospheric N2
SP_std	Site preference of nitrous oxide, standard deviation of replicates	per mil vs. atmospheric N2
N2O_Flag_1	Flag for first replicate N2O measurement: no flag=good, 3=questionable, 4=bad	unitless
N2O_Flag_2	Flag for second replicate N2O measurement: no flag=good, 3=questionable, 4=bad	unitless

[table of contents | back to top]

Instruments

Dataset-specific Instrument Name	Westco discrete analyzer system
Generic Instrument Name	Discrete Analyzer
Dataset-specific Description	Westco discrete analyzer system uses a cadmium column to convert NO3- to NO2- and colorimetric methods to quantify the concentration of NO2
Generic Instrument Description	Discrete analyzers utilize discrete reaction wells to mix and develop the colorimetric reaction, allowing for a wide variety of assays to be performed from one sample. These instruments are ideal for drinking water, wastewater, soil testing, environmental and university or research applications where multiple assays and high throughput are required.

Dataset-specific Instrument Name	Thermo Finnigan DELTA V isotope ratio mass spectrometer
Generic Instrument Name	Isotope-ratio Mass Spectrometer
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).

[table of contents | back to top]

Deployments

SR1805

Website	https://www.bco-dmo.org/deployment/833015
Platform	R/V Sally Ride
Start Date	2018-03-13
End Date	2018-04-16
Description	See additional cruise information from the Rolling Deck to Repository (R2R): https://www.rvdata.us/search/cruise/SR1805 Cruise DOI: 10.7284/908014

[table of contents | back to top]

Project Information

Collaborative Research: Mechanisms and Controls of Nitrous Oxide Production in the Eastern Tropical North Pacific Ocean (N2O in ETNP)

Coverage: Eastern Tropical North Pacific Ocean (oxygen minimum zone)

NSF Award Abstract:
Nitrous oxide (N2O) is present at very low concentrations in the atmosphere but is an important greenhouse gas and ozone destroying substance. As with other climate-active gases like methane and carbon dioxide, human activities are responsible for most of its production, either directly through fossil fuel burning or agricultural activities. However, about a third of natural N2O emissions come from the ocean, but even these emissions can be indirectly affected by human activities. About half of the ocean source is derived from three specific geographic regions in the Pacific Ocean and Arabian Sea. These three oceanic regions are places where oxygen concentrations are so low in the intermediate depths that metabolic processes requiring the absence of oxygen are able to occur. These regions are called Oxygen Minimum Zones (OMZs) and they have microbiological processes that occur nowhere else in global ocean waters. In the work proposed here, we will investigate how the microbiological pathways of N2O production and consumption are regulated by environmental conditions such as oxygen and nutrient concentration. This work will involve a research expedition to one of the OMZs, the Eastern Tropical Pacific Ocean off the coast of Mexico. On the cruise, we will perform experiments and collect samples for analysis in our home laboratories at Princeton and Stanford Universities. Advising of graduate students and teaching at the graduate and undergraduate levels at both institutions will be linked to this research. This work is particularly timely because global warming has already indirectly affected the size and geographic extent of the OMZs. Greater expanse of low oxygen water could cause N2O production to increase, leading to increased fluxes of N2O to the atmosphere. In the atmosphere, the role of N2O in ozone destruction and as a greenhouse gas could be critical elements of global change.

Nitrous oxide (N2O) is an important greenhouse gas and ozone destroying substance. About a third of natural N2O emissions come from the ocean, and about half of the ocean source is derived from waters with oxygen deficient intermediate waters (oxygen minimum zones, OMZs). Nitrification is recognized as the main source of N2O in the ocean, but denitrification also likely contributes to the net source in and around OMZs. Because nitrification and denitrification are performed by microbes with very different metabolisms and environmental controls, their contributions to N2O production are expected to differ in response to changes in oxygenation and nutrient inputs. Thus it is important to understand the regulation of N2O production by both processes. The main goal of this project is to quantify the environmental regulation of N2O production and consumption pathways in and around OMZs in order to obtain predictive understanding of N2O distributions and fluxes in the ocean. To do this, production and consumption of N2O will be measured using stable isotope tracer incubations at stations located within and outside one of the major OMZs in the Eastern Tropical North Pacific ocean. The dependence of the rate processes on substrate, product, and oxygen concentrations will be determined, and the composition of the microbial assemblages will be assessed to determine whether different microbial components are involved under different environmental conditions. Natural abundance stable isotope and isotopomer measurements of N2O will be interpreted in concert with measured rates to deduce the sources and pathways (nitrification, nitrifier-denitrification, denitrification, and ?hybrid? formation) involved in

N2O production and consumption. This work will also involve a novel application of isotopomer measurements of N2O from incubations to identify the placement of 15N from NH4+ and NO2- within labeled N2O pools.

OMZ regions are the sites of unique nitrogen cycling processes that are critical in determining the fixed nitrogen inventory of the ocean. If OMZs expand as predicted due to anthropogenic changes in the coming decades, changes in these chemical distributions may affect the atmospheric flux of nitrous oxide as well as modify overall ocean productivity via changes in the fixed nitrogen inventory. Understanding the regulation and environmental control of the processes responsible for N2O production and consumption is the foundation of understanding their response to global change.

[table of contents | back to top]

Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1657868

[table of contents | back to top]