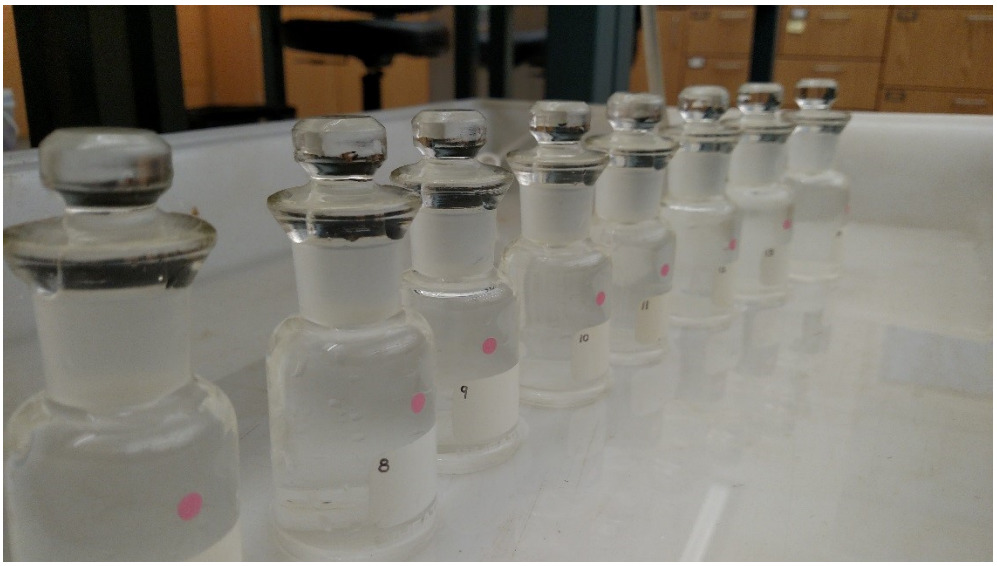
DEPARTMENT OF ECOLOGY
State of Washington

Quality Assurance Project Plan

Spatial and Seasonal Variability in Salish Sea Bottom-Water Microbial Respiration



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Cover: Samples at Padilla Bay National Estuarine Research Reserve Laboratory (photo by J. Apple).

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Spatial and Seasonal Variability in Salish Sea Bottom-Water Microbial Respiration

August 2019

Approved by:

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EAP: Environmental Assessment Program.
SEA: Shorelines and Environmental Assistance Program.

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2.0 Abstract

Water column microbial respiration is a key driver of carbon cycling, pH, and oxygen dynamics in marine ecosystems and a primary step for remineralization of organic matter in natural waters. However, *in situ* estimates of microbial respiration and its relative contribution to hypoxia and ocean acidification are seldom measured. For this reason, we have limited knowledge of regionally relevant rates for microbial respiration to include in water quality models. In 2014, we began reconnaissance monitoring of respiration rates at multiple sites in the central Salish Sea (San Juan Islands, Bellingham Bay) and routine monitoring of short-term respiration at multiple sites in Padilla Bay National Estuarine Research Reserve (NERR). Our investigation reveals that rates of respiration vary seasonally and appear to be associated with changes in organic matter supply. To a lesser extent, rates of respiration are also associated with temperature and incoming deeper waters of marine origin characterized by relatively low but consistent rates of respiration (i.e., ~5 µg O_2 L^{-1} h^{-1}).

The work proposed in this study will be incorporated into a larger-scale study to characterize the spatiotemporal variability of total dissolved inorganic carbon and total alkalinity in Puget Sound and the larger Salish Sea. Results from this work will help identify the seasonal variability of organic matter remineralization in Salish Sea near-bottom waters at stations ranging in depth from 15 to 200 m, improve the geographic scope of existing microbial respiration data sets, and provide empirical estimates for inclusion in the Salish Sea water quality model with the objective of optimizing model performance. Ultimately, these data and their use in water quality models will provide insight into the response of inland marine waters of the Pacific Northwest to a warmer, more acidified, and potentially more hypoxic ocean.

3.0 Background

3.1 Introduction and problem statement

Long-term observations of temperature, salinity, and dissolved oxygen reveal the spatial variability of water quality in Puget Sound. These patterns and trends have been captured by various long-term monitoring programs in our region, including Ecology (1975–2019), University of Washington (UW 1998–2016) and King County (1999–2019). Higher-resolution spatial variability of these parameters as they relate to water column structure have also been investigated using profiling buoy networks (Newton and Devol 2012). Factors influencing spatial variability include the morphology of the basins, bathymetric features, climatological and oceanographic patterns, marine and terrestrial influences, and site-specific seasonal patterns related to the cycling of elements, such as carbon, oxygen, nitrogen, phosphorus, and sulfur, between biotic and abiotic compartments.

Dissolved oxygen (DO) is an indicator of biological production, respiration, and degradation of organic compounds. It also is a useful parameter for understanding ecosystem health (Baumann and Smith 2017; Murrell et al. 2018; Caffrey 2004; Diaz and Rosenberg 2008). Low DO concentrations have been observed throughout Puget Sound, particularly in late summer and fall in embayments with limited water circulation (Albertson et al. 2002; Newton et al. 2011). Numerous areas within the Puget Sound do not meet the water quality standard for dissolved

oxygen (Ahmed et al. 2019). Human activity has been shown to have an impact in the DO levels experienced in Puget Sound (Roberts et al. 2008; Pelletier et al. 2017) and climate change is expected to exacerbate these conditions (Altieri and Gedan 2015).

The Salish Sea Model (SSM) was developed to evaluate the influence of human activity on ambient DO. Over nearly ten years, the model has been improved to more accurately simulate the physical and biogeochemical conditions of the Salish Sea. The SSM is being usedto quantitatively explore and evaluate the effect of meaningful options for nutrient reduction in Puget Sound. This modeling work contributes to the Puget Sound Nutrient Reduction Project (PSNRP 2019) – a project that will guide regional investments in point and nonpoint source nutrient controls so that Puget Sound will meet DO water quality criteria and aquatic life–designated uses by 2040. The goals of these reductions are 1) to meet water quality targets in Puget Sound, and 2) to provide increased resiliency to expected stresses associated with climate change and nutrient loading from future population growth. More information about PSNRP can be found at the project's website: Reducing Puget Sound Nutrients.[1]

The majority of respiration in marine environments is mediated by microbes via breakdown and metabolism of organic material. Heterotrophic microbial respiration is modeled as the dissolution of organic carbon via first-order kinetics, and it is one of the key processes that the SSM simulates. However, heterotrophic microbial respiration rate measurements at a Sound-wide scale have not been conducted to date. Domain-specific data on the spatial variability of heterotrophic microbial respiration rates will improve model performance. The SSM has the capability of using spatially distinct minimum heterotrophic respiration rate constants. The model is currently run with global minimum first-order rate constants (1/time units) for heterotrophic microbial respiration similar to those used for the Chesapeake Bay (Ahmed et al. 2019) – which is a very different estuarine ecosystem than the Salish Sea with respect to bathymetry, organic matter loading, residence times, and estimates of water column respiration (Apple et al. 2006; Smith and Kemp 1995). The assumed global minimum rates are adjusted automatically during the model simulation in every grid cell according to changing temperatures, phytoplankton concentrations, and DO levels, to represent the heterotrophic microbial respiration rates (mass/volume/time units) specific to the conditions in the Salish Sea.

This QAPP contains the scientific underpinnings and the details of a field study to fill the data gap, described above. The primary objective is to conduct a survey of microbial respiration rates for near-bottom waters at Ecology's long-term water quality monitoring sites. Observations will be compared to SSM model output and potentially used for calibration purposes. Other objectives include improving estimates of the seasonal and spatial variability in rates of pelagic microbial respiration in Salish Sea waters, identifying environmental parameters that drive changes in pelagic microbial respiration, and furthering our understanding of the contribution of pelagic microbial respiration to oxygen and carbon cycling.

The area of interest for this work is the Salish Sea, which comprises waters of Puget Sound, the Strait of Georgia, and the Strait of Juan de Fuca (Figure 1). Pacific Ocean water enters the Salish Sea primarily through the Strait of Juan de Fuca, with a lesser exchange around the north end of Vancouver Island in Canada, through Johnstone Strait. Although the SSM domain includes

[1] https://ecology.wa.gov/Water-Shorelines/Puget-Sound/Helping-Puget-Sound/Reducing-Puget-Sound-nutrients.

portions of the United States and Canada, including the Pacific Coast region, this study will focus on Puget Sound and waters of the eastern reaches of the Strait of Juan de Fuca (i.e., the Northwest Straits).

Puget Sound is the region south of Admiralty Inlet and receives varying freshwater inflows dependent on seasonal conditions. There are distinct regions and sub-basins within Puget Sound. The largest direct source of freshwater to Puget Sound is the Skagit River, which flows into Whidbey Basin, which also receives water from the Stillaguamish and Snohomish Rivers. The Fraser River, flowing from Canada and into the Salish Sea north of Admiralty Inlet, also influences Puget Sound (Banas et al. 2015; Khangaonkar et al. 2017, 2018). The major watersheds that drain into Central Puget Sound include the Cedar, Green, and Puyallup Rivers along with portions of the Puget Lowland to the east and west. Hood Canal receives water flowing from the eastern Olympic Mountains and the western Kitsap Peninsula. The Nisqually and Deschutes Rivers are the largest rivers that drain into South Puget Sound, in addition to freshwater from the Puget Lowlands. Puget Sound waters exhibit highly complex circulation patterns. Longer flushing times (the turnover time of freshwater in an estuary) occur in the inlets and contribute to low DO levels in these areas (Ahmed et al. 2017).

Figure 1. Map of Puget Sound within the Salish Sea.

3.2.1 History of study area

Sackmann (2009) details the history of the study area. In summary, low DO has been measured in several locations within Puget Sound, and these low levels of DO are influenced by nutrients, particularly nitrogen and carbon. Long-term measurement of water quality parameters in the study area are conducted as detailed in Bos et al. (2015). Various studies have investigated whether human activities contribute to the decline in oxygen levels or eutrophication and related parameters in Puget Sound (Mackas and Harrison 1997; Albertson et al. 2002; Newton et al. 2008; Ahmed et al. 2014; Roberts et al. 2014). Recent studies reveal that over-enrichment of nutrients from human sources contributes to dissolved oxygen and ocean acidification problems (Roberts et al. 2014; Pelletier et al. 2017). These excess nutrients may contribute to degradation of habitat quality, loss of biotic diversity, and increased harmful algal blooms (Howarth 2008).

3.2.2 Summary of previous studies

Water column microbial respiration represents one of the largest sinks for organic matter on the planet and is an important aspect of understanding oxygen and carbon dynamics in natural waters. Rates of oxygen consumption remain one of the most effective ways to estimate bulk microbial respiration rates, which in turn allow estimation of the rate of carbon remineralization in natural waters. Despite the importance of these measures, estimates of respiration remain relatively uncommon relative to other metrics of microbial growth, abundance, or production (Jahnke and Craven 1995; del Giorgio and Cole 1998; del Giorgio and Williams 2005).

Such oxygen-based measures of microbial respiration in natural waters have been used in numerous studies and reflect the bulk activity of heterotrophic metabolism of a wide suite of organisms. Although assays of biological oxygen demand (BOD; Standard Methods Committee 2001) have been used as proxies for microbial respiration, they are not equivalent. The consumption of oxygen and organic matter by planktonic communities is often a nonlinear response and can also be governed by zero order (i.e., substrate independent) or first order (i.e., substrate dependent) kinetics. For this reason, the two point (i.e., initial-final) approach used in the standard BOD protocol is inadequate to fully understand the nature of organic matter consumption and remineralization in natural waters and to quantify these rates in meaningful ways.

Early works by Carignan et al. (1998, 2000) were the first to describe the use of Winkler titrations to quantify the refined measure of microbial respiration in natural waters. Since then, others have developed and published methods for estimating microbial respiration in ways that reveal the nonlinearity of remineralization rates and more accurately reflect the kinetics of organic matter utilization in natural waters (e.g., Smith and Kemp 2003; Apple et al. 2006; Apple and del Giorgio 2007; Murrell et al. 2013). Although there is a growing body of knowledge and data representing the range and variability of microbial respiration in coastal waters, there is a dearth of these measures for waters of the Salish Sea. Indeed, in their review of over 30 published studies from across the globe reporting estimates of microbial metabolism in coastal waters, Apple et al. (2008) found none representing the west coast of the United States, let alone Puget Sound.

In 2010, as part of a collaborative project with Northwest Indian College (NWIC), Apple et al. (2011) began investigating hypoxia in Bellingham Bay. Over the next several years, numerous students participated in work focusing on oxygen dynamics and measuring water column respiration (e.g., DeLand et al. 2012). Through funding from Washington Ocean Acidification Center (WOAC) and Centers for Ocean Science Education Excellence (COSEE), this ongoing work was extended to also include waters of the San Juan Islands (e.g., Duarte and Apple 2013, Christman et al. 2015). Most recently, microbial respiration measurements were incorporated into the System Wide Monitoring Program routine water quality sampling at Padilla Bay National Estuarine Research Reserve (NERR). Syntheses of these data are reported elsewhere (e.g., PSEMP 2018; Apple et al. 2017). Collectively, this work represents the only effort being made towards Puget Sound–wide respiration measurements.

Organic carbon compounds play a central role in biogeochemical processes. Metabolism of organic carbon fuels heterotrophic microbial respiration. However, until recently, water column

organic carbon data with wide spatial coverage of the Puget Sound was not available. In 2016, Ecology commenced the collection of particulate (POC) and total organic carbon (TOC) data at marine long-term monitoring sites. Figure 2 shows the location of the Ecology long-term monitoring sites where organic measurements were taken at two depths (10 meters and near bottom) once per month starting in 2016. Figure 3 shows the distribution of TOC data collected near the bottom at each site, and Figure 4 shows the distribution of TOC data collected at 10 meters depth at each site (Figueroa-Kaminsky et al. 2018). We expect that collection of these data will continue during the time of this respiration rate study.

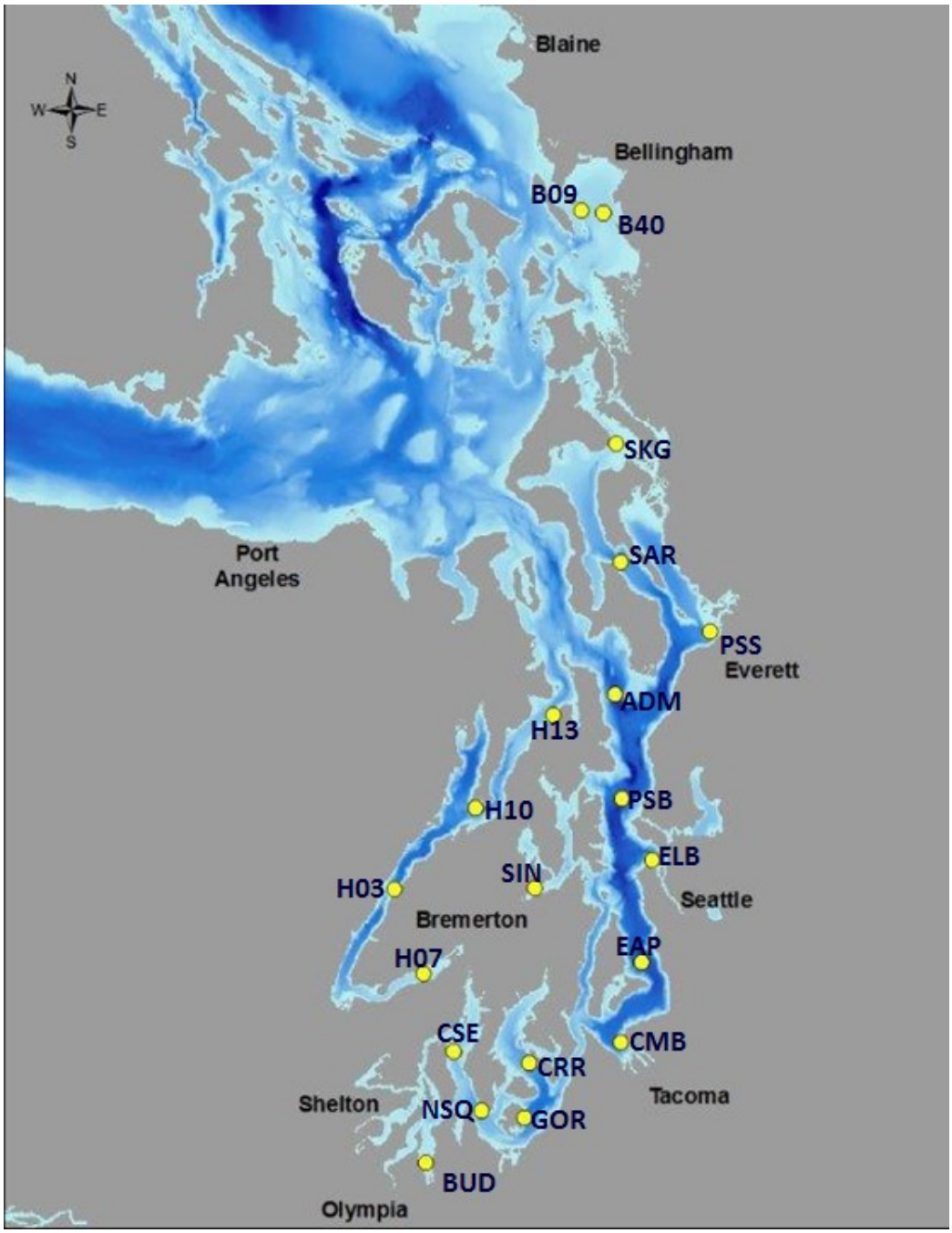

Figure 2. Long-term marine water quality monitoring stations with organic carbon data available since 2016.

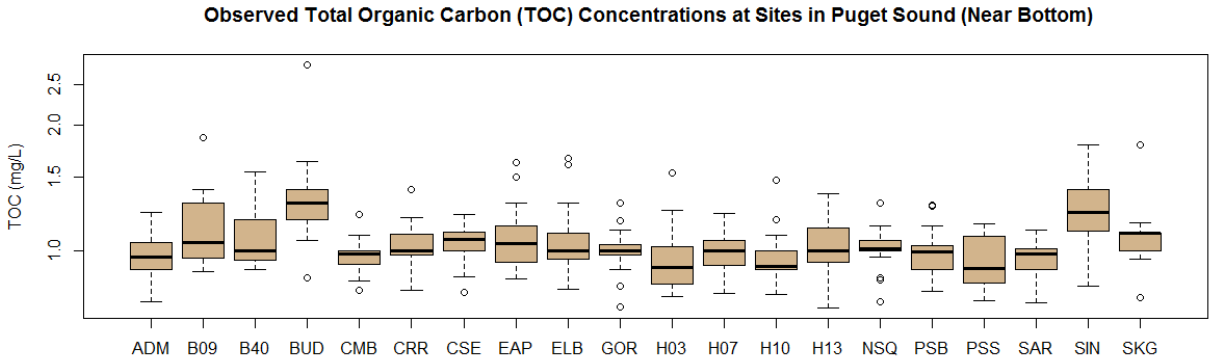

Figure 3. Distributions of near-bottom monthly total organic carbon concentrations for 2016–2017 at long-term monitoring stations.

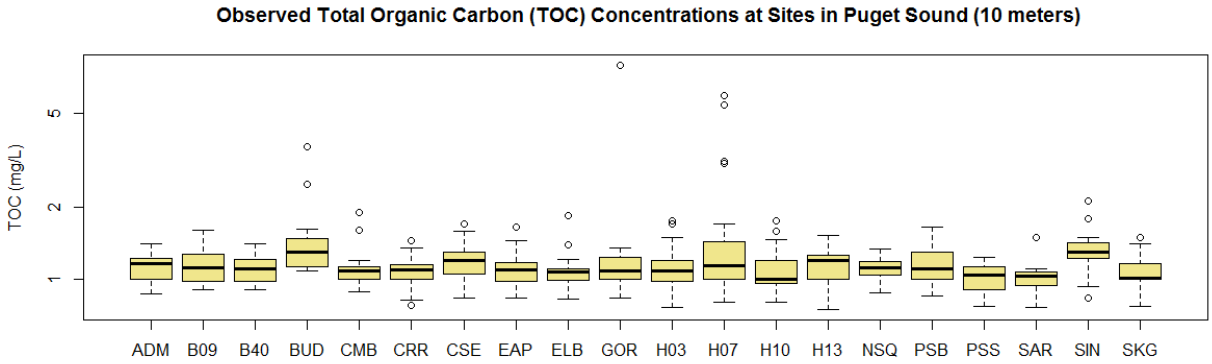

Figure 4. Distributions of monthly total organic carbon concentrations at 10 meter depths for 2016–2017 at long-term monitoring stations (Figueroa-Kaminsky et al. 2018, based on observations conducted by Ecology's Marine Monitoring Unit).

3.2.3 Utility of observed data to support modeling

These observational data may prove to be valuable for model calibration, if the values observed deviate greatly from the current values used. To isolate the heterotrophic microbial respiration component, the field study will focus on the bottom-water layer, which at deep sites is not in the euphotic zone. In some shallower stations, near-bottom waters may also be in the euphotic zone and include measurable and metabolically active algal biomass. In these situations, efforts will be made to quantify the relative contribution of phytoplankton to total microbial respiration. Incubations will be conducted at a constant reference temperature of 10°C to facilitate cross-seasonal comparisons.

Heterotrophic bacteria are a crucial component of the ecosystem due to the role they play in breaking down organic carbon via microbial respiration and nutrient cycling. In the Salish Sea Model (SSM), heterotrophic microbial respiration rate is equivalent to the rate of oxygen utilization for the conversion of dissolved organic carbon (DOC) to inorganic carbon. The

equation used in the SSM (Cerco and Cole 1995) for the change in the concentration of organic carbon substrate is shown below:

$$\frac{\delta}{\delta t} DOC = FCD \bullet R \bullet B + FCDP \bullet PR + Klpoc \bullet LPOC$$
$$+ Krpoc \bullet RPOC - \frac{DO}{KHodoc + DO} \bullet Kdoc \bullet DOC - DENIT \bullet DOC$$

In which:
DOC = dissolved organic carbon (g m^{-3})
LPOC = labile particulate organic carbon (g m^{-3})
RPOC = refractory particulate organic carbon (g m^{-3})
FCD = fraction of algal respiration released as DOC (0<FCD<1)
FCDP = fraction of predation on algae released as DOC (0<FCDP<1)
Klpoc = dissolution rate of LPOC (d^{-1})
Krpoc = dissolution rate of RPOC (d^{-1})
Kdoc = dissolution rate of DOC (d^{-1})
R = Algal respiration (d^{-1})
B = Algal biomass (g C m^{-3})
PR = rate of predation on algal groups (d^{-1})
DENIT =denitrification rate of dissolved organic carbon (d^{-1})
DO = dissolved oxygen (mg/L)
KHodoc = half-saturation DO concentration for oxic respiration (mg/L)

The heterotrophic microbial respiration rate is divided into both labile and refractory terms (KDOC and KRDC) for each model cell in the water column. It is represented as a first order rate constant that is applied to the availability of organic carbon substrate and modulated by temperature and DO concentrations at each model cell. The equation for KDOC used in SSM (from Cerco and Cole 1995) contains two terms, as shown below:

$$KDOC = KLDC + KDCALG \times ALGCAR$$

Where:
KDOC = the labile respiration rate in units of 1/days
KDC = the minimum respiration rate in units of 1/days (can be either KLDC or KRDC) where L indicates labile, R refractory; D indicates dissolved.
KDCALG = constant that relates DOC respiration to algal biomass (m^3 g^{-1} C d^{-1})
ALGCAR= Algal biomass for each algal group simulated (g C m^{-3})

An exponential function is used to adjust KDOC and KRDC to changing temperatures in each grid cell layer over time. In addition, a Monod-type ratio is applied using a constant for the half-saturation concentration of DO required for oxic respiration KHodoc and the DO concentration in each cell.

The first term in the equation above (KLDC) and the analogous term (KRDC) are global minimum constants currently used uniformly throughout the domain. The second term is based

on the established correlation between heterotrophic activity and algal biomass, as algae produce labile carbon that can fuel heterotrophic activity (Bird and Kalff 1984; Cole et al. 1988).

The observed microbial respiration rates will be compared with SSM respiration rates . We will make the necessary adjustments between predicted in-situ temperature and the reference temperature used during the laboratory measurements.

4.0 Project Description

4.1 Project goals

The main goals of this effort are (1) to obtain estimates of *in situ* microbial respiration rates, and (2) to compare these to values in current use by the SSM. The observed microbial respiration rates will be compared with SSM respiration rates, with necessary adjustments for *in situ* DO and differences between *in situ* temperature and the reference temperature used during laboratory measurements. This project will also provide additional insight into the seasonal and spatial variability in rates of pelagic microbial respiration in Salish Sea waters, assist in identifying environmental parameters that drive changes in pelagic microbial respiration, and further our understanding of the contribution of pelagic microbial respiration to oxygen and carbon cycling.

4.2 Project objectives

This QAPP will be used as guidance to meet the following project objectives:

- Conduct a study of heterotrophic respiration to obtain measurements at Ecology's long-term water quality monitoring sites.
- Ensure that this study complies with quality assurance methods and procedures and meets data quality objectives.
- Collect and document data for use in subsequent projects to integrate microbial respiration rate data into the SSM modeling framework.
- Improve estimates of the seasonal and spatial variability in rates of pelagic respiration in Salish Sea waters.
- Explore environmental parameters that drive changes in pelagic respiration.
- Further our understanding of the contribution of pelagic microbial respiration to oxygen and carbon cycling, hypoxia, and pH in Salish Sea waters.

Observations will be compared to SSM model output and potentially used for calibration purposes.

4.3 Field Methods

Refer to the field methods described in *Quality Assurance Project Plan: Ocean Acidification Monitoring at Ecology's Greater Puget Sound Stations* (Gonski et al. 2019). The work proposed in this QAPP will be conducted in concert with the field methods described therein. Cubitainers of near-bottom water column samples, obtained consistently using the methods described in Gonski et al. (2019) will be transported to the laboratory for incubation.

4.4 Tasks required

1. **Sample collection**: Water samples for respiration will be collected in coordination with Ecology's ongoing Marine Monitoring Unit (MMU) sampling at a subset of the sites identified in Gonski et al. (2019). Priority sites include those sampled as part of the South Sound cruises and a subset of those sampled as part of the North Sound cruises (e.g., Bellingham Bay, Skagit Bay). Strait of Juan de Fuca transect sites are also of interest, but not as high of a priority for this work. Thus, collection at Strait of Juan de Fuca transect sites will occur only if the opportunity presents itself from a logistical standpoint.
2. **Sample management**: Handling and transportation of water samples from shipboard collection to Padilla Bay NERR Laboratory will be the responsibility of Dr. Jude Apple and Environmental Technician Shauna Bjornson. Procedures for sample handling are outlined in Section 8: Field Procedures.
3. **Laboratory analysis**: Incubations to estimate whole-water microbial respiration will be conducted at the Padilla Bay NERR Laboratory. Details for these protocols are included in Section 9: Laboratory Procedures.
4. **Interim data reporting**: Monthly updates of data analyses from the most recent assays for microbial respiration will be provided to the Ecology modeling group (i.e., co-authors C. Figueroa-Kaminsky and G. Pelletier).
5. **Annual/final report**: A written report and data synthesis will be provided annually, or within 30 days of the end of a given funding period, whichever comes first.

4.5 Systematic planning process used

Field work and related logistics will require the coordination of multiple parties, including staff from Padilla Bay NERR, Shannon Point Marine Center, and Ecology's MMU. Planning and coordination of this work is led by the MMU and communicated through regular meetings.

5.0 Organization and Schedule

5.1 Key individuals and their responsibilities

Table 1 lists the individuals involved in this project. All are employees of Ecology unless otherwise noted.

Table 1. Organization of project staff and responsibilities.

Staff	Title	Responsibilities
Dustin Bilhimer Ecology Water Quality Program Phone: 360-407-7143	Client	Clarifies scope of the project. Provides internal review of the QAPP and approves the final QAPP. Also reviews final report and technical memos.
Dr. Jude Apple Padilla Bay National Estuarine Research Reserve (NERR), Ecology Shorelands and Environmental Assistance (SEA) Program Phone: 360-428-1089	Project Manager and Principal Investigator	Writes and reviews QAPP. Directs and manages project budget, sampling protocols, data collection and analysis, tracks progress. Writes and oversees preparation of final report.
Greg Pelletier and Cristiana Figueroa-Kaminsky Ecology Environmental Assessment Program (EAP) Marine Monitoring Unit (MMU) Modeling & TMDL Unit (MTU) Phone: 360-407-7392	Collaborating Scientists	Write and review QAPP. Review data as it becomes available. Review final report. Collaborate to meet science objectives and provide support, as needed. Compare and subsequently use respiration rate observations in SSM work—this will occur under the Salish Sea Model Applications QAPP.
Shauna Bjornson Padilla Bay NERR, SEA Program Phone: 360-428-1059	Environmental Technician	Provides assistance writing QAPP. Conducts sampling per QAPP and protocols, transports samples, conducts laboratory analyses, documents procedures, and collaborates on final report.
Mya Keyzers Ecology EAP Western Operations Section Marine Monitoring Unit (MMU) 360-407-6395	Marine Field Technician	Lead technician for MMU cruises. Logistical support and coordination for collaborative work on microbial respiration project. Communicates with Apple and Bjornson on a regular basis, as needed.
Stephen Gonski Ecology EAP Marine Monitoring Unit (MMU) 360-407-6517	Marine Scientist	Writes and reviews QAPP for Ocean Acidification Monitoring at Ecology's Greater Puget Sound Stations. Participates in project planning logistics.
Nate Schwarck Shannon Point Marine Center 360-650-7400 x229	Boat Captain	Boat captain for Shannon Point Marine Center (SPMC) cruises. Communicates with Apple and Bjornson on a regular basis, as needed.
Gene McKeen Shannon Point Marine Center 360-650-7400 x223	Facilities Manager	Organizes logistics for SPMC research cruises. Communicates with Apple and Bjornson on a regular basis, as needed.
Dale Norton Ecology EAP Western Operations Section Phone: 360-407-6596	EAP Section Manager for the Study Area	Reviews the draft QAPP and approves the final QAPP.

Staff	Title	Responsibilities
Tom Gries Ecology, EAP Statewide Coordination Section Phone: 360-407-6327	Ecology NEP Quality Coordinator	Reviews the draft QAPP and recommends approval of the final QAPP. May comment on draft project report
Arati Kaza Ecology, EAP	Quality Assurance Officer	Approves final QAPP

5.2 Special training and certifications

Key project personnel have extensive previous experience conducting respiration measurements and conducting oceanographic fieldwork. No special certifications are required.

5.3 Organization chart

Table 1 lists the key individuals, their current position, and their responsibilities for this project.

5.4 Proposed project schedule

Table 2 presents the proposed project schedule. This schedule depends on the Marine Monitoring Unit (MMU) and Shannon Point Marine Center (SPMC) research cruises, which are weather dependent. The schedule and data products may change as the project progresses. Due to funding constraints, sampling associated with this project will be completed by June 30, 2019. A final data report will be submitted by June 30, 2019, incorporating data that will be collected up to the final report date.

Table 2. Proposed schedule for sampling, analysis, and reporting.

Activities and Products	Expected Completion	Lead
Sample collection and analysis (in coordination with MMU and/or SPMC sampling cruises in South Sound, Bellingham Bay, Skagit Bay, Strait of Juan de Fuca)	Monthly (Sept. 2018 – June 2019)	Shauna Bjornson and Jude Apple
Monthly data report: submitted within 30 days of sample collection and processing. Includes all analyses of respiration data.	Monthly (Sept. 2018 – June 2019)	Shauna Bjornson
Mid-project data report	March 1, 2019	Jude Apple
Annual report	July 30, 2019	Jude Apple

5.5 Budget and funding

Funds from a National Estuarine Program grant (EPA Grant Agreement Nos. PC-00J20101 and PC00J89901) will be allocated to personnel support of 1.5 month for Dr. Jude Apple (PI) and 9 months for Shauna Bjornson (Environmental Technician). Support for travel to field sampling locations, registration for a regional scientific conference, and supplies and equipment for carrying out microbial respiration incubations are also detailed below.

Table 3. Project budget and funding.

	Rate	Year 1	Total
A. Senior Personnel and Salaries			
J. Apple, NRS-3 (mentoring/supervision - 0.25 mo.)	$6,202/ mo.	$1,551	$1,551
Environmental Technician (9 mo. @ Range 32K)	$3,044/mo.	$27,396	$27,396
Subtotal, salaries	–	$28,947	$28,947
B. Fringe Benefits			
Principal Investigator	36.0%	$558	$558
Environmental Technician	36.0%	$9,863	$9,863
Subtotal, fringe	–	$10,421	$10,421
Total personnel (salaries & fringe)	**–**	**$39,367**	**$39,367**
C. Travel			
Domestic (monthly sampling, state vehicle)	–	$1,000	$1,000
Attend meeting/present findings	–	$500	$500
Subtotal, travel	–	$1,500	$1,500
D. Supplies and Equipment			
Supplies (sensor arm, optode spots, BOD bottles)	–	$600	$600
Additional chiller/water incubation systems (2)	–	$600	$600
Subtotal, supplies and equipment	–	$1,200	$1,200
TOTAL DIRECT COSTS		$42,067	$42,067
Indirect (29.7% of salaries & fringe)		$11,692	$11,692
TOTAL COSTS		**$53,759**	**$53,759**

6.0 Quality Objectives

6.1 Data quality objectives

The main data quality objective (DQO) for this project is to collect samples to generate an accurate assessment of the range, variability, and seasonal change of *in situ* microbial respiration of near-bottom waters in the Salish Sea. We are also interested in capturing the spatial and geographic variability of rates within the Salish Sea by sample collection in terminal inlets (e.g., Budd Inlet, Case Inlet), central Salish Sea embayments (Bellingham Bay, Skagit Bay), and open waters (e.g., Strait of Juan de Fuca).

6.2 Measurement quality objectives

There are multiple levels of accuracy being addressed in our sampling and analysis protocols.

To ensure that microbial respiration rate measurements derived from any given water sample are reliable and representative, multiple levels of replication are performed.

Replicate estimates of respiration: During each CTD instrument cast, approximately 2 L of near-bottom water are collected with a single Niskin bottle and used to estimate bottom-water respiration. From that water sample, four 60 mL BOD bottles are filled as subsample replicates. Each BOD bottle is incubated for at least 21 days, with internal oxygen concentration measured periodically during the course of the incubation (i.e., two to six times per day, with more frequent measures earlier in the incubation). Oxygen concentrations for each bottle at each time point are determined by taking approximately ten direct measures of oxygen using the PreSens fiber optic cable, which interacts non-invasively with an oxygen-sensitive optode spot located inside of the borosilicate BOD bottle. The ten replicate measurements of oxygen are then used to calculate the mean oxygen concentration in each bottle at that time point, which are in turn used to calculate a slope of oxygen decline using standard least squares regression, or other decay equations as needed. The final reported rate of microbial respiration for any given station is determined by averaging slopes of oxygen decline measured in the four replicates.

Field replicates: Duplicate water samples (i.e., Niskin bottles) are collected at a minimum of one station each cruise cycle (e.g., Station BLL040 for the current study) and are used to estimate variability among field samples. Briefly, two side-by-side Niskin bottles are used to collect water samples during a single CTD instrument cast for bottom-water respiration. As described above, four replicate BOD bottles are collected from each Niskin, for a total of eight BOD bottles for this replicated comparison.

More details on the methodology and standard operating procedures (SOPs) are included in Section 9: Laboratory Procedures, as well as Appendix A (SOP for estimating *in situ* microbial respiration) and Appendix B (waiver for use of optodes to estimate BOD).

Measurement quality objectives for ambient temperature, salinity, and dissolved oxygen are described in Bos et al. (2015).

6.2.1 Targets for precision, bias, and sensitivity

The target coefficient of determination (R^2) derived from curve fitting of time series data during microbial respiration incubations will be >0.99.

6.2.1.1 Precision

Precision of the PreSens fiber optic measurement system equipment will be increased by using a two-point calibration of optode sensors (spots) for measuring oxygen concentrations in incubation bottles.

For evaluating precision among the ten repeated direct measures of individual BOD bottle oxygen concentrations at each time point measurement with the optode, a CV of 0.25% or less is acceptable, and those higher are flagged for investigation (e.g., occasionally incorrect individual optode readings occur as a result of incorrect positioning of the fiber optic wand; these can be easily determined by visualizing data before averaging values from each time point).

Precision among the four replicate bottles from which respiration rates are collected is evaluated using the mean of the slopes computed for each of the four replicate bottles. If the CV of the mean of the four replicate slopes is greater than 20%, that set of replicate bottles is investigated further. A replicate bottle is rejected if the slope is more than twice the mean of the four individual bottle slopes. The new mean slope is computed based on the three remaining replicates.

Based on historical long-term rate measurements from the Gong Deep System Wide Monitoring Program station, we generally expect a standard error of less than 0.6.

6.2.1.2 Bias

Bias (or drift) will be determined by 1) two-point calibration of optode sensor, 2) lab control samples (i.e., incubations with autoclaved filtered seawater and deionized water), and 3) comparison to standards (i.e., 100% oxygen-saturated water at standard pressure and temperature).

6.2.1.3 Sensitivity

The PreSens optode (PSt3) has a measurement range of 0 to 45 mg/L, and resolution depends on the ambient oxygen concentrations: 0.004 mg/L at 0.091 mg/L and 0.04 mg/L at 9.1 mg/L and limit of detection of 15 ppb or 0.03% dissolved oxygen. All of these parameters far exceed the sensitivity needs for detecting accurate rates of microbial respiration using long-term incubations. Incubation cooler target is set at 10°C and has a range of +/- 0.1°C accuracy.

6.2.2 Targets for comparability, representativeness, and completeness

6.2.2.1 Comparability

These methods for quantifying microbial respiration have been consistently used by Dr. Apple's research group for the past several years and are the only measures of water column microbial respiration being recorded in the Salish Sea. There are no other groups measuring microbial respiration, and thus no other values to which the values collected as part of this study could be compared.

6.2.2.2 Representativeness

Each sampling cruise, one site will be selected for collection of a duplicate water sample. A complete set of microbial respiration incubations will be conducted on the duplicate sample for comparison with the paired water sample collected at the same site and water depth.

6.2.2.3 Completeness

Reliable rates of microbial respiration will be determined and recorded for at least 95% of the samples collected as part of this study.

6.3 Acceptance criteria for quality of existing data

No data on microbial respiration exist for the study area. We are not aware of any published data for respiration rates in Puget Sound in the peer-reviewed literature. This study will not rely on any pre-existing data, and will only report new data that is collected per this QAPP.

6.4 Model quality objectives

The Salish Sea Model Applications QAPP (McCarthy et al. 2018) will guide the evaluation of separate Salish Sea Model quality objectives.

7.0 Study Design

Pacific Northwest National Laboratory (PNNL), in collaboration with Ecology, developed the Salish Sea Model (SSM) as predictive ocean-modeling tool for coastal estuarine research, restoration planning, water quality management, and assessment of response to future conditions (Khangaonkar et al. 2011, 2012) for the Salish Sea. It was developed using an unstructured grid framework specifically to function efficiently in a region dominated by the complex shorelines and fjord-like features. The model simulates hydrodynamics (tides, salinity, temperature) and water quality (biogeochemical variables such as algal biomass, nutrients, carbon, DO, and pH) including annual biogeochemical cycles. The Salish Sea Model Applications QAPP (McCarthy et al. 2018) will guide the evaluation of SSM output with microbial respiration rate observations obtained in this study.

7.1 Study boundaries

The study boundaries and site locations are described fully in *Quality Assurance Project Plan: Ocean Acidification Monitoring at Ecology's Greater Puget Sound Stations* (Gonski et al. 2019). Briefly, Ecology carries out long-term ambient monitoring at core marine stations in waters of the Salish Sea. Samples for microbial respiration will be collected at a subset of these stations in conjunction with those collected for total dissolved inorganic carbon and total alkalinity. Priority sites for microbial respiration measurements are located in South Sound (MF4, Figure 5) and a subset of the sites represented by Shannon Point Marine Center (SPMC) and MF2 (see Figure 5).

7.2 Field data collection

Location, schedule, and field data collection are detailed in Gonski et al. (2019) and are also part of the routine monitoring conducted by Ecology's Marine Monitoring Unit (MMU). The work proposed in this present QAPP will be conducted in concert with the field methods described in Gonski et al. (2019) and will be consistent with field methods used by Ecology's MMU.

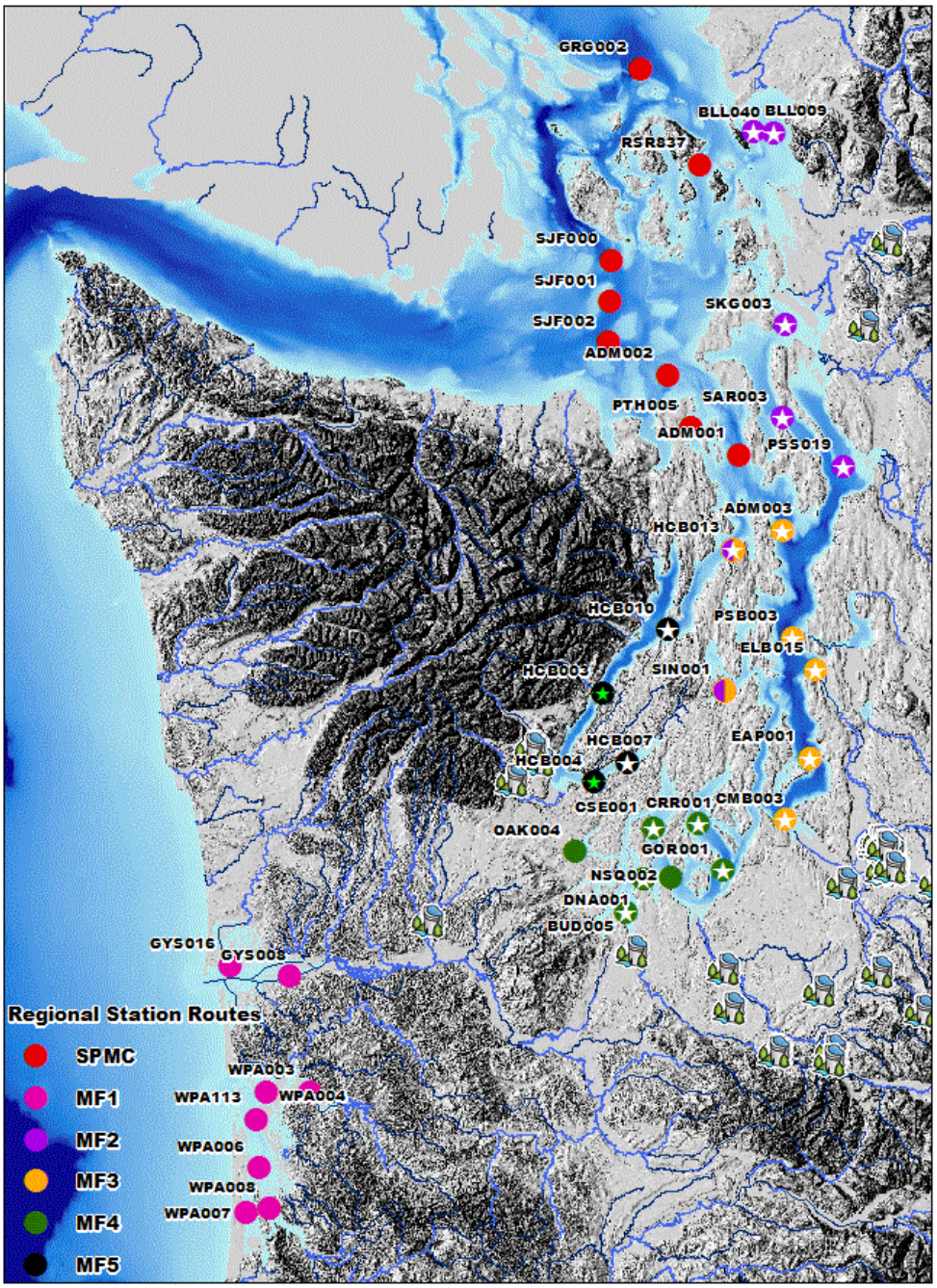

Figure 5. Map of project study area. SPMC = Shannon Point Marine Center; MF1 = Marine Flight 1, etc.

7.2.1 Sampling locations and frequency

This project will partner with existing sampling programs being conducted by the MMU and also detailed in Gonski et al. (2019). Samples will be collected at these sites:

- SKG003
- SAR003
- PSS019
- ADM001
- PTH005
- ADM002
- BLL009
- BLL040
- BUD005
- DNA001
- NSQ002
- GOR001
- CRR001
- CSE001
- OAK004

Other samples of interest, such as a Strait of Juan de Fuca transect or transects of inner inlets, if added to Gonski et al. (2019), may also be collected, contingent on staffing logistics.

7.2.2 Field parameters and laboratory analytes to be measured

In addition to the parameters measured as part of the MMU cruises and those detailed in Gonski et al. (2019), the proposed work will collect temperature, salinity, and dissolved oxygen concentrations (percent saturation and mg/L). Dissolved oxygen concentrations will be measured in incubations to estimate the *in situ* rate of microbial respiration for each sample. Measurement quality objectives (MQOs) for field measurements of temperature, salinity, and DO are found in Bos et al. (2015).

7.3 Modeling and analysis design

N/A

7.4 Assumptions in relation to objectives and study area

We are assuming that we can characterize rates of microbial respiration in near-bottom waters of the Salish Sea using the standard operating procedures (SOPs) and the sites specified in this and related QAPPs. We also assume that the rate measurements recorded in this study are proxies for representative *in situ* organic matter remineralization in waters of the Salish Sea and will thus improve assessment of skill of the Salish Sea Model. We are prioritizing and focusing on terminal inlets because of their flow dynamics and depositional characteristics.

7.5 Possible challenges and contingencies

Challenges of the proposed work include all the logistical and safety considerations inherent in boat-based research, as well as the challenges of coordinating fieldwork among multiple parties and stakeholders. Transportation of samples from South Sound cruises to the Padilla Bay NERR Laboratory represents a logistical challenge, but we are confident we can maintain sample integrity by controlling temperature and light conditions for the samples underway.

7.5.1 Logistical problems

Refer to *Quality Assurance Project Plan: Ocean Acidification Monitoring at Ecology's Greater Puget Sound Stations* (Gonski et al. 2019).

7.5.2 Practical constraints

Refer to *Quality Assurance Project Plan: Ocean Acidification Monitoring at Ecology's Greater Puget Sound Stations* (Gonski et al. 2019).

7.5.3 Schedule limitations

The sampling schedule is constrained by the routine monitoring of MMU cruises. The project termination date is June 30, 2019, and all sampling needs to be completed prior to this date.

8.0 Field Procedures

8.1 Invasive species evaluation

Refer to *Quality Assurance Project Plan: Ocean Acidification Monitoring at Ecology's Greater Puget Sound Stations* (Gonski et al. 2019).

8.2 Measurement and sampling procedures

Monthly sample collection will occur at the northern Puget Sound sites serviced by Shannon Point Marine Center (SPMC), including the two Bellingham Bay sites, and at the South Sound stations (green dots in Figure 5). Central Puget Sound and Hood Canal stations will not be sampled as part of this project. Sampling at the northern stations will commence as soon as it is feasible after the approval of this QAPP. Sampling at the southern stations is expected to commence in September or October of 2018 after receiving permission from Ecology's Quality Assurance Officer to begin work and after approval of a waiver from laboratory accreditation requirements.

Sampling procedures are detailed in the Padilla Bay NERR SOP for microbial respiration (see Appendix A) and shown in Table 4. Briefly, and with minor modifications for the present project, this method entails:

1) Collect water at each site using a Niskin bottle or other water column sampler. Waters are collected from the near-bottom water mass (i.e., within 0.5 meters of the sediment).
2) Affix a length of Tygon tubing (12–18 inches long) with a pinch clamp to the spigot on the Niskin bottle.
3) If sample temperature, salinity, and dissolved oxygen concentrations are not available for the target depth (e.g., CTD instrument profile data), collect this information by inserting a pre-rinsed handheld YSI probe (or other comparable hand-held sampling device) into the cubitainer and recording these parameters.
4) Open spigot to dispense sample and rinse all air bubbles from the tubing. Flicking the tube can help remove persistent bubbles. Use this water to pre-rinse a labeled 4-L LDPE cubitainer by dispensing approximately 25 mL of water. Cap the cubitainer and invert several times. Dump out rinse and repeat two more times for a total of three rinses.
5) Fill cubitainer from the bottom, preventing splashing and making sure there are no bubbles in the tubing. Avoid introduction of bubbles into sample by closing the pinch clamp before the Niskin bottle has been completely drained.

6) Compress the cubitainer to remove headspace. Place in cooler at target temperature.

Table 4. Measurement methods (field).

Analyte	Sample Matrix	Samples (Number/ Arrival Date)	Expected Range of Results	Accuracy	Sample Prep Method	Analytical (Instrumental) Method
Dissolved oxygen	liquid (sample water)	varies	5 to 15 mg/L	±0.2 mg/L	see above	SeaBird CTD or YSI Pro2030 handheld
Salinity	liquid	varies	0 to 35 PSU	±0.1 PSU	see above	SeaBird CTD or YSI Pro2030 handheld
Temperature	liquid	varies	0 to 25°C	±0.3°C	see above	SeaBird CTD or YSI Pro2030 handheld

8.3 Containers, preservation methods, holding times

Table 5. Sample containers, preservation, and holding times.

Parameter	Matrix	Minimum Quantity Required	Container	Preservative	Holding Time
Whole water	liquid	1.5 liters	LDPE cubitainer	none	~1 hour

When each cubitainer is filled, it is placed immediately in the dark in a water bath or cooler and kept at target temperature (10ºC) until BOD bottles can be filled with sample water for incubations (see Section 9: Laboratory Procedures).

8.4 Equipment decontamination

None necessary, although 10% HCL is used as a precautionary decontaminant on all bottles, tubing, and sampling materials.

At least one day prior to sampling, sterilize tubing and BOD bottle caps with 10% HCL solution (overnight soak). Rise with deionized (DI) water prior to use. Wash all BOD bottles prior to each incubation using the following steps:

1) Triple rinse and vigorous shake with warm tap water.
2) Place in a DI water bath for at least 24 hours.
3) Rinse with DI water before use.

8.5 Sample ID

Each sample will be given a unique alphanumeric identification indicating the date, location, and depth where the sample was collected. This will be linked to other parameters (e.g., salinity, temperature) measured at the time of sample collection.

8.6 Chain of custody

Samples will always be in the possession of the lead scientist or environmental technician.

8.7 Field log requirements

Field data sheets printed on Rite-in-Rain paper are used to record data in the field. Upon return to the laboratory, field data are entered into 1) a bound laboratory notebook dedicated to the microbial respiration project, and 2) project database. Field datasheets include the following:

- Date, time, and ID for each sample
- Location (i.e., site, basin, depth of sample collection)
- CTD filename for profile and Niskin bottle number from which sample was collected
- Temperature, salinity, and dissolved oxygen of each sample
- Comment section for circumstances that might affect interpretation of results

8.8 Other activities

Always rinse field equipment (i.e., Niskin bottle, Niskin attachments, weights, messenger, line) with warm tap water after use in the field.

9.0 Laboratory Procedures

9.1 Lab procedures table

Table 6. Measurement methods (laboratory).

Analyte	Sample Matrix	Samples (Number/Arrival Date)	Expected Range of Results	Detection or Reporting Limit	Sample Prep Method	Analytical (Instrumental) Method
Dissolved oxygen	liquid (sample water)	varies	0.5 to 15 mg/L	15 ppb	see below	Presens optode (fiber optic spot and sensor)

9.2 Sample preparation method(s)

Sample water from cubitainers is dispensed into BOD bottles dockside or at the laboratory. The procedure for filling BOD bottles is described below.

1. Unscrew lid of cubitainer and attach the presterilized lid and Tygon tubing with pinch clamp.
2. Invert cubitainer to thoroughly mix contents and elevate for gravity-filling of BOD bottles.
3. While water is flowing, insert tube into the appropriately labeled 70 mL BOD bottle, invert bottle, and flush with sample for 3 to 5 seconds. Gently invert again and allow bottle to fill.
4. Slowly dispense sample into the BOD bottle, filling from the bottom and allowing it to overflow until the bottle has been fully flushed three times (i.e., approx. 300 mL). Gently remove tubing.
5. Inspect the bottle and make sure there are NO bubbles (they will sometimes collect around the optical spot or in the shoulder of the bottle). If bubbles are present, repeat step 4.
6. Cap securely with the appropriately labeled, presterilized stopper. Store bottle fully submerged in a 10°C (or other specified water temperature) water bath.
7. Repeat steps 3 through 6 until the desired number of replicates are collected.
8. Repeat steps 1 through 7 for each cubitainer of sample water.
9. Place BOD bottles into incubation chamber and begin microbial respiration assay.
10. Microbial respiration assays for each sample will continue until one of the following conditions is met: 1) oxygen consumption (i.e., change over time) reaches or approaches zero, 2) oxygen concentrations drop below 2 mg/L, or 3) time elapsed since the start of the incubation reaches 3 weeks.

9.3 Special method requirements

Nonstandard methods for microbial respiration are described in Appendix A (Microbial Respiration SOP) and Appendix B (Accreditation Waiver).

9.4 Laboratories accredited for methods

Nonstandard methods for microbial respiration are described in Appendix A (Microbial Respiration SOP) and Appendix B (Accreditation Waiver).

10.0 Quality Control Procedures

Quality control procedures are detailed in Appendices A and B and described in Section 6: Quality Objectives.

10.1 Table of field and laboratory quality control

See Appendix B.

Table 7. Quality control samples, types, and frequency.

Parameter	Field		Laboratory			
	Blanks	Replicates	Check Standards	Method Blanks	Analytical Duplicates	Matrix Spikes
Microbial respiration rates	N/A	2	yes	4	4	N/A

10.2 Corrective action processes

If conditions of the proposed study are not met, the project team and all collaborating parties will convene to decide on steps to be taken to improve performance or remedy errors.

11.0 Data Management Procedures

11.1 Data recording and reporting requirements

Refer to *Quality Assurance Project Plan: Ocean Acidification Monitoring at Ecology's Greater Puget Sound Stations* (Gonski et al. 2019). Briefly, data from microbial respiration incubations are captured using the PreSens optode fiber-optic cable and recorded on the PreSens Fibox sampling device. After each incubation, all optode measurements are transferred from the PreSens Fibox to Ecology network servers at Padilla Bay. Periodic downloads from PreSens Fibox every week will also ensure that no data is lost.

11.2 Laboratory data package requirements

There is no external analytical laboratory involved in sample processing. Standardized lab bench sheets will be used to record all DO results used to calculate respiration rates.

11.3 Electronic transfer requirements

N/A

11.4 EIM/STORET data upload procedures

Microbial respiration rates will be transferred to Environmental Assessment Program team members (C. Figueroa-Kaminsky, G. Pelletier) for the option of transferring to the EIM/STORET database.

11.5 Model information management

N/A

12.0 Audits and Reports

12.1 Field, laboratory, and other audits

None planned.

12.2 Responsible personnel

N/A

12.3 Frequency and distribution of reports

A final report focused on the observational data obtained in this study will be prepared.

12.4 Responsibility for reports

The authors of the final report will be Jude Apple and Shauna Bjornson.

13.0 Data Verification

13.1 Field data verification, requirements, and responsibilities

Microbial respiration data will be reviewed by Ecology's Salish Sea Modeling Team.

13.2 Laboratory data verification

All dissolved oxygen data will be reviewed by independent lab staff. This will consist of reviewing field records, chain of custody forms, all raw data, and calculated results presented in the electronic data deliverable.

13.3 Validation requirements, if necessary

N/A

13.4 Model quality assessment

N/A

14.0 Data Quality (Usability) Assessment

14.1 Process for determining project objectives were met

The Salish Sea Modeling Team and Padilla Bay NERR Research Staff will work together to evaluate the success of the study and evaluate if the objectives have been met. These findings will be communicated in the final report submitted at the end of the project.

14.2 Treatment of nondetects

Statistical significance of the findings will be evaluated as part of the final data synthesis and project summary report.

14.3 Data analysis and presentation methods

Microbial respiration rates will be determined by simple linear regression for short-term 36- to 48-hour respiration rates. Statistical packages may include R, JMP, and Excel. Means comparisons will be used to explore differences among locations and seasons.

14.4 Sampling design evaluation

Statistical significance of the findings will be evaluated as part of the final data synthesis and project summary report.

14.5 Documentation of assessment

Reported as part of the final data synthesis and project summary report.

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16.0 Appendices

Appendix A. Padilla Bay NERR Standard Operating Procedure for Determination of Microbial Oxygen Consumption (Microbial Respiration) in Marine Waters

Overview

Water column respiration is a critical component of oxygen and carbon cycling in marine waters and an essential rate measurement for quantifying remineralization or organic matter. A number of methods have been developed to estimate water column microbial respiration using consumption of oxygen, including membrane inlet mass spectrometry (MIMS), Winkler titrations, oxygen probes, and fiber optic sensors. The following protocol describes a nondestructive, non-invasive method for determining rates of oxygen consumption in natural water samples using the PreSens fiber optic oxygen measuring system to collect multiple time points in each single bottle incubation.

Equipment and supplies

1. **Biological oxygen demand (BOD) bottles with glass fritted stoppers and optode "spots" installed**. Either 70 mL or 300 mL BOD bottles can be used. Smaller bottles are helpful when running large numbers of replicates or when the total available sample volume is limited. Plan on four replicate BOD bottles per sample, plus an additional four bottles for reference blanks. Sample water from the Niskin bottle can be dispensed directly into the BOD bottles, or collected in small (4 L) cubitainers for transport and filling of BODs at the lab.
2. **Immersion water bath.** Once filled, bottles need to be incubated while fully immersed in water at the target temperature and in the absence of light. Stable temperature water baths, coolers with chillers and circulators, biological incubators (e.g., Percival), or environmental chambers can be used to provide stable temperature for the incubations.
3. **Equilibrated water for reference samples.** Prior to field sampling, equilibrate approximately 1 L of deionized (DI) water by aerating a beaker for several hours. Room-temperature water is adequate, as long as room temperature is warmer than the temperature at which the bottles will be incubated. Prior to starting sample incubations, fill four reference bottles with equilibrated DI water following the filling procedure outlined below. These will serve as check standards (known oxygen concentration), as well as method blanks for the incubations.

Sample collection

The following is for collection of water samples in the field:

1. Collect water from the target depth using a Niskin bottle or other water column sampler. Bring on deck.
2. Affix a length of Tygon tubing (12–18 inches long) to spigot on Niskin bottle. Open spigot to dispense sample and rinse all air bubbles from the tubing. Flicking the tube can help remove persistent bubbles.
3. While water is flowing, insert tube into BOD bottle, invert bottle and flush with sample for 3 to 5 seconds. Gently invert again and allow bottle to fill.

4. Slowly dispense sample into the BOD bottle, filling from the bottom and allowing it to overflow for at least 5 seconds. Gently remove tubing.
5. Inspect the bottle and make sure there are NO bubbles (they will sometimes collect in the shoulder of the bottle). If bubbles are present, repeat step 4.
6. Cap securely with the stopper. Store bottle fully submerged in water at the target incubation temperature. (NOTE: A temporary incubation chamber can be created by filling a cooler with water from the Niskin bottle to achieve an *in situ* immersion bath).
7. Repeat steps 3 through 6 until the desired number of replicates for each water sample are collected.
8. Transport samples back to the lab or location of the incubation chamber.

NOTE: If water samples are being collected to fill BOD bottles at a later time (e.g., dockside or laboratory), dispense sample water into a 4-L LDPE cubitainer, filling the cubitainer from the bottom to avoid splashing and introduction of oxygen or bubbles into the sample. When full, measure temperature, salinity and dissolved oxygen concentration using a hand-held YSI probe or other comparable device. Once at the dock or laboratory, fill bottles following steps 3 through 7 above.

Incubations for estimating microbial respiration

1. After sample collection is complete and all BOD bottles are in the incubation chamber at the target temperature, take ten initial readings for each bottle using the optode. For best results, keep the BOD bottle fully immersed in the incubation water and make sure the fiber optic cable is centered on the "spot" sensor and perpendicular to the side of the bottle. Record the time. This is the initial time point (t_0) and the oxygen concentration on which respiration rates will be based.
2. Incubate all bottles in the dark at the target temperature. Take oxygen measurements (ten per bottle for each time point) at regular intervals during the course of the incubation. The frequency of these will depend on the needs of the study. More frequent (e.g., every 3 hours for the first 24 hours) will be helpful to identify nonlinearity of microbial respiration as different labilities of carbon are consumed. If long-term BOD is of interest, less frequent measurements can be made.
3. At the end of the incubation, take final measurements and export data from the Fibox unit for analysis. The length of the incubation will vary among studies and also depend on the research objectives. For eutrophic waters with warmer water temperatures (e.g., Chesapeake Bay, Gulf of Mexico), measureable changes suitable for estimating *in situ* microbial respiration rates may be achieved in as few as 6 to 8 hours. In colder water systems, or where planktonic abundance is relatively low, it may take 12 to 24 hours to get reliable estimates of *in situ* microbial respiration.
4. Rates of microbial respiration in milligrams of oxygen per liter per hour are calculated based on the decrease in oxygen concentration during the course of the incubation, relative to the initial oxygen concentration. These are generally estimated using linear regression or exponential decay, depending on the length of the incubation or research question. Alternatively, for periods during the incubation where the decline of oxygen is generally linear (e.g., the first 24 hours), the difference between the initial and final oxygen concentrations, divided by the duration of the incubation (in hours), is used to estimate the

respiration rate. This calculation is represented by the following equation, which is used to generate a numerical estimate of the rate of oxygen consumption.

$$\textit{Microbial respiration rate (mg } O_2 \textit{ } L^{-1} \textit{ } h^{-1}) = \frac{[O_2]_{final} - [O_2]_{initial}}{\textit{incubation length (h)}}$$

5. The mean of the four replicate measures of microbial respiration (i.e., BOD bottles filled with the same sample water) is used to generate a representative *in situ* microbial respiration rate for that location or water mass.

QA/QC standards:

1. Oxygen concentrations at each time point: Ten (10) replicate measures of dissolved oxygen concentration are collected for each BOD bottle at each time point. The standard deviation and CV are calculated for each set of replicate measures and those with a CV >0.15% are flagged and investigated.
2. Microbial respiration rates: Linear regression (or exponential decay) are used to generate a rate of loss of oxygen over time. In practice, the coefficient of determination (R^2) for these relationships is generally very strong (i.e., >0.95).
3. Replicated rate measures: Each water sample is incubated in four replicate BOD bottles under identical conditions, each of which has its own intrinsic microbial respiration rate. The mean of these four bottles is calculated and used as a representative rate for that water sample. Rates of microbial respiration among these four replicate bottles are generally in strong agreement, with very low variability (i.e., SE <0.1). A replicate can be rejected if the slope is more than twice the mean ratio averaged from all four replicates. If a replicate is rejected a new mean slope should be calculated from the three remaining replicates.

Appendix B. Waiver for Pelagic Microbial Respiration Derived From Modified Biological Oxygen Demand (BOD) Protocol

Date: 7/19/18 **Program**: SEA/Padilla Bay

Name of Requester: Dr. Jude Apple **Work Phone**: 360-630-1839

Project Name: **Quantifying pelagic microbial respiration in Salish Sea waters**

Test(s) for which a waiver is requested:
Pelagic microbial respiration derived from modified biological oxygen demand (BOD) protocol

Justification for Request:
☑ There is no lab accredited to do the test(s)
☑ There is no accredited lab in a reasonable distance
☑ Other (explain in narrative)

Narrative (explain the above checked boxes):

Water column microbial respiration — also referred to as community, pelagic, planktonic, or microbial respiration — represents one of the largest sinks for organic matter on the planet and is an important aspect of understanding oxygen and carbon dynamics in natural waters. The need for *in situ* measures of microbial respiration has been outlined in many texts and articles, most prominent being *Respiration in Aquatic Ecosystems* by del Giorgio and Williams (2005). Oxygen-based measures of microbial respiration remain one of the most effective ways to estimate rates of oxygen consumption and carbon remineralization in natural waters.

Although assays of biological oxygen demand (BOD; Standard Methods 2001) have been used as proxies for microbial respiration, it is important not to conflate the two. The consumption of oxygen and organic matter by planktonic communities is often a nonlinear response, and can also be governed by zero-order (i.e., substrate independent) or first-order (i.e., substrate dependent) kinetics. For this reason, the two point (i.e., initial–final) approach used in the standard BOD protocol is inadequate to fully understand the nature of organic matter consumption and remineralization in natural waters and to quantify these rates in meaningful ways.

Oxygen-based measures of microbial respiration in natural waters have been used in numerous studies. Early works by Carignan et al. (1998, 2000) were the first to describe the use of Winkler titrations to quantify respiration in natural waters. Since then, numerous others have developed and published methods for estimating respiration in ways that reveal the nonlinearity of remineralization rates and more accurately reflect these processes in natural waters. Methods include the use of Winkler titrations (Smith and Kemp 2003), membrane inlet mass spectrometry (Apple and del Giorgio 2007; Apple et al. 2006), O_2 sensors (Murrell et al. 2013), and PreSens optode spots (Warkentin et al. 2007).

Over the past several years, a number of marine microbial ecologists (including myself and colleagues listed above, e.g., Mike Murrell, Erik Smith, Paul del Giorgio) have been implementing optode fiber optic technology as a nondestructive, high-resolution, and high-throughput means of estimating *in situ* respiration and have adopted it as a means of understanding short-term carbon cycling and oxygen dynamics in natural waters. A protocol for estimating respiration using the PreSens Fibox fiber optic system is attached.

Associated Quality Control Tests: Exemption from the requirement to use an accredited lab does not relieve the data user from the requirement to assure lab data meets quality objectives. Reviewing quality control (QC) test results, routinely performed as part of the lab accreditation process, becomes an especially important issue for the user of data coming from a non-accredited lab. Mark below the QC tests the lab will be required to report along with the environmental data. Refer to this description of QC tests, if needed, to determine the significance of each test.

Below are quality controls implemented with our existing protocol.

1. Reference samples: Equilibrated DI water (i.e., 100% DO saturated) is used to fill replicated BOD bottles and used as a reference sample and accuracy check for the optode (i.e., check standards). These are measured at the same time and frequency as the natural water samples.
2. Methods blanks: The same equilibrated DI samples above are used in the incubations to serve as method blanks and identify any systematic perturbations or drift.
3. Replication for each time point: Ten (10) replicate measures of dissolved oxygen concentration are collected for each BOD bottle at each time point. The standard deviation and CV are calculated for each set of replicate measures. Those with a CV >0.25% are flagged and investigated.
4. Calculating respiration rates: Linear regression (or exponential decay) is performed on each of the time series generated by a single BOD bottle over the course of the incubation. In practice, the coefficient of determination (R^2) for these relationships is generally very strong (i.e. >0.95).
5. Replicated rate measures: Each water sample is incubated in four replicate BOD bottles under identical conditions, each of which has its own intrinsic microbial respiration rate (see #4 above). The mean of these four bottles is calculated and used as a representative rate for that water sample. Rates of microbial respiration among these four replicate bottles are generally in strong agreement, with very low variability (i.e., SE <0.1).

Appendix C. Glossaries, Acronyms, and Abbreviations

Glossary of General Terms

Ambient: Background or away from point sources of contamination. Surrounding environmental condition.

Anthropogenic: Human-caused.

Clean Water Act: A federal act passed in 1972 that contains provisions to restore and maintain the quality of the nation's waters.

Designated uses: Those uses specified in Chapter 173-201A WAC (Water Quality Standards for Surface Waters of the State of Washington) for each water body or segment, regardless of whether or not the uses are currently attained.

Diel: Of, or pertaining to, a 24-hour period.

Dissolved oxygen (DO): A measure of the amount of oxygen dissolved in water.

Eutrophic: Nutrient rich and high in productivity resulting from human activities, such as fertilizer runoff and leaky septic systems.

Sediment: Soil and organic matter that is covered with water (for example, river or lake bottom).

Watershed: A drainage area or basin in which all land and water areas drain or flow toward a central collector, such as a stream, river, or lake at a lower elevation.

303(d) list: Section 303(d) of the federal Clean Water Act, requiring Washington State to periodically prepare a list of all surface waters in the state for which beneficial uses of the water – such as for drinking, recreation, aquatic habitat, and industrial use – are impaired by pollutants. These are water quality–limited estuaries, lakes, and streams that fall short of state surface water quality standards and are not expected to improve within the next two years.

90th percentile: An estimated portion of a sample population based on a statistical determination of distribution characteristics. The 90th percentile value is a statistically derived estimate of the division between 90% of samples, which should be less than the value, and 10% of samples, which are expected to exceed the value.

Acronyms and Abbreviations

BMP	Best management practice
BOD	Biological oxygen demand
CTD	Conductivity, temperature, and depth
CV	Coefficient of variation
DI	Deionized
DO	(see Glossary above)
DOC	Dissolved organic carbon
Ecology	Washington State Department of Ecology
EIM	Environmental Information Management database
EPA	U.S. Environmental Protection Agency
GIS	Geographic Information System software
GPS	Global Positioning System
MEL	Manchester Environmental Laboratory
MIMS	Membrane inlet mass spectrometry
MQO	Measurement quality objective
NERR	National Estuarine Research Reserve
POC	Particulate organic carbon
QA	Quality assurance
QC	Quality control
R^2	Coefficient of determination
SOP	Standard operating procedures
SPMC	Shannon Point Marine Center
SRM	Standard reference materials
TIR	Thermal infrared radiation
TOC	Total organic carbon
TSS	Total suspended solids
USGS	United States Geological Survey
WAC	Washington Administrative Code
WQA	Water Quality Assessment
WRIA	Water Resource Inventory Area
WSTMP	Washington State Toxics Monitoring Program
WWTP	Wastewater treatment plant

Units of Measurement

°C	degrees centigrade
cfs	cubic feet per second
cfu	colony forming units
cms	cubic meters per second, a unit of flow
dw	dry weight
ft	feet
g	gram, a unit of mass
kcfs	1000 cubic feet per second
kg	kilograms, a unit of mass equal to 1,000 grams
kg/d	kilograms per day
km	kilometer, a unit of length equal to 1,000 meters
l/s	liters per second (0.03531 cubic foot per second)
m	meter
mm	millimeter
mg	milligram
mgd	million gallons per day
mg/d	milligrams per day
mg/kg	milligrams per kilogram (parts per million)
mg/L	milligrams per liter (parts per million)
mg/L/hr	milligrams per liter per hour
mL	milliliter
mmol	millimole or one-thousandth of a mole
mole	an International System of Units (IS) unit of matter
ng/g	nanograms per gram (parts per billion)
ng/Kg	nanograms per kilogram (parts per trillion)
ng/L	nanograms per liter (parts per trillion)
NTU	nephelometric turbidity units
pg/g	picograms per gram (parts per trillion)
pg/L	picograms per liter (parts per quadrillion)
psu	practical salinity units
s.u.	standard units
µg/g	micrograms per gram (parts per million)
µg/Kg	micrograms per kilogram (parts per billion)
µg/L	micrograms per liter (parts per billion)
µg O_2 L^{-1} h^{-1}	micrograms oxygen per liter per hour
µm	micrometer
µM	micromolar (a chemistry unit)
µmhos/cm	micromhos per centimeter
µS/cm	microsiemens per centimeter, a unit of conductivity
ww	wet weight

Quality Assurance Glossary

Accreditation: A certification process for laboratories, designed to evaluate and document a lab's ability to perform analytical methods and produce acceptable data. For Ecology, it is "Formal recognition by (Ecology)…that an environmental laboratory is capable of producing accurate analytical data." [WAC 173-50-040] (Kammin 2010).

Accuracy: The degree to which a measured value agrees with the true value of the measured property. USEPA recommends that this term not be used, and that the terms *precision* and *bias* be used to convey the information associated with the term *accuracy* (USGS 1998).

Analyte: An element, ion, compound, or chemical moiety (pH, alkalinity) which is to be determined. The definition can be expanded to include organisms, e.g., fecal coliform, *Klebsiella* (Kammin 2010).

Bias: The difference between the sample mean and the true value. Bias usually describes a systematic difference reproducible over time and is characteristic of both the measurement system and the analyte(s) being measured. Bias is a commonly used data quality indicator (DQI) (Kammin 2010; Ecology 2004).

Blank: A synthetic sample, free of the analyte(s) of interest. For example, in water analysis, pure water is used for the blank. In chemical analysis, a blank is used to estimate the analytical response to all factors other than the analyte in the sample. In general, blanks are used to assess possible contamination or inadvertent introduction of analyte during various stages of the sampling and analytical process (USGS 1998).

Calibration: The process of establishing the relationship between the response of a measurement system and the concentration of the parameter being measured (Ecology 2004).

Check standard: A substance or reference material obtained from a source independent from the source of the calibration standard; used to assess bias for an analytical method. This is an obsolete term, and its use is highly discouraged. See Calibration Verification Standards, Lab Control Samples (LCS), Certified Reference Materials (CRM), and/or spiked blanks. These are all check standards but should be referred to by their actual designator, e.g., CRM, LCS (Kammin 2010; Ecology 2004).

Comparability: The degree to which different methods, data sets, and/or decisions agree or can be represented as similar; a data quality indicator (USEPA 1997).

Completeness: The amount of valid data obtained from a project compared to the planned amount. A data quality indicator, usually expressed as a percentage (USEPA 1997).

Continuing Calibration Verification Standard (CCV): A quality control (QC) sample analyzed with samples to check for acceptable bias in the measurement system. The CCV is usually a midpoint calibration standard that is re-run at an established frequency during the course of an analytical run (Kammin, 2010).

Data integrity: A qualitative DQI that evaluates the extent to which a data set contains data that is misrepresented, falsified, or deliberately misleading (Kammin 2010).

Data quality indicators (DQI): Commonly used measures of acceptability for environmental data. The principal DQIs are precision, bias, representativeness, comparability, completeness, sensitivity, and integrity (USEPA 2006).

Data quality objectives (DQO): Qualitative and quantitative statements derived from systematic planning processes that clarify study objectives, define the appropriate type of data, and specify tolerable levels of potential decision errors that will be used as the basis for establishing the quality and quantity of data needed to support decisions (USEPA 2006).

Data set: A grouping of samples organized by date, time, analyte, etc. (Kammin 2010).

Data validation: An analyte-specific and sample-specific process that extends the evaluation of data beyond data verification to determine the usability of a specific data set. It involves a detailed examination of the data package, using both professional judgment and objective criteria, to determine whether the MQOs for precision, bias, and sensitivity have been met. It may also include an assessment of completeness, representativeness, comparability, and integrity, as these criteria relate to the usability of the data set. Ecology considers four key criteria to determine if data validation has actually occurred. These are:

- Use of raw or instrument data for evaluation.
- Use of third-party assessors.
- Data set is complex.
- Use of EPA Functional Guidelines or equivalent for review.

Examples of data types commonly validated would be:

- Gas Chromatography (GC).
- Gas Chromatography-Mass Spectrometry (GC-MS).
- Inductively Coupled Plasma (ICP).

The end result of a formal validation process is a determination of usability that assigns qualifiers to indicate usability status for every measurement result. These qualifiers include:

- No qualifier – data are usable for intended purposes.
- J (or a J variant) – data are estimated, may be usable, may be biased high or low.
- REJ – data are rejected, cannot be used for intended purposes (Kammin 2010; Ecology 2004).

Data verification: Examination of a data set for errors or omissions, and assessment of the Data Quality Indicators related to that data set for compliance with acceptance criteria (MQOs). Verification is a detailed quality review of a data set (Ecology 2004).

Detection limit (limit of detection)**:** The concentration or amount of an analyte which can be determined to a specified level of certainty to be greater than zero (Ecology 2004).

Duplicate samples: Two samples taken from and representative of the same population, and carried through all steps of the sampling and analytical procedures in an identical manner. Duplicate samples are used to assess variability of all method activities, including sampling and analysis (USEPA 1997).

Field blank: A blank used to obtain information on contamination introduced during sample collection, storage, and transport (Ecology 2004).

Initial Calibration Verification Standard (ICV): A QC sample prepared independently of calibration standards and analyzed along with the samples to check for acceptable bias in the measurement system. The ICV is analyzed prior to the analysis of any samples (Kammin 2010).

Laboratory Control Sample (LCS): A sample of known composition prepared using contaminant-free water or an inert solid that is spiked with analytes of interest at the midpoint of the calibration curve or at the level of concern. It is prepared and analyzed in the same batch of regular samples using the same sample preparation method, reagents, and analytical methods employed for regular samples (USEPA 1997).

Matrix spike: A QC sample prepared by adding a known amount of the target analyte(s) to an aliquot of a sample to check for bias due to interference or matrix effects (Ecology 2004).

Measurement Quality Objectives (MQOs)**:** Performance or acceptance criteria for individual data quality indicators, usually including precision, bias, sensitivity, completeness, comparability, and representativeness (USEPA 2006).

Measurement result: A value obtained by performing the procedure described in a method (Ecology 2004).

Method: A formalized group of procedures and techniques for performing an activity (e.g., sampling, chemical analysis, data analysis), systematically presented in the order in which they are to be executed (EPA 1997).

Method blank: A blank prepared to represent the sample matrix, prepared and analyzed with a batch of samples. A method blank will contain all reagents used in the preparation of a sample, and the same preparation process is used for the method blank and samples (Ecology 2004; Kammin 2010).

Parameter: A specified characteristic of a population or sample. Also, an analyte or grouping of analytes. Benzene and nitrate + nitrite are all "parameters" (Kammin 2010; Ecology 2004).

Population: The hypothetical set of all possible observations of the type being investigated (Ecology 2004).

Precision: The extent of random variability among replicate measurements of the same property; a data quality indicator. (USGS, 1998)

Quality assurance (QA): A set of activities designed to establish and document the reliability and usability of measurement data (Kammin 2010).

Quality Assurance Project Plan (QAPP): A document that describes the objectives of a project and the processes and activities necessary to develop data that will support those objectives (Kammin 2010; Ecology 2004).

Quality control (QC): The routine application of measurement and statistical procedures to assess the accuracy of measurement data (Ecology 2004).

Replicate samples: Two or more samples taken from the environment at the same time and place, using the same protocols. Replicates are used to estimate the random variability of the material sampled (USGS 1998).

Representativeness: The degree to which a sample reflects the population from which it is taken; a data quality indicator (USGS 1998).

Sample (field): A portion of a population (environmental entity) that is measured and assumed to represent the entire population (USGS 1998).

Sample (statistical): A finite part or subset of a statistical population (USEPA 1997).

Sensitivity: In general, denotes the rate at which the analytical response (e.g., absorbance, volume, meter reading) varies with the concentration of the parameter being determined. In a specialized sense, it has the same meaning as the detection limit (Ecology 2004).

Spiked blank: A specified amount of reagent blank fortified with a known mass of the target analyte(s); usually used to assess the recovery efficiency of the method (USEPA 1997).

Spiked sample: A sample prepared by adding a known mass of target analyte(s) to a specified amount of matrix sample for which an independent estimate of target analyte(s) concentration is available. Spiked samples can be used to determine the effect of the matrix on a method's recovery efficiency (USEPA 1997).

Split sample: A discrete sample subdivided into portions, usually duplicates (Kammin 2010).

Standard Operating Procedure (SOP): A document which describes in detail a reproducible and repeatable organized activity (Kammin 2010).

Surrogate: For environmental chemistry, a surrogate is a substance with properties similar to those of the target analyte(s). Surrogates are unlikely to be native to environmental samples. They are added to environmental samples for quality control purposes, to track extraction

efficiency and/or measure analyte recovery. Deuterated organic compounds are examples of surrogates commonly used in organic compound analysis (Kammin 2010).

Systematic planning: A stepwise process that develops a clear description of the goals and objectives of a project and produces decisions on the type, quantity, and quality of data that will be needed to meet those goals and objectives. The DQO process is a specialized type of systematic planning (USEPA 2006).

References for QA Glossary

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