Recommended Guidelines for Measuring Conventional Marine Water-Column Variables in Puget Sound

For

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LIST OF ACRONYMS

APHA	American Public Health Association
EDTA	ethylenediaminetetraacetic acid
EPA	U.S. Environmental Protection Agency
MF	membrane filter
MPN	most probable number
NIST	National Institute of Standards and Technology
NTU	nephelometric turbidity units
OSHA	Occupational Safety and Health Administration
PSEP	Puget Sound Estuary Program
TSS	total suspended solids

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INTRODUCTION

Recommended guidelines for measuring the following 15 conventional water-column variables in Puget Sound are presented in this chapter:

- pH
- Salinity
- Temperature
- Transparency
- Turbidity (transmissivity)
- Total suspended solids
- Dissolved oxygen (modified Winkler method)
- Dissolved oxygen (probe method)
- Nitrogen (ammonia)
- Nitrogen (nitrite)
- Nitrogen (nitrate)
- Phosphate
- Silicate
- Chlorophyll *a*
- Total and fecal coliform bacteria.

Each guideline is based on the results of a workshop sponsored by the Puget Sound Estuary Program (PSEP) and written reviews by representatives from most organizations that fund or conduct environmental studies in Puget Sound (Table 1). The purpose of developing these recommended guidelines is to encourage all Puget Sound investigators conducting monitoring programs, baseline surveys, and intensive investigations to use standardized methods whenever possible. If this goal is achieved, most data collected in Puget Sound should be directly comparable and thereby capable of being integrated into a sound-wide database. Such a database is necessary for developing and maintaining a comprehensive water quality management program for Puget Sound.

TABLE 1. CONTRIBUTORS TO THE MARINE WATER-COLUMN GUIDELINES

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Each recommended guideline describes field collection and processing methods and laboratory analytical, quality assurance/quality control, and data reporting procedures. Each recommended analytical procedure was based on standardized techniques from Parsons et al. (1984), U.S. EPA (1983), or APHA (1989). Much of this report was based on U.S. EPA (1987). These techniques are summarized in Table 2. The general collection and holding recommendations for each water-column variable are presented in Table 3.

Although the following guidelines are recommended for most studies conducted in Puget Sound, departures from these methods may be necessary to meet the special requirements of individual projects. If such departures are made, however, the funding agency or investigator should be aware that the resulting data may not be comparable with most other data of that kind. In some instances, data collected using different methods may be compared if the methods are intercalibrated adequately.

TABLE 2. RECOMMENDED METHODS FOR MEASURING WATER-COLUMN CONVENTIONAL VARIABLES

Variable	Method Reference		
	U.S. EPA[a]	APHA[b]	Other[c]
pH	150.1	4500-H B	*In situ*[d]
Salinity	--	2520 B	Salinometer[e]; *in situ*[d]
Temperature	170.1	2550 B	*In situ*[d]
Transparency	--	--	Secchi disk
Turbidity	180.1	2130 B	--
Transmissivity	--	--	*In situ*[d]
Total suspended solids	160.2[f]	2540 D[f]	--
Dissolved oxygen			
Probe method	360.1	4500-0 G	*In situ*[d];
Winkler method	--	--	Parsons et al. (1984)
Nitrogen			
Ammonia-N	--	--	Parsons et al. (1984); autoanalyzer[g]
Nitrite-N	--	--	Parsons et al. (1984); autoanalyzer[g]
Nitrate-N	--	--	Parsons et al. (1984); autoanalyzer[g]
Phosphate	--	--	Parsons et al. (1984); autoanalyzer[g]
Silicate	--	--	Parsons et al. (1984)
Chlorophyll *a*	--	--	Parsons et al. (1984); *in situ*
Total coliform bacteria	--	9221 B[h] 9222 B[i]	--
Fecal coliform bacteria	--	9221 C[h] 9222 D[i]	--

[a] Methods recommended in U.S. EPA (1983).

[b] Methods recommended in APHA (1989).

[c] Methods recommended in sources other than U.S. EPA (1983) or APHA (1989).

TABLE 2. (Continued)

[d] This variable can be measured using an *in situ* instrument. The operating manual for the instrument should provide all necessary information for proper instrument calibration and measurement of this variable.

[e] The instruction manual for the salinometer should provide all necessary information for instrument calibration and salinity determination.

[f] A 0.40- or 0.45-µm membrane filter should be used instead of the glass fiber filter recommended in the method.

[g] The instruction manual for the autoanalyzer should provide all necessary information for instrument calibration and nutrient determinations. A general discussion of the use of autoanalyzers is presented by Grasshoff et al. (1983).

[h] This method can be used whether or not chlorine is present.

[i] This method cannot be used when chlorine is present.

TABLE 3. RECOMMENDED SAMPLE SIZES, CONTAINERS, PRESERVATION, AND HOLDING TIMES FOR WATER-COLUMN SAMPLES[a]

Measurement	Minimum Sample Size[b]	Container[c]	Preservative	Maximum Holding Time
pH	25 mL	P,G	None	Analyze immediately[d]
Salinity	200 mL	G	None	Indefinitely
Temperature	1 L	P,G	None	Measure immediately[d]
Turbidity	100 mL	P,G	Cool, 4°C, dark	24 hours
Total suspended solids	1-4 L[f]	P,G	Cool, 4°C, dark	7 days
Dissolved oxygen				
Probe method	125 mL	G bottle & top only	None	Analyze immediately[d]
Modified Winkler method	125 mL	G bottle & top only	Fix onsite; store in dark	8 hours
Nitrogen				
Ammonia-N	100 mL	P,G	Freeze[e] at -20°C	7 days
Nitrite-N	100 mL	P,G	Freeze[e] at -20°C	28 days
Nitrate-N	200 mL	P,G	Freeze[e] at -20°C	28 days
Phosphate	200 mL	P,G	Freeze[e] at -20°C	28 days
Silicate	100 mL	P only	Freeze[e] at -20°C	28 days
Chlorophyll *a*	100-300 mL[f]	P,G	Freeze filter at -20°C in the dark in a desiccator	30 days
Total and fecal coliform bacteria	500 mL	P,G	Cool, 4°C 0.008% $Na_2S_2O_3$[g]	24 hours

[a] Reference: Adapted from U.S. EPA (1983, 1984).
[b] Recommended field sample sizes for one laboratory analysis. If additional laboratory analyses are required (e.g., replicates), the field sample size should be adjusted accordingly.
[c] Polyethylene (P) or Glass (G).
[d] Immediately means as soon as possible after the sample is collected, generally within 15 minutes (U.S. EPA 1984). However, reversing thermometers must equilibrate at depth prior to making measurements.
[e] After filtration.
[f] The volumes specified are only estimates; the actual volume filtered depends on concentration and may be larger than those presented in the table.
[g] Should be used in the presence of chlorine residual.

COLLECTION AND ANALYSIS OF WATER-COLUMN SAMPLES

For most studies in Puget Sound, water-column variables are sampled using water bottles, *in situ* instrumentation, or a combination of both of these techniques. Both kinds of sampling equipment can be deployed from a variety of sampling vessels. For some purposes, the equipment can also be deployed from a seaplane. The key consideration in selecting a sampling platform is that samples can be collected in a manner that ensures the safety of the sampling personnel and the quality of the resulting data. The remainder of this section discusses the use of water bottles and *in situ* instrumentation to measure marine water-column variables.

WATER BOTTLES

Water-bottle samplers are relatively simple devices that generally consist of some type of cylindrical tube with stoppers at each end and a closing device that is activated by a messenger or an electrical signal. The most commonly used samplers of this description are the Van Dorn, Niskin, Nansen, and Go-Flo samplers. Each device samples a discrete parcel of water at any designated depth. Each sampler can be attached directly to a hydrographic wire or cable and lowered to the desired sampling depth. Frequently, multiple water samplers are fixed on a rosette frame so that several depths can be sampled during one cast or replicate samples can be taken at the same depth using an electronic release.

Prior to deployment, the stoppers of water-bottle samplers are cocked open on the sampling vessel. At this step, it is critical that the interior of the sampler and stoppers remain free from contamination. All members of the sampling team should therefore avoid touching the insides of the sampler and stoppers. The interior of each water bottle sampler should be washed periodically with 10-percent hydrochloric acid (i.e., before each cruise at a minimum).

After cocking, the sampler is lowered to a designated depth. The sampler must be open at both ends so that water is not trapped within the device as it is being lowered through the water column. Once the sampler reaches the desired depth, it should be allowed to equilibrate with ambient conditions for 2-3 minutes before being closed. Equilibration time should be extended to 5 minutes if thermometers are used for temperature measurements.

After equilibration, the closing device can be activated by messenger or electrical signal, and the sampler can be retrieved. In some cases (e.g., for deep water), two samplers can be used simultaneously for each depth. A second sampler provides a backup to the primary sampler in case the latter device misfires or will not trigger. This may eliminate the need for an additional cast. A second sampler can also supplement the primary sampler if the volume collected by the latter device is too small for all required subsampling and rinsing. To ensure that all subsamples at a particular depth are collected from the same water parcel, it is essential that they all be taken from a single cast.

Multiple casts using a single water sampler will not meet this objective. Sample water must therefore be used conservatively after collection.

Once the water sampler is brought on board the sampling vessel, the stoppers should be checked immediately for complete seals. If a stopper is not properly sealed, water from the sampled depth may have been replaced by water from another depth. Because this kind of contamination can bias results, the entire water sample should be rejected.

Accepted water samples should be subsampled as soon as possible (i.e., within 15 minutes) because appreciable delay may result in unrepresentative subsamples. For example, measurement of variables sensitive to biological alteration (e.g., dissolved oxygen, turbidity, color, nutrients) or settlement within the water sampler (e.g., total suspended solids, settleable solids, phytoplankton) can be biased substantially by subsampling delays. Samples for dissolved oxygen measurements should be the first samples collected. If samples for other variables are not collected within 15 minutes after sample collection, the sampling bottles should be shaken prior to subsampling to homogenize the sample water.

IN SITU INSTRUMENTATION

A wide variety of instruments capable of measuring water column variables *in situ* are available. Most are deployed from the sampling vessel using a hydrographic wire or conducting cable. Sensors housed within the instruments measure the variables of interest and transmit data in the form of electrical signals to recorders on the survey vessel or within the instruments. The simplest instruments measure conductivity (i.e., for conversion to salinity), temperature, and water pressure (i.e., for conversion to depth). Additional sensors and instrumentation can be included to measure a variety of additional water-column variables such as dissolved oxygen, pH, transmissivity (i.e., an index of turbidity), and oxidation-reduction potential. Water transparency can be measured using a quantum meter. Chlorophyll *a* can be measured *in situ* using an *in situ* fluorometer. Generally, the operating manuals supplied with these instruments provide detailed descriptions of how to calibrate, operate, and maintain the equipment. If a particular manual lacks sufficient detail, the manufacturer should be contacted for specific guidance. An annual or semiannual intercalibration effort among the various groups that measure water-column variables in Puget Sound would be useful for ensuring the comparability and quality of those measurements.

A major advantage of *in situ* instrumentation is the ability to measure continuous depth profiles of water-column variables. Continuous profiling eliminates the need to arbitrarily select discrete sampling depths. In addition, continuous profiling can identify water-column discontinuities and plumes that may not be detected if measurements are restricted to discrete depths. This information can be used to guide sampling at discrete depths by water bottle samplers.

Although the instrument operating manuals should be consulted for specific instructions, several general procedures for operating *in situ* instruments apply to all or most devices and have a direct influence on data quality. When acquiring *in situ* instruments for use in marine and estuarine waters the following features are highly recommended:

- Instruments should be of rugged construction, corrosion-resistant, and waterproof
- Instruments should be capable of operating with acceptable accuracy within the range of expected environmental conditions
- Cables should be of adequate length and strength
- Electrical connectors should be easy to use, waterproof when connected, and capable of being locked after connection
- External sensors should be protected by housings or other means
- Instruments should be easy to calibrate on board the survey vessel in the case of extended cruises
- Ideally, a service center should be located nearby so that an instrument can be repaired rapidly, if necessary
- If sample contamination by the sampling equipment may be a problem, all such equipment should be made of noncontaminating material.

When using *in situ* instruments it is critical that they be protected from rough handling and adverse environmental conditions. The following precautions should always be taken:

- Instruments should be transported and stored in specially designed shipping boxes. During transport, care should be taken to ensure that the instruments are secure and subjected to a minimal amount of agitation.
- Instruments should be surrounded by a "birdcage" when being deployed. Frequently, instruments are attached within a rosette frame when they are used in conjunction with water bottles. Caution should be taken to ensure that the birdcage or rosette frame does not create sampling artifacts.
- Instruments should be securely lashed down in a safe area when on deck, and preferably stored in shipping boxes when not being used.
- Instruments should be rinsed with fresh water, and probes and sensors should be rinsed with distilled water after each submersion.

- Optical surfaces should be cleaned with alcohol and lens tissue (or as recommended by the instrument manufacturer) after each submersion.

- Instruments should be protected from direct sunlight and excessive heat, as plastic components may be damaged by heat.

- External sensors and optical ports should be covered and protected whenever the instrument is not being used for extended periods (i.e., >1 hour).

When operating *in situ* instruments, the following procedures should be followed to ensure that instruments are prepared, deployed, and retrieved properly:

- Instruments should be allowed to warm up for a sufficient length of time prior to calibration or deployment. This time should be specified in the instrument manuals.

- Instruments should be field-calibrated at the beginning and end of each day of sampling and 3-4 times during the day. All circuits should be tested at the same time. Calibration should be conducted more frequently if equipment malfunctioning is suspected. Instruments should also be calibrated in the laboratory before and after each field sampling event. Calibration should be conducted with standard solutions, whenever possible.

- Upon instrument deployment, the survey vessel should be anchored or drifting slowly. The extent of vessel drift and wave motion should be noted if they could influence the representativeness or quality of measurements.

- Instruments should be deployed relative to vessel construction and sea conditions so that cables will not tangle in the propeller or rudder assemblies and measurements will not be influenced by physical or chemical artifacts from the vessel.

- When measuring continuous profiles, the lowering speed through the water column should not exceed the equilibration rate of the sensor having the slowest response time (usually the dissolved oxygen sensor).

- Excessive strain should not be placed on the cable(s), as it could disrupt electrical connections.

Routine maintenance and inspection of *in situ* instruments should follow the manufacturer's recommendations. Detailed records of all maintenance activities should be kept for quality assurance purposes. General maintenance and inspection procedures include:

- All rubber parts of underwater connectors should be coated with silicone grease to ensure proper lubrication

- Plugs should be inspected for bent or broken pins, which may cause faulty connections and flooded cables

- Cables should be inspected for nicks, cuts, abrasions, or other signs of physical damage

- Seals should be inspected and periodically cleaned and greased to ensure a waterproof fit

- Desiccant should be inspected and replaced with fresh or reactivated desiccant when necessary

- Battery voltages and conditions should be checked periodically.

To facilitate shipboard repair of *in situ* instruments, it is recommended that critical spare parts be stored on the sampling vessel. Factory inspection and recalibration at recommended intervals is essential to ensure that the *in situ* instruments are functioning properly and will continue to function properly during future cruises. It is strongly recommended that factory service be conducted at least once per year. Factory service should always be conducted when instrument malfunctions cannot be corrected by following the operating manual. Factory service may also be required when part of an *in situ* system is replaced, as all components are not interchangeable without factory recalibration.

SHIPBOARD LABORATORY ANALYSES

Depending upon the size and capabilities of the survey vessel, many of the water-column variables described in this document can be analyzed on board. In general, the laboratory procedures described in this document are applicable to both shipboard and land-based laboratories. This consistency is important to ensuring that analytical results will be comparable regardless of which kind of laboratory generates them.

Although most laboratory procedures are similar between shipboard and land-based laboratories, a number of additional factors must be considered when analyzing samples at sea. These factors relate primarily to the remoteness of the shipboard laboratory from land-based support, the movement and limited space of the survey vessel, and the potential for sample contamination. The major considerations are:

- The design of the laboratory should be efficient, with convenient equipment locations and adequate storage space.

- The vessel should be equipped with an uninterruptible power supply that is adequate for operation of all scientific instruments.

- The laboratory should be well-ventilated to remove any toxic vapors created by chemicals. All laboratory conditions should be consistent with the specifications of the Occupational Safety and Health Administration (OSHA).

- The temperature of the laboratory should be well-controlled, especially if variations in ambient temperature can influence particular analyses.

- Adequate lighting is necessary, especially for analyses requiring color discrimination (e.g., titration endpoints). Fluorescent lights of the daylight type are often recommended.

- The laboratory should have adequate water purification apparatus or be capable of storing pre-purified water.

- For storing many kinds of samples, adequate refrigeration and freezing capabilities are required.

- The laboratory should never be used as a general passageway or lounge. Smoking should be prohibited in the laboratory.

- The laboratory should be off-limits to unauthorized personnel.

- Adequate safety and first aid equipment should be on board, preferably including an overhead quick-pull safety shower.

- Extreme care must be taken when handling samples (for quality purposes) and hazardous reagents (for safety purposes), as vessel movement can sometimes be unpredictable.

- Backup supplies and instruments should be on board so that sampling can continue if a piece of equipment is broken or will not operate properly. A continuously updated inventory tracking system is useful for maintaining backup equipment.

- All equipment should be properly secured to compensate for predictable and unpredictable vessel movements. Specially designed racks are useful for this purpose.

- Instruments should be checked and calibrated before sailing so that problems requiring land-based assistance can be solved quickly.

- Whenever possible, plastic containers or plastic-coated glass containers should be

used instead of glass, because plastic is less susceptible to breakage.

- Preprinted data sheets should be used to ensure that all required information is recorded. Data sheets should be made of waterproof paper if they will be exposed to the weather.

MEASUREMENT OF SPECIFIC VARIABLES

Recommended procedures are presented in this section for measuring the following 15 water-column variables:

- pH*
- Salinity*
- Temperature*
- Transparency*
- Turbidity (transmissivity)*
- Total suspended solids
- Dissolved oxygen (Winkler method)
- Dissolved oxygen (Probe method)*
- Nitrogen (ammonia)
- Nitrogen (nitrite)
- Nitrogen (nitrate)
- Phosphate
- Silicate
- Chlorophyll *a**
- Total and fecal coliform bacteria.

Samples to be analyzed for these variables generally will be collected using water-bottle samplers. Those variables followed by an asterisk (*) may also be measured (or estimated in the case of turbidity) using *in situ* instruments. Operation of water-bottle samplers and *in situ* instruments are described in the preceding general methods section.

It is recommended that all measurements of nutrients (i.e., ammonia, nitrite, nitrate, phosphate, and silicate) be made on the dissolved fraction of samples, to represent the nutrients with the highest degree of bioavailability. Results of all analyses should be identified accord-ingly (i.e., that they represent dissolved nutrients). All samples for nutrient analyses should therefore be filtered in the field, using filters with a pore size of approximately 0.40 or 0.45 µm. Filters should be rinsed in sequence with 10-percent hydrochloric acid and ammonium-free distilled water (i.e., to remove ammonia) prior to use. This filtration step will separate the dissolved and particulate fractions of the samples.

pH

Field Procedures

Collection—Samples for pH determination should be collected in polyethylene or glass bottles with airtight screw caps. Because pH can be unstable (especially when influenced by fresh water) and cannot be preserved, these samples should be collected immediately after the sampler is brought on deck. Only dissolved oxygen samples should be collected before pH samples. Because pH of waters not at equilibrium with the atmosphere may change upon exposure to the atmosphere, sample containers should be completely filled and tightly sealed during collection.

Prior to sample collection, each sample bottle and cap should be rinsed thoroughly with sample water. This can be achieved by rinsing the bottle three times with a small volume of sample water, and rinsing the stopper as the wash water is discarded each time.

A piece of soft-walled rubber tubing should be attached to the outlet valve of the sampler. This tubing should then be inserted to the bottom of the sample bottle and at least one full volume allowed to overflow the bottle. With the water still flowing, the tubing should be withdrawn slowly from the sample bottle. Contamination of the sample with air bubbles should be avoided.

After the tubing has been removed from the sample bottle, the stopper should be put in place carefully to avoid trapping air bubbles. Once stoppered, the sample should be checked for bubbles. If they are present, the sample should be discarded and a new one taken. The stopper on each accepted sample should be double-checked to ensure a tight seal.

Processing—Because pH cannot be preserved, samples should be analyzed immediately after collection (i.e., within 15 minutes from the time the water bottle is brought on deck).

Laboratory Procedures

Analytical Procedures—Analytical procedures are given in EPA Method 150.1 and APHA Method 4500-H B. As noted previously, pH samples should be analyzed as soon as possible following collection.

Several potential sources of interference with pH measurements should be avoided. Because the response of the electrode can be impaired if it is coated with oily or particulate material, the electrode should be gently blotted or washed periodically with a detergent. Treatment with hydrochloric acid may be necessary to remove some kinds of film. Temperature can influence pH measurements by altering electrode output and changing the pH inherent in the sample. The first source of temperature interference can be controlled by using a pH meter with temperature compensation or by calibrating the meter at the temperature of the samples. Because the second kind of temperature interference cannot be controlled, the temperature at which the pH determination of each sample is made should be logged and reported.

When pH measurements are being made, it is critical that the sample be stirred at a constant rate to provide drift-free (<0.1 pH units) measurements. The rate of stirring should minimize air transfer at the surface of the sample. At least 30 seconds should be allowed for each measurement to stabilize.

Calibration and Preventive Maintenance—Calibration procedures for the pH meter should follow specifications of the manufacturer. General guidelines are given in U.S. EPA (1983) and APHA (1989).

Calibration buffers for pH can be purchased as solutions that are traceable to buffer solutions prepared from buffer salts available from the National Institute of Standards and Technology (NIST). The pH meter should be calibrated at a minimum of two points that bracket the expected pH value of the samples and that are three or more pH units apart (U.S. EPA 1983). Prepare fresh buffer solutions at least every month to avoid erroneous calibration as a result of mold growth or contamination.

Preventive maintenance procedures should follow specifications given by the manufacturer of the pH meter. In general, verification of electrode performance and meter performance is the only operator service recommended. Maintenance records should document all maintenance activities including performance checks and equipment replacement. An electrode should be replaced when it no longer meets span requirements and does not improve with rejuvenating procedures.

Quality Control Checks—The pH meter should be calibrated at the beginning of each series of samples and after each group of 10 successive measurements. It is recommended that duplicate pH determinations be made on at least 10 percent of the total number of samples. As an independent check, an EPA reference sample should be analyzed at a minimum of every 3 months.

Corrective Action—If the pH meter does not appear to be operating correctly, consult the manufacturer's troubleshooting guide. Some common problems include a dirty electrode, failure to fill the reference portion of the electrode with internal solution, and inadequate stirring.

Data Quality and Reporting—A precision of ±0.02 pH unit and an accuracy of ±0.05 pH unit can be achieved under the best circumstances. However, the limit of accuracy under most circumstances is ±0.1 pH unit (APHA 1989). A precision of 0.1 pH unit is considered acceptable (U.S. EPA 1983).

Measurements of pH are reliable only when the instrument has been calibrated by standard buffers bracketing the desired range. Samples having a pH greater than 10 may require a special probe to correct for "sodium" error. However, pH values as high as 10 are not likely to be encountered in most coastal waters.

It is recommended that pH values be reported to the nearest 0.1 unit. In addition, the ambient temperature at the time of measurement of each sample should be reported to the nearest degree Celsius. Results of all determinations should be reported, including quality assurance replicates. Any factors that may have influenced sample quality should also be reported.

SALINITY

Field Procedures

Collection—The bottles used for collecting salinity samples should be made of borosilicate glass and have an airtight septum stopper. Soft glass bottles, polyethylene bottles, and ground glass stoppers should not be used.

During storage and prior to use, the collection bottles should be "seasoned" by filling them with seawater. Bottles should remain upside down in the case until the sample is taken. Bottles with chipped edges or loose caps should not be used.

Each collection bottle and cap should be rinsed at least three times with sample water before the sample is collected. No salt crystals should remain on the bottle or stopper. The bottle and stopper should not be contaminated by contact with any surface. If contamination occurs, the rinsing step should be repeated.

After the bottle and stopper have been rinsed thoroughly, the bottle should be filled to the shoulder of the bottle with sample (leaving headspace) and sealed. The stopper should be double-checked for a tight fit. The external label on each bottle should be filled out completely.

Processing—No reagents are necessary to preserve the salinity samples. Bottles should be stored upright after samples have been collected. If necessary, properly sealed salinity samples can be stored indefinitely before analysis (Parsons et al. 1984).

Laboratory Procedures

Analytical Procedures—Analytical procedures are given in APHA Method 2520 B. It is recommended that salinity determinations be made using a salinometer (including a wheatstone bridge) . The salinometer manufacturer should provide a detailed description of how to use the instrument. To avoid heating the sample bottles, they should be propped up rather than gripped by hand during the salinity determination. The sample cell bowl should be filled slowly to avoid introducing bubbles. The cell should be rinsed thoroughly with sample water before each sample is analyzed. Prior to analysis, samples should be allowed to equilibrate to close to the temperature of the salinometer water bath. Samples should also be shaken vigorously prior to analysis to eliminate potential stratification.

Calibration and Preventive Maintenance—Calibration procedures should follow the specifications given by the manufacturer of the salinometer. It is recommended that the primary standard be Copenhagen seawater and that all secondary standards be based on this primary

standard. Secondary standards should consist of filtered seawater and should be periodically checked against the primary standard to guard against contamination or drift. The secondary standards should be equilibrated to the temperature of the samples before calibration begins. Secondary standards should be stored in glass containers and protected from evaporation. The calibration standards should span the range of salinity expected to be encountered in a study.

Preventive maintenance procedures should follow the salinometer manufacturer's recommendations. These include periodic cleaning of the sample cell bowl, greasing the threads on the water-trap jar, tightening all water connections, checking the temperature circuit calibration, and lubricating the pump, pump motor, and stirrer motor. It is critical that the sample cell bowl be kept clean. Normally, the bowl should be cleaned daily. However, if the sample water is very dirty, hourly cleaning may be necessary.

Quality Control Checks—Two standards should be analyzed before the start of each series of samples. In addition, one standard should be analyzed after each group of 10 successive samples to monitor instrument drift. It is recommended that duplicate determinations be made for at least 10 percent of the samples analyzed.

Corrective Action—If the salinometer does not appear to be operating properly, the manufacturer's troubleshooting guide should be consulted. Several common problems include failure of the null indicator to show a deflection, failure of the thermometer circuit, excessive salinity balance drift, inability to fill the sample cell completely, and failure of the stirrer to operate properly.

Data Quality and Reporting—A precision of ±0.1 ppt is possible using an induction salinometer. Conductivity measurements should be converted to salinity values using standard tables corrected for temperature. Salinity concentrations should be reported in ppt to the nearest 0.01 unit. Results of all determinations should be reported, including quality assurance replicates and standards. Any factors that may have influenced sample quality should also be reported.

TEMPERATURE

Field Procedures

Collection—Temperature can be measured using a mercury-filled Celsius thermometer or a digital thermometer on samples collected in glass or plastic containers. The former kind of thermometer should have a scale etched on capillary glass for 0.1°C increments and a minimal thermal capacity to permit rapid equilibration. Temperature can be measured *in situ* using a reversing thermometer or a thermistor. Of these two *in situ* instruments, the thermistor is usually more accurate, but also more expensive.

Processing—Because temperature can change rapidly after a sample is removed from ambient conditions, temperature determinations by thermometer should be made immediately after sample collection. However, temperature of samples from deep water under stratified conditions should be measured *in situ*. When using a reversing thermometer, the thermometer should be allowed to equilibrate to ambient conditions before it is inverted in the water column. The thermometer should also be allowed to equilibrate on board the sampling vessel before temperature measurements are made.

Laboratory Procedures

Analytical Procedures—Methods for making temperature measurements are described in EPA Method 170.1 and in APHA Method 2550 B. It is critical that the measuring device be adequately immersed in the sample and allowed to completely equilibrate (i.e., the temperature reading stabilizes) before temperature is determined. The sample should be agitated if temperature stratification within the sample is suspected.

Calibration and Preventive Maintenance—Each kind of temperature-measuring instrument should be calibrated frequently against an NIST-certified thermometer that is used with its certificate and correction chart. To prevent breakage, it is recommended that each thermometer be enclosed in a metal case. If a mercury thermometer is broken, samples or containers in the vicinity of the exposed area may be contaminated by the mercury.

Quality Control Checks—Each temperature-measuring instrument should be periodically calibrated against an NIST standard thermometer.

Corrective Action—If the temperature-measuring instrument cannot be calibrated consistently against the NIST thermometer, it should be repaired or replaced.

Data Quality and Reporting—Precision and accuracy have not been determined for temperature measurements (U.S. EPA 1983). If possible, temperature measurements should be reported to the nearest 0.01°C. Results of all determinations should be reported, including quality assurance replicates and standard checks. Any factors that may have influenced sample quality should also be reported (e.g., unusually hot or cold air temperature, proximity to a river plume or other kind of discharge).

TRANSPARENCY

Transparency is measured using a Secchi disk. This device is usually a circular plate with a standard diameter of 30 cm (although plates having a diameter of 20 cm are also used). The top side of the disk should be white. A ring attached at the center of the disk allows a graduated line to be secured. A 2-4 kg weight should be attached centrally to the underside of the disk to ensure that the device will sink rapidly and vertically. The deployment line should be made of material that will not stretch or shrink substantially after repeated use (e.g., braided Dacron).

Transparency measurements should be made by lowering the Secchi disk from the shaded side of the survey vessel until the disk is barely perceptible. This depth should be recorded to the nearest 0.5 meters and the disk should continue to be lowered until it is no longer visible. The disk should then be raised slowly until it is again barely visible. This second depth should also be recorded. The average of the two depth readings (i.e., downward and upward) should be reported to the nearest 0.5 meters as the measured transparency value.

Because Secchi disk readings are dependent upon the available illumination, they vary with time of day, cloud formation, and cloud cover. Secchi disk readings may also vary with the observer because of interpersonal differences in visual acuity. Thus, to standardize these readings, repeated measurements should be made by one individual under similar conditions of illumination. Because these criteria are not always achievable, associated meteorological data at the time of measurement and the name of the person making the determination should be included on the log sheet with the Secchi disk readings.

TURBIDITY

Field Procedures

Collection—Turbidity samples can be collected in glass or plastic containers. Samples should be removed from the sampler as soon as possible after collection to minimize settling of suspended material within the sampler. Sample containers and lids should be rinsed thoroughly with sample water before samples are collected. A gross approximation of turbidity can be made *in situ* using an appropriately calibrated transmissometer. A discussion of such *in situ* instrumentation is provided earlier in the general methods section.

Processing—Because turbidity samples cannot be preserved adequately, they should be analyzed as soon as possible after collection. If a delay occurs, samples should be held at 4°C for no more than 24 hours to minimize microbial decomposition of solids. The length of delay should be noted on the log sheet.

Laboratory Procedures

Analytical Procedures—The nephelometric method is described in EPA Method 180.1 and in APHA Method 2130 B. For turbidities greater than 40 nephelometric turbidity units (NTU) (an unlikely value for most of Puget Sound), samples should be diluted with one or more volumes of turbidity-free water until the turbidity falls below 40 NTU. Turbidity-free water is distilled water passed through a membrane filter with a 0.2-µm pore size. Samples should be shaken vigorously to thoroughly disperse solids, and resulting air bubbles should be allowed to dissipate before the sample is analyzed.

Interference with turbidity measurements arises from several sources. Because the presence of floating debris and coarse sediments that settle out rapidly will give low readings, readings should be made as soon as possible after sample agitation. Small air bubbles will tend to result in high readings. If present, the bubbles should be allowed to dissipate before a reading is made. Finally, dissolved substances that absorb light in the sample will interfere with turbidity readings. Any color in the sample should therefore be noted.

Calibration and Preventive Maintenance—It is recommended that a standard suspension of formazin be used to calibrate the nephelometer. Formazin provides a more reproducible turbidity standard than do other materials used in the past. The formazin standard suspension should be prepared daily (APHA 1989). Commercially available standards such as styrene divinylbenzene beads (trade name AMCO-AEPA-1) can be substituted for formazin if they are demonstrated to be equivalent to freshly prepared formazin (APHA 1989). Standards measured on the nephelometer should cover the range expected for the samples. The instrument should give stable readings in all sensitivity ranges used. Calibration curves should be prepared for each instrument range to be used. This information should be included with the maintenance records.

Quality Control Checks—The nephelometer should be calibrated at the start of each series of analyses and between all samples. Duplicate analyses should be conducted on at least 10 percent of the total number of samples.

Corrective Action—If the nephelometer will not stabilize in any of the relevant ranges or if the instrument does not appear to be functioning properly in any other aspect, the manufacturer's troubleshooting guide should be consulted. Sample tubes that become scratched or etched should be replaced.

Data Quality and Reporting—Limited precision data indicate that standard deviations of measurements vary directly with the level of turbidity (APHA 1989). Accuracy data are not available at present. The sensitivity of the nephelometer should allow detection of a turbidity difference of 0.05 units or less in waters with turbidities less than 1.0 unit. Results should be reported in NTU. APHA (1989) recommends the nearest reporting unit as a function of the range of measured values as follows:

NTU	Record to Nearest
0.0-1.0	0.05
1-10	0.1
10-40	1
40-100	5
100-400	10
400-1,000	50
>1,000	100

Results of all determinations should be reported, including quality assurance replicates. Any factors that may have influenced sample quality should also be reported (e.g., disturbed bottom sediment, proximity to a river plume).

TOTAL SUSPENDED SOLIDS

Field Procedures

Collection—Samples should be collected in glass or plastic bottles. Samples should be collected soon after the sampler is brought on board to minimize settling of suspended material within the sampler. Nonrepresentative particulates such as leaves and sticks should be noted, and then excluded from the sample.

Processing—Total suspended solids (TSS) samples cannot be preserved adequately and should therefore be analyzed as soon as possible after collection (APHA 1989). If a delay occurs, samples should be held at 4°C to minimize microbiological decomposition of solids. The length of delay should not exceed 7 days and should be noted on the log sheet.

Laboratory Procedures

Analytical Procedures—Suspended solids determination should be made according to procedures described in EPA Method 160.2 and APHA Method 2540 D. These procedures entail the measurement of material retained on filters. However, a 0.40- or 0.45-µm membrane filter should be used to remove suspended solids instead of the glass fiber filter specified in the EPA and APHA methods, because the pores of the membrane filters are more uniform in size than those of the glass fiber filters. Membrane filters are among those commonly used for oceanographic work in coastal waters. However, if ashed weights are to be determined, a membrane filter cannot be used.

The drying temperature of the filtered residue can influence results because temperature and time of heating affect weight losses due to volatilization of organic matter, mechanically occluded water, water of crystallization, and gasses from heat-induced chemical decomposition, as well as weight gains due to oxidation (APHA 1989). Thus, drying temperature must be carefully controlled and not allowed to deviate from the recommended range of 103-105°C. It is recommended that drying time be uniform and not exceed 6 hours.

To avoid contamination, filters should be handled with forceps during all steps from initial to final weight determinations. Filters should always be stored in a desiccator when cooling. When filtering the samples, it is critical that the filter is sealed tightly on the surface of the filtration apparatus and that all holes in the filter holder are covered. To ensure complete removal of salts after filtering the sample, the filter should be rinsed by filtering a minimum of three successive 20-mL portions of distilled water. It is recommended that enough sample be filtered to ensure that at least 5 mg of residue is collected. Because excessive residue on the filter may form a water-entrapping crust, the sample size should be limited to that which yields less than 200 mg of residue (APHA 1989). Prolonged filtration times resulting from filter clogging may produce high results due to the capture of particles that would pass through an unclogged filter. Therefore, filtering should be terminated before any evidence of clogging is noted.

Calibration and Preventive Maintenance—The analytical balance should be calibrated weekly using standard weights according to the manufacturer's instructions. It is recommended that the balance have a minimum accuracy of 0.1 mg. The manufacturer's preventive maintenance procedures should be followed carefully.

Quality Control Checks—For each TSS weight determination, filters should be run repeatedly through the drying/cooling cycle until the weight loss is less than 4 percent of the previous weight or 0.5 mg, whichever is less (APHA 1989). Duplicate analyses should be conducted on at least 10 percent of the total number of samples. Two filter blanks should be taken through the preparation, drying, and desiccation steps for each batch of samples to monitor changes in filter tare weight. If necessary, blank corrections should be applied to data in the range of 0-10 mg/L. EPA reference samples should be analyzed quarterly to check the overall accuracy of the method.

Corrective Action—If the analytical balance will not produce repeatable measurements within 0.1 mg, the manufacturer's troubleshooting guide should be consulted. If the filter becomes clogged during filtration, it should be discarded and the analysis should be repeated using a clean filter. To prevent clogging of the second filter, the volume of sample analyzed should be reduced.

If the weight of the filter blank is not consistent after drying and cooling (4 percent of previous weight or 0.5 mg, whichever is less), the desiccant should be checked. A color-indicating desiccant is recommended, so that spent desiccant is easily detected. Also, the seal on the desiccator should be checked and, if necessary, ground glass rims should be greased or "O" rings replaced. Cooling time should be closely monitored, so weighing times are consistent between batches of samples, thereby minimizing the need for multiple weighings.

Data Quality and Reporting—Precision of results varies directly with the concentration of suspended matter and, at low levels, the ratio of the weight of the suspended matter to the weight of the filter. There are no procedures for determining the accuracy of field measurements of suspended matter. Total suspended solids measurements should be reported as mg/L to a minimum of two significant figures. Results of all determinations should be reported including quality assurance replicates and filter blanks. Any factors that may have influenced sample quality should also be reported.

DISSOLVED OXYGEN (MODIFIED WINKLER METHOD)

Field Procedures

Collection—Prior to sample collection, the fixing reagents should be prepared and the dispensing apparatus should be filled. Because the fixing agents are sensitive to light, they should be stored in dark bottles. The accuracy of the volumes being dispensed should be checked and no air should be trapped in the system. Packets of premeasured reagents can be used instead of the dispensing apparatus if it is demonstrated that use of the packets provides results comparable to those achieved using the dispensing apparatus. It is recommended that 125-mL glass BOD bottles or 125-mL Erlenmeyer flasks with ground glass stoppers be used for the modified Winkler method.

Oxygen samples must be the first ones collected from the sampler, and they should be collected immediately after the sampler is brought on board. Temperature and salinity should be measured in conjunction with all oxygen measurements to allow calculation of percent saturation. Collection of samples should be conducted by experienced personnel, because most errors in measuring this variable are the result of poor collection techniques. It is recommended that a piece of soft-walled rubber tubing with a glass tip be connected to the discharge valve of the sampler to prevent air bubbles from contaminating the sample during collection. The tubing should be soaked in seawater prior to use to prevent air bubbles from collecting inside.

After being attached to the sampler, the tubing should be flushed with sample water to remove air bubbles. The sample bottle and stopper should then be rinsed thoroughly with sample water. After rinsing, the tubing should be inserted to the bottom of the sampling bottle. The bottle should be filled slowly until at least half full, and then filled rapidly thereafter. A least one full bottle volume of sample should overflow the bottle before the tubing is removed. After the tubing is removed slowly, the stopper should be carefully put in place with a twisting motion while water is displaced from the bottle. Once stoppered, the sample should be checked for air bubbles. If bubbles are present, the sample should be discarded and a new sample collected. Acceptable samples should be fixed as soon as possible after collection.

Processing—The stopper should be carefully removed from the bottle without agitating the sample. Each fixing reagent (i.e., manganous chloride and alkaline iodide) should be added by gently placing the tip of the pipette slightly below the surface of the sample and gently pushing the plunger. The plunger should not be released until the pipette has been removed from the sample. The pipette tip should be rinsed with distilled water before being returned to the reagent bottle.

After the fixing reagents have been added, the bottle should be carefully stoppered without introducing air bubbles. Excess fluid around the outside of the stopper should be poured off and the sample bottle should be inverted 5-10 times to thoroughly disperse the precipitate. After the precipitate has settled, the sample bottle should be inverted another 5-10 times.

After allowing the precipitate to settle for 10-15 minutes, the stopper should be removed and sulfuric acid should be added to the sample in the same manner as the fixing reagents. The stopper should then be replaced and the bottle inverted several times until all of the precipitate has dissolved. If the precipitate fails to dissolve, it should be allowed to settle again and additional sulfuric acid should be added to the sample. It is critical that all of the precipitate be dissolved before samples are stored. Also, it is critical that samples not be allowed to stand longer than 8 hours before sulfuric acid is added, as erroneous measurements may result.

Preserved dissolved oxygen samples should be stored in the dark (i.e., to prevent color from bleaching in sunlight) at 10-20°C. Fixed samples should be analyzed as soon as possible after collection, and it is recommended that storage time should not exceed 8 hours. The length of storage should be recorded on the log sheet.

Laboratory Procedure

Analytical Procedures—The recommended modified Winkler method is described in detail in Parsons et al. (1984). The method is applicable for dissolved oxygen concentrations of 0.1-10 mg/L. The method involves the addition of divalent manganese and strong alkali solutions to the sample. The precipitated manganous hydroxide is dispersed evenly throughout the sample, and any dissolved oxygen rapidly oxidizes an equivalent amount of divalent manganese to basic hydroxides of higher valency states. The solution is then acidified in the presence of iodine, the oxidized manganese reverts to the divalent state, and iodine (equivalent to the original dissolved oxygen content of the sample) is liberated. The amount of the iodine is then measured by titration with standardized thiosulfate solution. The titration equipment should be capable of making duplicate measurements of standards that agree within ±0.05 mg/L. An alternate method of measuring the amount of iodine is through use of a spectrophotometer.

Calibration and Preventive Maintenance—Methods of standardizing the thiosulfate solution are presented by Parsons et al. (1984). It is recommended that one person perform the standard and sample titrations for each set of samples because of subjectivity in the color of the endpoint.

Preventive maintenance is generally limited to ensuring that reagent dispensing and titrating equipment are clean and function properly. Thiosulfate should be stored in a dark bottle to prevent light-induced changes. Thiosulfate solution that has remained in the buret for longer than 2 hours should be replaced.

Quality Control Checks—It is recommended that triplicate thiosulfate titration analyses be conducted on at least 10 percent of the total samples. Four replicate reagent blanks should be run daily during a cruise, or whenever a reagent is changed.

Corrective Action—If any of the results obtained by running triplicate standard titrations of the thiosulfate solution do not agree within ±0.05 mL, the titrations should be repeated until agreement is achieved. All reagent dispensers should be checked for bubbles, and the amounts of reagents delivered should be verified.

Any substances with oxidizing or reducing potential are possible sources of interference with measurements of dissolved oxygen. Examples include nitrite, ferrous iron, and organic matter. If problems related to these substances are suspected, Parsons et al. (1984) provide references that describe precautions that can be taken.

Data Quality and Reporting—Using the modified Winkler method, reproducibility for field samples is approximately 0.2 mg/L of dissolved oxygen at the 7.5 mg/L level (U.S. EPA 1983). Duplicate titrations made during standardization of reagents should agree within ±0.05 mL. With careful collection and treatment of samples, dissolved oxygen as low as 1 percent of saturation can be measured. Dissolved oxygen concentrations should be reported in mg/L to the nearest 0.1 unit. Results should be reported for all determinations, including quality assurance replicates and reagent blanks. Any factors that may have influenced sample quality should also be reported (e.g., bubbles in sample, all reagent not dissolved or used).

DISSOLVED OXYGEN (PROBE METHOD)

Field Procedures

Collection—Water samples for oxygen measurements should be the first ones collected from the sampler, and they should be collected immediately after the sampler is brought on board. Temperature and salinity should be measured in conjunction with all oxygen measurements to allow calculation of percent saturation. Collection of samples should be conducted by experienced personnel, because most errors in measuring this variable are the result of poor collection techniques. It is recommended that a piece of soft-walled rubber tubing with a glass tip be connected to the discharge valve of the sampler to prevent air bubbles from contaminating the sample during collection. The tubing should be soaked in seawater prior to use to prevent air bubbles from collecting outside.

After being attached to the sampler, the plastic or rubber tubing should be flushed with sample water to remove air bubbles. The sample bottle and stopper should then be rinsed thoroughly with sample water. After rinsing, the tubing should be inserted to the bottom of the sampling bottle. The bottle should be filled slowly until at least half full, and then filled rapidly thereafter. At least one full bottle volume of sample should overflow the bottle before the tubing is removed. After the tubing is slowly removed, the stopper should be carefully put in place with a twisting motion while water is displaced from the bottle. Once stoppered, the sample should be checked for air bubbles. If bubbles are present, the sample should be discarded and a new sample collected.

Processing—Because no reagents are used to preserve the oxygen samples, analyses should be conducted immediately after collection (i.e., within 15 minutes). If a delay occurs, it should be noted on the log sheet.

Laboratory Procedures

Analytical Procedures—Detailed analytical procedures should be provided by the manufacturer of the dissolved oxygen meter. General procedures are listed in EPA Method 360.1 and APHA Method 4500-0 G. The data provided by this method are generally lower in quality than the data provided by the modified Winkler method. The probe method is not commonly used for oceanographic studies in which measurements are made on discrete samples of seawater. Probes are used mainly on *in situ* instruments for providing continuous water-column profiles of dissolved oxygen.

Several precautions should be taken when making measurements with a membrane electrode. First, constant turbulence should be provided by a stirrer or pump to ensure precise measurements. Second, adequate time should be allowed for the instrument to warm up before measurements are started and, when individual samples are analyzed, for the probe to stabilize to sample temperature and dissolved oxygen. Third, reactive gases, such as chlorine and hydrogen sulfide, pass through the membrane probes and may interfere with the analysis or desensitize the probe. Finally, broad variations in the kinds and concentrations of salts in samples can influence the partial pressure of oxygen in samples and thereby affect measurement accuracy.

Calibration and Preventive Maintenance—Calibration procedures should follow the instruction manual for the dissolved oxygen meter. The meter generally can be calibrated using one of four methods: Winkler titration, saturated water calibration, sodium sulfite zero calibration, or air calibration. The air method is simplest, but the Winkler method is preferred. Overall error is diminished when the probe and instrument are calibrated under conditions of temperature and dissolved oxygen that match those of the samples. Calibration can be disturbed by physical shock, touching the membrane, or desiccation of the electrolyte.

Preventive maintenance procedures should follow the manufacturer's recommendations. The oxygen probe should always be stored in a humid environment to prevent drying out and the need to frequently replace membranes.

Quality Control Checks—The instrument should be calibrated at the beginning of each series of measurements and after each group of 10 successive samples. Duplicate measurements should be made on at least 10 percent of the total number of samples.

Corrective Action—If the dissolved oxygen meter does not appear to be operating correctly, consult the manufacturer's troubleshooting guidelines for remedial actions.

Data Quality and Reporting—Repeatability of dissolved oxygen measurements using a membrane electrode should be 0.1 mg/L and accuracy should be ±1 percent (U.S. EPA 1983). Sensitivity of the electronic readout meter for the output from the dissolved oxygen probes should normally be 0.05 mg/L (U.S. EPA 1983). Dissolved oxygen concentrations should be reported in mg/L to the nearest 0.1 unit. Results should be reported for all determinations, including quality assurance replicates. Any factors that may have influenced sample quality should also be reported.

NITROGEN (AMMONIA)

Field Procedures

Collection—Samples for dissolved ammonia should be filtered in the field and can be collected in glass or plastic containers with leak-proof caps. Prior to collection, each container and cap should be rinsed with sample water. If plastic containers are used more than once, they should be acid-washed to remove bacteria. Filters should be washed in sequence with 10-percent hydrochloric acid and ammonium-free distilled water. Filters should be washed in the laboratory and brought to the field in sealed containers.

Processing—Ammonia samples should be analyzed immediately after collection. However, samples can be stored for up to 7 days by freezing the filtrate at -20°C immediately after collection. It is recommended that stored samples be frozen as quickly as possible, preferably using dry ice or liquid nitrogen. Sufficient headspace should be left in the sample container to accommodate increases in sample volume during freezing.

Laboratory Procedures

Analytical Procedures—The recommended analytical method for ammonia is described by Parsons et al. (1984). The method is applicable for ammonia concentrations of 0.04-10 µg-at N/L. The method involves the oxidation of ammonia in the sample to nitrite with hypochlorite in alkali, using a large excess of potassium bromide as a catalyst. The precipitation of metal hydroxide in saline water in an alkaline medium is prevented by the addition of a complexing reagent prior to the oxidation step. Nitrite produced from the oxidation of ammonia is then determined. Ammonia can also be measured using automated procedures and instruments. The instrument manual should describe all relevant procedures for analyzing samples. Automated procedures are therefore not considered further in this section.

Calibration and Preventive Maintenance—Calibration procedures should follow those specified for the method being used. Calibration should be conducted using filtered seawater in which the concentration of ammonia has been reduced by boiling. Alternatively, calibration can be conducted using deep ocean water that has been depleted of ammonia by phytoplankton. At least three concentrations of the calibration standards should be used, and they should bracket the sample concentrations. If a sample concentration is outside the range of calibration, then an additional calibration standard should be analyzed to check if the result is within the linear range of the method. Alternatively, the sample should be diluted to within the calibration range and then reanalyzed.

Because sample contamination is a particularly common problem for ammonia analyses, the following precautions should be taken:

- Frozen samples should not be stored with potential sources of contamination (e.g., fish, meat, cleaning products)
- The deionized water used for standards and reagents should be passed through a strong cation exchange resin immediately before use (the resin should preferably be in lithium or sodium form)
- All reagents should be stored in well-stoppered containers which have been washed with dilute hydrochloric acid
- All glassware used for the laboratory analysis of ammonia should be acid-washed
- Ammonium hydroxide should not be used in the laboratory during ammonia analysis
- Ammonium-free seawater is desirable in the calibration. If it is not available, then 1 liter of seawater should be boiled after addition of 5 mL of 1*N* NaOH and the volume reduced until it is about 0.7 liters. The volume is replaced to 1 liter with deionized water after neutralization with equivalent HCl and is filtered through a glass fiber filter.

Quality Control Checks—Duplicate analyses should be conducted on a minimum of 5 percent of the total number of samples, with an additional 5 percent of the samples spiked and analyzed for percent recovery. Duplicate seawater and reagent blanks should be analyzed with each batch of samples or every 20 samples (whichever is less). The seawater blank should be comprised of ammonium-free seawater and the reagent blank should consist of high-quality deionized water. At least one (preferably two) check standards prepared independently of the calibration standards should be analyzed with each batch of samples. An EPA performance sample should be analyzed at least once per quarter.

Corrective Action—Contamination of ammonia samples can occur easily (particularly on vessels) due to the volatile nature of ammonia. To prevent possible cross-contamination, reagents should be isolated from samples and standards used for ammonia determinations. In addition, cleaning preparations that contain significant quantities of ammonia (e.g., PineSol®, wax removers, hand cleaners) should not be used in the laboratory area where ammonia determinations are performed. Because tobacco smoke can be a source of ammonia contamination, smoking should not be allowed near samples.

Prior to use, glassware should be rinsed once with 10-percent hydrochloric acid and then three times with distilled water. To check for contamination, blanks should be analyzed whenever a new reagent is prepared. It may also be advisable to designate specific glassware for use with low, moderate, and high concentrations of ammonia.

Data Quality and Reporting—Detection and accurate quantification of ammonia in receiving water is routinely attainable, although method detection limits can vary widely because of methods or instrumentation. The analytical instructions should be consulted to determine expected detection limits, precision, and accuracy. In general, ammonia concentrations in seawater are very low. Data should be reported in µM as N [µM=mg/L×(1,000/14)] to a maximum of three significant figures. Results should be reported for all determinations, including quality assurance replicates and spiked samples. Any factors that may have influenced sample quality should also be recorded.

NITROGEN (NITRITE)

Field Procedures

Collection—Samples for dissolved nitrite should be filtered in the field and can be collected in glass or plastic containers with leak-proof caps. Prior to collection, each container and cap should be rinsed with sample water. If plastic containers are used more than once, they should be acid-washed to remove bacteria.

Processing—Nitrite samples should be analyzed immediately after collection. However, samples can be stored for up to 28 days by freezing the filtrate at -20°C immediately after collection. It is recommended that stored samples be frozen as quickly as possible, preferably using dry ice. Sufficient headspace should be left in the sample container to accommodate increases in sample volume during freezing.

Laboratory Procedures

Analytical Procedures—The recommended analytical method for nitrite is described by Parsons et al. (1984). This method is applicable for nitrite concentrations of 0.01-2.5 µg-at N/L. The method involves the reaction of nitrite in the sample with sulfanilamide in an acid solution. The resulting diazo compound is reacted with N-(l-naphthyl)-ethylenediamine and forms a highly colored azo dye, which can be measured spectrophotometrically. Nitrite can also be measured using automated procedures and instruments. The instrument manual should describe all relevant procedures for analyzing samples. Automated procedures are therefore not considered further in this section.

Calibration and Preventive Maintenance—Calibration procedures should follow those specified in the method. At least three concentrations of the calibration standards (prepared using distilled water) should be used and they should bracket the sample concentrations. If a sample concentration is outside the range of calibration, then an additional calibration standard should be analyzed to check if the result is within the linear range of the method. Alternatively, the sample should be diluted to within the calibration range and reanalyzed.

Quality Control Checks—Duplicate analyses should be conducted on a minimum of 5 percent of the total number of samples, with an additional 5 percent of the samples spiked and analyzed for percent recovery. Duplicate blanks comprised of distilled water should be analyzed with each batch of samples or every 20 samples (whichever is less). At least one (preferably two) check standards prepared independently of the calibration standards should be analyzed with each batch of samples. An EPA performance sample should be analyzed at least once per quarter.

Corrective Action—Various components of a sample can interfere with the analysis. The method should be reviewed for ways to remove possible interferences prior to analysis. Possible interferences include suspended solids, residual chlorine, oil and grease, and high concentrations of iron, copper, or other metals.

The area where nitrite analyses are performed should be well isolated from exposure to nitric acid or nitric acid fumes.

Data Quality and Reporting—The detection and accurate quantification of nitrite in seawater are routinely attainable, although method detection limits can vary because of methods or instrumentation. The analytical method should be consulted to determine expected detection limits, precision, and accuracy. In general, nitrite concentrations in seawater are very low. Data should be reported in µM as N [µM=mg/L×(1,000/14)] to a maximum of three significant figures. Results of all determinations should be reported, including quality assurance replicates, blanks, and spiked samples. Any factors that may have influenced sample quality should also be reported.

NITROGEN (NITRATE)

Field Procedures

Collection—Samples for dissolved nitrate should be filtered in the field and can be collected in glass or plastic containers with leak-proof caps. Prior to collection, each container and cap should be rinsed with sample water. If plastic containers are used more than once, they should be acid-washed to remove bacteria.

Processing—Nitrate samples should be analyzed immediately after collection. However, samples can be stored for up to 28 days by freezing the filtrate at -20°C immediately after collection. It is recommended that stored samples be frozen as quickly as possible, preferably using dry ice. Sufficient headspace should be left in the sample container to accommodate increases in sample volume during freezing.

Laboratory Procedures

Analytical Procedures—The recommended analytical method for nitrate is described by Parsons et al. (1984). This method is applicable for nitrate concentrations of 0.05-45 µg-at N/L. The method involves the quantitative reduction of nitrate to nitrite by running the sample through a column containing cadmium filings coated with metallic copper. The resulting nitrite is then determined by diazotizing with sulfanilamide and coupling with N-(l-naphthyl)-ethylenediamine dihydrochloride to form a highly colored azo dye which can be measured spectrophotometrically. Any nitrite initially present in the sample must be accounted for by a separate nitrite analysis. Nitrate can also be measured using automated procedures and instruments. The instrument manual should describe all relevant procedures for analyzing samples. Automated procedures are therefore not considered further in this section.

Calibration and Preventive Maintenance—Calibration procedures should follow those specified in the method. Efficiency of each reduction column should be checked by comparing a nitrate standard to a nitrite standard at the same concentration. This efficiency check should be made at the beginning and the end of each sample run and at a minimum frequency of every 10 samples. Reactivate the copper-cadmium granules when reduction falls below 95 percent. Standard values should not be compared among different columns because each column may have unique characteristics.

Because of a small salt effect, standard nitrate solutions should be prepared in synthetic seawater or a low nitrate seawater sample should be "spiked" with a standard amount of nitrate. The standard nitrate solution should be prepared fresh before each use.

Because the cadmium column can be destroyed by hydrogen sulfide, samples containing hydrogen sulfide (e.g., from anoxic areas) should be aerated prior to analysis.

Quality Control Checks—Duplicate analyses should be conducted on a minimum of 5 percent of the total number of samples, with an additional 5 percent of the samples spiked and analyzed for percent recovery. Duplicate blanks comprised of dilute ammonium chloride that have been run through the reduction column should be analyzed with each batch of samples or every 20 samples (whichever is less). A blank should be applied only to the column with which it was determined, as each column may have unique characteristics. At least one (preferably two) check standards prepared independently of the calibration standards should be analyzed with each batch of samples. An EPA performance sample should be analyzed at least once per quarter.

Corrective Action—Various components of a sample can interfere with the analysis. The method should be reviewed for ways to remove possible interferences prior to analysis. Possible interferences include suspended solids, residual chlorine, oil and grease, and high concentrations of iron, copper, or other metals.

The area where nitrate analyses are performed should be well isolated from exposure to nitric acid or nitric acid fumes.

Data Quality and Reporting—The detection and accurate quantification of nitrate in seawater are routinely attainable, although method detection limits can vary because of methods or instrumentation. The analytical method should be consulted to determine expected detection limits, precision, and accuracy. Data should be reported in µM as N [µM=mg/L×(1,000/14)] to a maximum of three significant figures. Results of all determinations should be reported, including quality assurance replicates, blanks, and spiked samples. Any factors that may have influenced sample quality should also be reported.

PHOSPHATE

Field Procedures

Collection—Samples for dissolved phosphate should be filtered in the field and can be collected in glass or plastic containers with leak-proof caps. Containers should be rinsed with 1*N* HCl followed by several rinses with distilled water. Detergents containing phosphate (most detergents contain phosphate) should never be used on containers or other laboratory equipment that will be used for phosphate analysis. Sample containers and lids should be rinsed thoroughly with sample water before sample collection.

Processing—Phosphate samples should be analyzed immediately after collection. However, samples can be stored for up to 28 days by freezing the filtrate at -20°C immediately after collection. It is recommended that stored samples be frozen as quickly as possible, preferably using dry ice. Sufficient headspace should be left in the sample container to accommodate increases in sample volume during freezing.

Laboratory Procedures

Analytical Procedures—The recommended analytical method for phosphate is described by Parsons et al. (1984). This method is applicable for phosphate concentrations of 0.03-5 µg-at/L. The method involves the reaction of the sample with a composite reagent containing molybdic acid, ascorbic acid, and trivalent antimony. The resulting complex is reduced to yield a blue solution that is analyzed spectrophotometrically. Phosphate can also be measured using automated procedures and instruments. The instrument manual should describe all relevant procedures for analyzing samples. Automated procedures are therefore not considered further in this section.

Calibration and Preventive Maintenance—Calibration procedures should follow those specified in the method. At least three of the calibration standards should be used, and they should bracket the sample concentrations. If a sample concentration is outside the range of calibration, then an additional calibration standard should be analyzed to check if the result is within the linear range of the method. Alternatively, the sample should be diluted to within the calibration range and reanalyzed.

Quality Control Checks—Duplicate analyses should be conducted on a minimum of 5 percent of the total number of samples, with an additional 5 percent of the samples spiked and analyzed for percent recovery. Duplicate blanks consisting of distilled water should be analyzed with each batch of samples or every 20 samples (whichever is less). At least one (preferably two) check standards prepared independently of the calibration standards should be analyzed with each batch of samples. An EPA performance sample should be analyzed at least once per quarter.

Corrective Action—Because phosphorus contamination can occur from a variety of sources, it is recommended that a clearly marked set of labware be dedicated to only phosphorus analysis. This labware should never be exposed to phosphorus detergents or reagents containing phosphate. If high blank values are experienced, the ammonium molybdate should be made up again and tested.

Various components of a sample can interfere with the analysis. The method should be reviewed for ways to remove interferences or adjust for interferences from components that cannot be removed. Silica and arsenic are possible positive interferences, while hexavalent chromium and nitrite can cause low recovery.

For highly colored or turbid samples, additional sample preparation (e.g., further filtration) may be required prior to color development. In any case, blanks should be prepared by adding all the reagents except the coloring reagents to the sample. Measure absorbance in the sample blank at the wavelength used for the phosphorus determination and subtract this absorbance value from the sample absorbance prior to calculation of phosphorus concentration.

Quality Control and Reporting—Detection and accurate quantification of phosphate in seawater is routinely attainable. Actual method detection limits can vary because of methods or instrumentation. The analytical method should be consulted to determine expected detection limits, precision, and accuracy. All glassware used for phosphate analysis should be cleaned with hot 1:1 hydrochloric acid. If the glassware is rinsed thoroughly after each use, the hydrochloric-acid treatment is needed only occasionally. Data should be reported in µM as P [µM = mg/L×(1,000/31)] to a maximum of three significant figures. Results of all determinations should be reported, including quality assurance replicates and spiked samples. Any factors that may have influenced sample quality should also be reported.

SILICATE

Field Procedures

Collection—Samples for dissolved silicate should be filtered in the field and should be collected in plastic containers. Glass containers (particularly borosilicate glass) should not be used. Prior to collection, each container should be rinsed thoroughly with sample water.

Processing—Silicate samples should be analyzed immediately after collection. However, samples may be stored for up to 28 days by freezing the filtrate at -20°C immediately after collection. If the silica concentration of samples exceeds 100 µM (e.g., near freshwater sources), the samples should not be frozen and should be analyzed as soon as possible. It is recommended that stored samples be frozen as quickly as possible, preferably using dry ice. Sufficient headspace should be left in the sample container to accommodate increases in sample volume during freezing.

Laboratory Procedures

Analytical Procedures—The recommended analytical method for silicate is described by Parsons et al. (1984). The method is applicable for silicate concentrations of 0.1-140 µg-at/L. The method involves the reaction of the sample with molybdate under conditions that result in the formation of silicomolybdate, phosphomolybdate, and arsenomolybdate complexes. A reducing solution containing metal and oxalic acid is then added to reduce the silicomolybdate complex and give a blue color. Simultaneously, any phosphomolybdate or arsenomolybdate in the sample is decomposed. The resulting extinction is then measured spectrophotometrically. Silicate can also be measured using automated procedures and instruments. The instrument manual should describe all relevant procedures for analyzing samples. Automated procedures are therefore not considered further in this section.

Calibration and Preventive Maintenance—Calibration procedures should follow those specified by Parsons et al. (1984). The standard silicate solution should be stored in a plastic container and mixed with synthetic seawater to achieve the desired concentrations. At least three concentrations of the calibration standards should be used, and they should bracket the sample concentrations. If a sample concentration is outside the range of calibration, an additional calibration standard should be analyzed to determine if the result is within the linear range of the method. Alternatively, the sample should be diluted to within the calibration range and reanalyzed.

Quality Control Checks—Duplicate analyses should be conducted on a minimum of 5 percent of the total number of samples, with an additional 5 percent of the samples spiked and analyzed for percent recovery. A blank consisting of distilled water collected in a plastic container should be analyzed with each batch of samples or every 20 samples (whichever is less). At least one (preferably two) check standards prepared independently of the calibration standards should be analyzed with each batch of samples.

Corrective Action—Various components of a sample can interfere with the analysis. The method should be reviewed for ways to remove possible interferences prior to analysis. Possible interferences include excessive coloration or turbidity and large amounts of iron and sulfide. Sample contact with glass should be minimized and silica-free reagents should be used as much as possible.

Data Quality and Reporting—The detection and accurate quantification of silicate in seawater are routinely attainable, although method detection limits can vary depending on methods and instrumentation. The analytical method should be consulted to determine expected detection limits, precision, and accuracy. Data should be reported in µM as Si [µM=mg/L×(1,000/28.1)] to a maximum of three significant figures. Results of all determinations should be reported, including quality assurance replicates, blanks, and spiked samples. Any factors that may have influenced sample quality should also be reported.

CHLOROPHYLL *a*

Field Procedures

Collection—Chlorophyll *a* samples can be collected in glass or plastic containers. Because acids will decompose chlorophyll *a* to phaeo-pigments, it is critical that the water sampler and sample containers remain free of acids (including acids from fingerprints). In addition, sample containers should be rinsed three times before sample collection.

Processing—Chlorophyll *a* samples should be filtered immediately after collection. Two or three drops of magnesium carbonate suspension should be added to the filter before filtration to prevent the sample from becoming acidic (which causes chlorophyll *a* to decompose). Filters can be stored for up to 30 days by holding them in the dark in a desiccator at -20°C (Parsons et al. 1984). However, storage usually leads to low results and makes the extraction of chlorophyll more difficult. It is therefore recommended that filters be extracted immediately after filtration. If filters are stored, the length of delay until analysis should be recorded on the log sheet.

Laboratory Procedures

Analytical Procedures—Chlorophyll *a* determinations using the spectrophotometric and fluorometric methods are described in detail in Parsons et al. (1984). It is recommended that chlorophyll *a* concentrations be determined using the fluorometric method rather than the spectrophotometric method, because the former technique is 5-10 times more sensitive, requires less sample, and can be used for *in vivo* measurements (APHA 1989). However, the fluorometric method may be less accurate than the spectrophotometric method. Although the fluorometric method does not distinguish between chlorophyll *a* and *b*, the chlorophyll *b*:*a* ratio in seawater is usually well below the value (i.e., 0.2) at which chlorophyll *b* will affect measurement of chlorophyll *a*.

All work with chlorophyll extracts should be conducted in subdued light to avoid degradation. Opaque containers or containers wrapped in aluminum should be used to protect samples from light. Glass fiber filters (Grade GF/F) with a pore size of approximately 0.5 µm are recommended because they are inexpensive and result in a low blank (Parsons et al. 1984). To improve extraction efficiency, a sonification or cell-grinding step should be included prior to extraction. For sonification, the filter should be placed in a tube with 90-percent acetone, immersed in a water/ice bath, sonicated for 7 minutes, extracted for 10 minutes, shaken, centrifuged, and analyzed.

Calibration and Preventive Maintenance—The fluorometer should have a red-enhanced photodetector and the appropriate filters. The fluorometer should be calibrated spectrophotometrically using samples from the same standard chlorophyll solution. This solution should have a known concentration of chlorophyll *a* that was extracted from a marine phytoplankton. Parsons et al. (1984) recommend that a mixed culture of equal amounts (by pigment) of *Skeletonema costatum, Coccolithus huxleyii,* and *Peridinium trochoidium* be used as the sources of chlorophyll. If natural phytoplankton populations are used, phaeopigments may be present.

A series of dilutions of the standard chlorophyll solution should be made so that concentrations of 2, 6, 20, and 60 µg/L are achieved. Readings of each dilution should be made at sensitivity settings of 1x, 3x, 10x, and 30x and linearity should be checked for each scale. This will allow derivation of calibration factors to convert fluorometric readings in each sensitivity level to chlorophyll *a* concentrations (for details, see APHA 1989).

Quality Control Checks—To correct for scatter, the fluorometer should be zeroed against a cuvette of 90-percent acetone for each sample. Duplicate analyses should be conducted on at least 10 percent of the total number of samples.

Corrective Action—If the fluorometer does not appear to be functioning properly, the manufacturer's troubleshooting guide should be consulted. Scratched or etched cuvettes should be replaced. Care should be taken to keep fingerprints off the cuvette. All acid should be well rinsed from the cuvette between samples to avoid creating an erroneously low initial fluorescence reading for the later samples.

Data Quality and Reporting—Precision of the recommended method varies as a function of the amount of pigment being measured. For chlorophyll *a* concentrations exceeding 0.5 mg/m^3, a precision of ±8 percent is possible. The sensitivity of detection has been estimated as 0.01 mg/m^3 for a 2-liter sample (Parsons et al. 1984). Chlorophyll *a* concentrations should be reported as mg/m^3 or µg/L to the nearest 0.01 units. Results of all determinations should be reported, including quality assurance replicates. Any factors that may have influenced sample quality should also be reported (e.g., time of filtering, types of filters).

TOTAL AND FECAL COLIFORM BACTERIA

The recommended protocols for total and fecal coliform bacteria are included in the PSEP microbiology protocols (PSEP 1986). However, the primary source of information should be APHA (1989) rather than the earlier edition of that document (i.e., 1985) cited in PSEP (1986).

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