PHYTOPLANKTON METHODS SUMMARY

I. Phytoplankton Sampling

Phytoplankton – defined as:

- minute, free-floating aquatic plants;
- photosynthetic or plant constituent of plankton; mainly unicellular algae.

Appendix A consists of the following:

1) *FDEP protocols* in Phytoplankton collection, including the equipment and supplies required for sampling, method description, sample preservation and handling, and laboratory quality control for sample identification.
2) Phytoplankton Methods Summary Table, and
3) Methods description

FDEP Protocols:

FS 7100 - Phytoplankton Sampling is outlined in DEP-SOP-001/01. For more information on sample collection, see also FS 2100 (Surface water sampling). Refer also to QASR – **Appendix B**, for discussion on equipment use and construction and sample preservation, handling and holding times, if applicable.

1) Equipment and Supplies

- 1 L Nalgene wide-mouth dark sample bottle
- Van Dorn Bottle
- Lugol's solution
- Buffered formalin
- Cooler with ice

2) Methods

1) Rinse the Nalgene sample bottle three times with ambient water. When sampling from a boat, rinse the bottle on the side of the boat opposite from where samples are collected in order to avoid disturbance of the surface algal community. When not sampling from a boat, collect samples upstream from where you are standing.

2) When the algae are mostly near the surface, collect the grab sample by scooping the bottle through the top 0.3 m of the surface water. Swirl the bottle to evenly mix the contents. Collect samples from the top 0.3 meters to estimate algal populations that would represent the "worst case" scenario. Algal populations are usually most dense near the surface. When the algae are dispersed through the water column, compositing samples from different depths is acceptable. When a fuel-powered boat is used, collect samples from the bow to avoid contamination from the motor or from sediment sampling activities at the rear of the boat.

3) You may collect samples directly with the sample bottles for surface collection or with the additional equipment for various water depths.

PHYTOPLANKTON METHODS SUMMARY

3) Sample Preservation and Handling

1) Place sample on ice.

2) Preserve the 1 L sample with 3 mL of Lugol's solution (see FS 7001, section 2) within 8 hours of collection time. For long-term storage, add buffered formalin (see FS 7001, section 1) to achieve a minimum of 2.5% final concentration (approximately 25 mL). Depending on the study objectives and the organisms present, samples can be initially preserved with buffered formalin.

4) *Laboratory Quality Control for Algal Identification (LQ 7100)* – For more information on quality control procedures for Algal identification, refer to: LQ7100, visit ftp://ftp.dep.state.fl.us/pub/labs/assessment/sopdoc/2004sops/lq1000.doc

1) *General Requirements:* Perform the following quality control activities for all taxonomic identifications:

- Maintain copies of appropriate taxonomic identification keys
- Follow the quality control procedures to insure a new analyst has achieved a minimum proficiency of Algal Identification.
- Achieve 60% correct at the genus level for soft algae or 60% correct at the species level for Diatoms in the Consistency Identifications procedure (LQ 7130). A recommended target level of 80% correct is suggested.
- The new analyst should re-identify a minimum of 10 samples previously identified by a proficient analyst.

II. Phytoplankton Methods Summary

Some of the methodologies used for Phytoplankton sampling and monitoring, including the parameters being measured, the required reporting units, references, and the detailed method's description are presented below:

II. Phytoplankton Methods Summary Table

Method #	Parameter	Method	Reference	Reporting Units
101	Phytoplankton 1^{o} Productivity	Light and Dark Bottle- Change in dissolved oxygen	Koch, M.S. and Madden, C. J., 2001. Patterns of primary production and nutrient availability in a Bahamas lagoon with fringing Mangroves. Mar. ecol. Prog. Ser., 219:109-119.	g C m^{-2} d^{-1} (converted from mg l^{-1} O_2 d^{-1}

PHYTOPLANKTON METHODS SUMMARY

II. Phytoplankton Methods Summary Table

Method #	Parameter	Method	Reference	Reporting Units
102-a	Phytoplankton a) cell density and b) biovolume	Standard Procedures for Phytoplankton Collection and Enumeration	Downing, J.A. and Rigler, F.H., 1984. A Manual on Methods for the Assessment of Secondary Productivity in Freshwaters (2nd ed.) Blackwell Scientific Publications, Oxford.	a) cells ml^{-1} b) μm^3 ml^{-1}
102-b	Phytoplankton a) cell density and b) biovolume	Inverted Microscope and Modified Utermohl Sedimentation Technique	1) Hasle, G. R., 1978. The inverted microscope method. Chapter 5.2.1 in Phytoplankton Manual. A. Sournia, ed. United Nations Educational, Scientific and Cultural Organization. Paris. 337 pp.	a) cells ml^{-1} b) μm^3 ml^{-1}
102-c	Phytoplankton a) cell density b) biovolume	Palmer-Maloney Counting Cells	Palmer, C.M. and T. E. Maloney, 1954. A new counting slide for nanoplankton. American Society of L&O Spec. Pub. 21. 6 pp.	a) cells ml^{-1} b) μm^3 ml^{-1}
103	Phytoplankton a) cell density, b) biovolume and c) taxonomy	Gravimetric sedimentation technique and counting cells	Hotzel, G. and R. Croome. 1999. A Phytoplankton Methods Manual for Australian Freshwaters. LWRRDC Occasional Paper 22/99.	a) cells ml^{-1} b) μm^3 ml^{-1} a) c) Identification (ID) to lowest taxonomic level
104-a	a) Phytoplankton: Subsurface Grab Sampling in Rivers	Discreet Depth Sampling Using Van Dorn, Ruttner, or Niskin Bottle	Hotzel, G. and R. Croome. 1999. A Phytoplankton Methods Manual for Australian Freshwaters. LWRRDC Occasional Paper 22/99.	NA (collection only)
104-b	Phytoplankton: Subsurface Grab Sampling in Standing Waters	Discreet depth sampling using Van Dorn, Ruttner, or Niskin Bottle	Hotzel, G. and R. Croome. 1999. A Phytoplankton Methods Manual for Australian Freshwaters. LWRRDC Occasional Paper 22/99.	NA (collection only)
104-c	Phytoplankton: Subsurface Grab Sampling in Estuaries	discreet depth sampling using Van Dorn, Ruttner, or Niskin Bottle	Hotzel, G. and R. Croome. 1999. A Phytoplankton Methods Manual for Australian Freshwaters. LWRRDC Occasional Paper 22/99.	NA (collection only)
105	Marine and Estuarine Chlorophyll-a and Phaeophytin	Acetone extraction and fluorescence	Arar, E.J. and G.B. Collins, 1997. In Vitro Determination of Chlorophyll a and Phaeophytin a in Marine and Freshwater Algae by Fluorescence. In Methods for the Determination of Chemical Substances in Marine and Estuarine Environmental Samples, USEPA. Method	Chl-a in µg/L Pheo-a in µg/L

PHYTOPLANKTON METHODS SUMMARY

II. Phytoplankton Methods Summary Table

Method #	Parameter	Method	Reference	Reporting Units
			445.0-1.	
106-a	Phytoplankton: Algal Chlorophyll-a and Phaeophytin	Spectrophoto-metric determination of chlorophyll (phaeophytin corrected)	Eaton, A.D., L.S. Clesceri and A.E. Greenberg (Eds.) 1995. Standard Methods for the Examination of Water and Wastewater. 19th ed. American Public Health Association, Washington, D.C. Section 10200H.	Chl a in µg l^{-1} with pheo correction (µg l^{-1})
106-b	Phytoplankton: Algal Chlorophyll-a and Phaeophytin	Fluorometric determination of chlorophyll (phaeophytin corrected)	Eaton, A.D., L.S. Clesceri and A.E. Greenberg (Eds.) 1995. Standard Methods for the Examination of Water and Wastewater. 19th ed. American Public Health Association, Washington, D.C. Section 10200H.	Chl-a in µg/L Pheo-a in µg/L
106-c	Phytoplankton Chlorophyll a (used as biomass estimate)	Chlorophyll a- a) spectrophoto-metric, b) fluorometric c) HPLC	Hotzel, G. and R. Croome. 1999. A Phytoplankton Methods Manual for Australian Freshwaters. LWRRDC Occasional Paper 22/99.	Chl-a in µg l^{-1}
107	Phytoplankton: (a) density and (b) indirect density estimate by light transmission	a) Phytoplankton Collection and Enumeration (Method 102) b) Secchi depth	Havens, K.E., East, T.L., Beaver, J.R., 1996. Experimental studies of zooplankton-phytoplankton interactions in a large subtropical lake (Lake Okeechobee, FL, USA) Freshwater Biology 36:579-597.	a) cells ml^{-1} b) cm
108	Phytoplankton a) productivity and b) biomass	a) Light and dark bottle and b) Filtration	Havens, K.E., East, T.L., Beaver, J.R., 1996. Experimental studies of zooplankton-phytoplankton interactions in a large subtropical lake (Lake Okeechobee, FL, USA) Freshwater Biology 36:579-597.	a) g C $m^{-2}d^{-1}$ b) mg l^{-1} (dry weight)

PHYTOPLANKTON METHODS SUMMARY

III. Phytoplankton Methods Description

Group Name	Phytoplankton	
Parameter	Phytoplankton Primary (1º) Productivity	
Method	Light and Dark Bottles- Change in dissolved oxygen	
Method #	101	
Method Description		
Phytoplankton and epiphyte metabolism is determined under in-situ conditions in 300 ml BOD bottles under light and dark conditions. Epiphytes scraped from 10 cm sections of Thalassia testudium leaves and phytoplankton were incubated for 4 h, and initial and final oxygen concentrations were measured. Photosynthetic rates were determined by converting oxygen fluxes to carbon equivalents using a photosynthetic quotient of 1.2 and a respiration quotient of 1.0. Daily respiration rates were calculated over 24 h, and photosynthesis over a 10 h light cycle based on PAR above 500 umol m^{-2} s^{-1}.		
Reference		
Koch, M.S. and C.J. Madden, 2001. Patterns of Primary production and Nutrient Availability in a Bahamas Lagoon with Fringing Mangroves. Mar. Eco. Prog. Ser., 219:109-119.		
Quality Control		
Reporting	Name	Community production/respiration
	Units	g C m^{-2} d^{-1} (converted from mg l^{-1} O_2 d^{-1})
Database Elements	NA	

PHYTOPLANKTON METHODS SUMMARY

Group Name	Phytoplankton	
Parameter	Phytoplankton: (a) cell density (b) biovolume	
Method	Standard Procedures for Phytoplankton Collection and Enumeration	
Method #	102-a	

Method Description

Collection: An integrated water column sampler is used to collect phytoplankton. A large plastic container (20 L) is filled using the integrated water sampler. The composite sampler is mixed and a pre-labeled 125-ml amber bottle is manually immersed into the container. The bottle should be pre-labeled with the date, station id, sample type, replicate number, and preservative type. The sample is immediately preserved with 2-ml Lugol's solution per 100-ml of sample (Vollenweider 1974) and stored in the dark.

Enumeration: Phytoplankton are counted using the inverted microscope procedure of Utermohl as described by Lund et al. (1958). Sub-samples are settled for at least 24 hours in a sedimentation chamber prior to counting. Replicate areas are enumerated at a magnification of no less than 500X, until at least 400 animals have been counted (Lund et al. 1958). For enumeration of rare, large taxa, the entire chamber is subsequently scanned and counted at low magnification. Results are expressed as cells/ml and then converted to um3/ml using appropriate geometric formulae (Downing and Rigler 1984) for all algal taxa.

References

Downing, J. A. and Rigler, F.H. 1984. A manual on Methods for the Assessment of Secondary Productivity in fresh Waters (2nd ed.) Blackwell Scientific Publications, Oxford.

Additional References

Lund, J.W. Kipling, G.C., and Le Cren, E. E., 1958. The inverted microscope method of estimating algal numbers and the statistical basis of estimation by counting.

Vollenweider, R. A. 1974. A Manual on Methods for Measuring Primary Production in Aquatic Environments (2nd Ed.). Blackwell Scientific, Oxford.

Quality Control		
Reporting	Name	a) cell density b) biovolume
	Units	a) cells ml^{-1} b) um3 ml^{-1}
Database Elements	NA	

PHYTOPLANKTON METHODS SUMMARY

Group Name	Phytoplankton
Parameter	Phytoplankton: (a) cell density and (b) biovolume
Method	Inverted microscope and a modified Utermohl sedimentation technique
Method #	102-b

Method Description

The U.S. Geological Survey's (USGS) National Water-Quality Assessment Program (NAWQA) samples phytoplanktonic algae by collecting whole-water samples (Porter et al. 1993, Moulton et al. 2002). This protocol describes quantitative procedures for analyzing the soft-algal component of phytoplankton and counting the total number of diatoms. This procedure is quantitative and designed to provide data on algal densities (as cells per ml) and amount of algal biovolume *(um3* per m]) at a sampling site.

Pre-Concentrate Subsamples. The initial concentration should be approximately 5-10 times the original whole-water sample, leaving about 20 ml of concentrate for analysis. Samples are concentrated by settling and decanting (settle for at least 2 days) or by centrifugation (1000 g for 20 min). It is important to measure and record the original and final volumes, before and after concentration. This concentrated sample is then divided into at least two subsamples one for soft-algae or phytoplankton analysis and one for diatom analysis.

Prepare Palmer-Maloney Counting Cell.
a. Spread a small drop of Rose Bengal solution on the base of the chamber of a clean Palmer-Maloney counting cell and let dry.
b. Place a rectangular cover slip (#1 thickness, 22 x 50 mm) at 45° to the counting cell, covering about 1/3 of the chamber, but not across the center of the cell.
c. Thoroughly mix the Palmer-Maloney fraction and draw it into an elongated Pasteur pipette (5.25 inch). Quickly add the fraction drop-wise into the center of the chamber. When the surface tension starts to draw the cover slip across the chamber, adjust the sides of the cover slip so that the ends of the chamber are covered and the cover slip hangs over both sides of the ceramic portion of the counting cell. Then add glycerin to the area where the cover slip hangs over the ceramic portion. This seals the cover slip to the counting cell temporarily; without excess heat or vibration, the counting cell can be used for a week or more.

Choose to count random fields or along transects. Both methods (inverted microscope and Palmer-Maloney counting cell) involve counting phytoplankton cells in a chamber, by counting either random fields or along transects. Enumerate 300 natural algal units.

a) Enumerate algal forms using natural counting units. Natural counting units are defined as one for each colony, filament, diatom cell (regardless if colonial or filamentous) or unicell. With the exception of diatoms, identify algal forms to the lowest possible taxonomic level. Differentiate diatoms to the lowest practical taxonomic level. Categorize diatoms as either "living" or "dead" at the time of collection and quantify them separately.

b) Measure cell biovolumes. For each group of samples, measure the dimensions of the taxa hat contribute most to sample biovolume. For each taxon requiring biovolume measurements, select a simple geometric figure matching the shape of the taxon as best as possible, and determine the dimensions that must be measured (see the "Shapes" table in the NADEDdat database).

Reference

Hasle, G.R. 1978. The inverted-microscope method. Chapter 5.2.1 in Phytoplankton Manual. A. Sournia, ed. United Nations Educational, Scientific and Cultural Organization. Paris. 337 pp.

Additional References

Moulton, S.R., II, 1.G. Kennen, RM. Goldstein, lA. Hambrook. 2002. Revised protocols for sampling algal,

PHYTOPLANKTON METHODS SUMMARY

invertebrate, and fish communities in the National Water-Quality Assessment program, U.S. Geological Survey Open-File Report 02-150. In Press.

Palmer, C.M. and T.E. Maloney. 1954. A new counting slide for nanoplankton. American Society of Limnology and Oceanography Special Publication Number 21. 6 pp.

PCER, ANSP. 2002. Analysis of Diatoms in USGS NAWQA Program Quantitative Targeted-Habitat (RTH and DTH) Samples. Protocol No. P-I3-39.

PCER, ANSP. 2002. Preparation of Algal Samples for Analysis Using Palmer-Maloney Cells. Protocol P-13-50.

PCER, ANSP. 2002. Sub sampling Procedures for USGS NAWQA Program Periphyton Samples. Protocol P-13-48.

Porter, S.D., T.F. Cuffney, M.E. Gurtz, M.R. Meador.] 993. Methods for Collecting Algal Samples as Part of the National Water Quality Assessment Program. U.S. Geological Survey Open-File Report 93-409, Raleigh, NC [39 pp] http//water.usgs.gov/nawqalnawqa_home.html.

United States Geological Survey, National Water-Quality Assessment Program. 1997. Procedures for Processing NAWQA Algal Samples. Draft Manuscript. February 1997.

Weber, C.I. 1973. Biological Field and Laboratory Methods for Measuring the Quality of Surface Waters and Effluents. EPA-670/4- 73-001. National Environmental Research Center, Office of Research & Development, U. S. Environmental Protection Agency. Cincinnati, OH.

Quality Control		
Reporting	Name	a) cell density b) biovolume
	Units	a) cells ml^{-1} b) μm^3 ml^{-1}
Database Elements	Enter data recorded on the bench sheets into the three tables of the PHYCLGY database.	

PHYTOPLANKTON METHODS SUMMARY

Group Name	Phytoplankton
Parameter	Phytoplankton: (a) cell density and (b) biovolume
Method	Palmer-Maloney Counting Cells
Method #	102-c

Method Description

The U.S. Geological Survey's (USGS) National Water-Quality Assessment Program (NAWQA) samples phytoplanktonic algae by collecting whole-water samples (Porter et al. 1993, Moulton et al. 2002). This protocol describes quantitative procedures for analyzing the soft-algal component of phytoplankton and counting the total number of diatoms. This procedure is quantitative and designed to provide data on algal densities (as cells per ml) and amount of algal biovolume *(um3* per m]) at a sampling site.

Pre-Concentrate Subsamples. The initial concentration should be approximately 5-10 times the original whole-water sample, leaving about 20 ml of concentrate for analysis. Samples are concentrated by settling and decanting (settle for at least 2 days) or by centrifugation (1000 g for 20 min). It is important to measure and record the original and final volumes, before and after concentration. This concentrated sample is then divided into at least two subsamples one for soft-algae or phytoplankton analysis and one for diatom analysis.

Prepare Utermohl Sedimentation Chamber.
a. Attach a glass cover glass to the bottom of an Uterm6hl sedimentation chamber. For tubular varieties of settling chambers, seal a cover glass to the threaded end of the tube and screw the tube into the base assembly. Assemble the plate chamber type of settling chambers by sealing a cover glass on the bottom of the base, locking it into place with the metal ring, and sealing the cylinder on top of the base. Use a light amount of vacuum grease to seal the cover glasses and cylinders. It is critical that the cover glass be clean and grease-free.
b. Homogenize the concentrated samples by repeatedly inverting the sample bottle. Place a 10-ml aliquot of the sample into the assembled settling chamber. Let the sample settle for at least 8 hours. For the plate chamber type ofUterm6hl chamber, drain the volumetric cylinder by sliding over the drainage hole. Slide the cover plate over the chamber without allowing air bubbles to form. Analysis should proceed within a few hours of removing the cylinder.

Choose to count random fields or along transects. Both methods (inverted microscope and Palmer-Maloney counting cell) involve counting phytoplankton cells in a chamber, by counting either random fields or along transects.

(a) Enumerate algal forms using natural counting units. Natural counting units are defined as one for each colony, filament, diatom cell (regardless if colonial or filamentous) or unicell. With the exception of diatoms, identify algal forms to the lowest possible taxonomic level. Differentiate diatoms to the lowest practical taxonomic level. Categorize diatoms as either "living" or "dead" at the time of collection and quantify them separately.

(b) Measure cell biovolumes. For each group of samples, measure the dimensions of the taxa hat contribute most to sample biovolume. For each taxon requiring biovolume measurements, select a simple geometric figure matching the shape of the taxon as best as possible, and determine the dimensions that must be measured (see the "Shapes" table in the NADEDdat database).

PHYTOPLANKTON METHODS SUMMARY

Reference

Palmer, C.M. and T.E. Maloney. 1954. A new counting slide for nanoplankton. American Society of Limnology and Oceanography Special Publication Number 21. 6 pp.

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Hasle, G.R. 1978. The inverted-microscope method. Chapter 5.2.1 in Phytoplankton Manual. A. Sournia, ed. United Nations Educational, Scientific and Cultural Organization. Paris. 337 pp.

Moulton, S.R., II, 1.G. Kennen, RM. Goldstein, lA. Hambrook. 2002. Revised protocols for sampling algal, invertebrate, and fish communities in the National Water-Quality Assessment program, U.S. Geological Survey Open-File Report 02-150. In Press.

PCER, ANSP. 2002. Analysis of Diatoms in USGS NAWQA Program Quantitative Targeted-Habitat (RTH and DTH) Samples. Protocol No. P-I3-39.

PCER, ANSP. 2002. Preparation of Algal Samples for Analysis Using Palmer-Maloney Cells. Protocol P-13-50.

PCER, ANSP. 2002. Subsampling Procedures for USGS NAWQA Program Periphyton Samples. Protocol P-13-48.

Porter, S.D., T.F. Cuffney, M.E. Gurtz, M.R. Meador.] 993. Methods for Collecting Algal Samples as Part of the National Water Quality Assessment Program. U.S. Geological Survey Open-File Report 93-409, Raleigh, NC [39 pp] http//water.usgs.gov/nawqalnawqa_home.html.

United States Geological Survey, National Water-Quality Assessment Program. 1997. Procedures for Processing NAWQA Algal Samples. Draft Manuscript. February 1997.

Weber, C.I. 1973. Biological Field and Laboratory Methods for Measuring the Quality of Surface Waters and Effluents. EPA-670/4- 73-001. National Environmental Research Center, Office of Research & Development, U. S. Environmental Protection Agency. Cincinnati, OH.

Quality Control		
Reporting	Name	a) cell density b) biovolume
	Units	a) cells ml^{-1} b) μm^3 ml^{-1}
Database Elements	Enter data recorded on the bench sheets into the three tables of the PHYCLGY database.	

PHYTOPLANKTON METHODS SUMMARY

Group Name	Phytoplankton	
Parameter	Phytoplankton: (a) cell density (b) biovolume (c) taxonomy	
Method	Gravimetric sedimentation technique and counting cells	
Method #	103	

Method Description

While other methods are available, the concentration of algae by sedimentation is the preferred method.

After mixing, a subsample of between 5 mL and 1,000 mL (depending on cell density) is taken and sedimented in a measuring cylinder of suitable size, allowing two hours for each 1 cm of water column at 20°e. If small diatoms are present, a settling time of six hours for every 1 cm of water column is recommended (Furet and Benson-Evans, 1982). For example, if a 100 mL subsample is sedimented in a standard 100 mL measuring cylinder (18.5 cm in height), sedimentation will take 2.3 days for average plankton and 4.6 days for small centric diatoms. For most algal populations a sedimentation time of 48 hours for a standard 100 mL measuring cylinder is recommended. Such a sedimentation time will also settle 'difficult' species such as Cylindrospermopsis and Planktolyngbya. There are shorter 100 mL measuring cylinders available which will reduce average sedimentation time to 24 hours. For occupational health and safety reasons, place sedimentation cylinders under a fume hood or use stoppered cylinders. If these options are not available, at least cover the top of the cylinders with a protective film or foil.

After sedimentation, the top 90% of volume is carefully siphoned off without disturbing the sedimented algae, the remainder is shaken gently and a subsample of appropriate volume (see sections 4.1.7 to 4.1.9) is transferred to the counting chamber and allowed to settle before counting. From time to time, examine the supernatant for cells that have not sedimented. If the supernatant is siphoned off from a point well below the meniscus, cells floating on the surface will be pulled down into the concentrate and will mix with the other cells when the sample is shaken before taking the subs ample for counting.

Full details are available in the complete method text.

Reference

Hotzel, G. and R. Croome. 1999. A Phytoplankton Methods Manual for Australian Freshwaters. LWRRDC Occasional Paper 22/99.

Quality Control		
Reporting	Name	a) cell density b) biovolume c) taxonomy
	Units	a) cells ml^{-1} b) μm^3 ml^{-1} c) Identification to the lowest taxonomic level
Database Elements	NA	

PHYTOPLANKTON METHODS SUMMARY

Group Name	Phytoplankton	
Parameter	Phytoplankton: Subsurface Grab Sample in Rivers	
Method	Discreet depth sampling using Van Dorn, Ruttner or Niskin Bottle	
Method #	104-a	

Method Description

Field Collection

Site selection will depend on the objectives of the individual phytoplankton program. Two aspects to be considered: choosing the site along the stream or within the river basin and. choosing the exact location of sampling at the sampling site.

The sampling method chosen should guarantee a representative sample and be easy to handle.
The method will vary according to in-stream conditions. The preferred method is a depth-integrated sample taken from the main current. Water to be analyzed for all variables (e.g. algae and chemical and physical data) should be collected in a single grab then sub sampled.
If the river appears well-mixed vertically and horizontally (presence of turbulence, lack of temperature variability), a sample taken at mid-Stream 0.5 m below the surface (APHA, 1995) will suffice to characterize the phytoplankton present. For specific purposes, for example sampling at a water supply off take for drinking water, samples from a particular depth may be needed. Avoid sampling backwaters or back currents when sampling for phytoplankton.

Full details are available in the complete method text.

Reference

Hotzel, G. and R. Croome. 1999. A Phytoplankton Methods Manual for Australian Freshwaters. LWRRDC Occasional Paper 22/99.

Quality Control		
Reporting	Name	NA
	Units	Collection only
Database Elements	NA	

PHYTOPLANKTON METHODS SUMMARY

Group Name	Phytoplankton	
Parameter	Phytoplankton: Subsurface Grab Sampling in Standing Waters	
Method	Discreet depth sampling using Van Dorn, Ruttner or Niskin Bottle	
Method #	104-b	

Method Description

Field Collection

Standing waters refer to lakes, reservoirs, lagoons and weir pools as well as rivers under unusual low-flow conditions. Sampling sites may be chosen following a statistical approach or according to a set of criteria related to the aim of the program.

Sampling methodology includes a vertical temperature profile should be taken at 1 m intervals to verify the presence of stratification (Bartram and Ballance, 1996). The presence of stratification is best recognized if the rate of temperature change with depth at the thermocline is significant (0.2C m-1, B. Sherman, pers. comm,). In shallow waters, a temperature difference between the top and bottom layers of 1oC or greater under low wind conditions may be taken as an indication of stratification.

The specific sampling method for phytoplankton depends on the depth at the water body. In shallow waters with a depth of less than 2 m, a subsurface grab sample at 0.5 m is taken if the water body is well mixed. However, if the water body is stratified or motile algae are present, a depth-integrated sample or discrete depth samples are taken.

At each site, three samples are taken within an ecologically relevant sized area, mixed at equal volumes in a clean container and the required volume subsampled. For work on large reservoirs, where usually only a limited number of sites are sampled, at each site take five integrated samples within an area of 100 m by 100 m and mix even volumes into a composite sample, In this way, the sampling regime takes into account the horizontal patchiness of the phytoplankton without increasing the number of samples.

Full details are available in the complete method text.

Reference

Hotzel, G. and R. Croome. 1999. A Phytoplankton Methods Manual for Australian Freshwaters. LWRRDC Occasional Paper 22/99.

Quality Control		
Reporting	Name	NA
	Units	Collection only
Database Elements	NA	

PHYTOPLANKTON METHODS SUMMARY

Group Name	Phytoplankton	
Parameter	Phytoplankton: Subsurface Grab Sampling in Estuaries	
Method	Discreet depth sampling using Van Dorn, Ruttner or Niskin Bottle	
Method #	104-c	

Method Description

Field Collection

When deciding where and when to sample phytoplankton in an estuary, consideration needs to be given to the physico-chemical condition of the system. Salinity stratification needs to be assessed in addition to temperature stratification and integrated samples taken from layers of interest. When samples are integrated across salinity layers, the total biomass sampled will include both fresh and marine species. The vertical and horizontal salinity gradients move upstream or downstream within the estuary according to changes in seasonal flow and weather conditions.

It is recommended that an experienced ecologist and/or statistician be consulted before commencing a phytoplankton monitoring program in an estuary.

When sampling phytoplankton in an estuary, consideration should be given to both salinity stratification and temperature stratification. It is therefore necessary to measure the temperature and electrical conductivity profile at the sampling site and determine which depths of the water column are to be sampled in accordance with project objectives. It may be that only the freshwater or saltwater layer is of interest, or it may be that both are. Samples are then taken accordingly. To obtain an integrated sample, use a hosepipe sampler of appropriate length. To obtain samples from different depths employ a discrete depth sampler. The discrete depth samples may then be pooled into a composite sample if required.

Full details are available in the complete method text.

Reference

Hotzel, G. and R. Croome. 1999. A Phytoplankton Methods Manual for Australian Freshwaters. LWRRDC Occasional Paper 22/99.

Quality Control		
Reporting	Name	NA
	Units	collection only
Database Elements	NA	

PHYTOPLANKTON METHODS SUMMARY

Group Name	Phytoplankton	
Parameter	Marine and Estuarine Chlorophyll-a and Phaeophytin	
Method	Acetone extraction and fluorescence	
Method #	105	

Method Description

This method provides a procedure for low level determination of chlorophyll *a* (chl *a*) and its magnesium free derivative, phaeophytin *a* (pheo *a*), in marine and freshwater phytoplankton using fluorescence detection.

Chlorophyll-containing phytoplankton in a measured volume of sample water are concentrated by filtering at low vacuum through a glass fiber filter. The pigments are extracted from the phytoplankton in 90% acetone with the aid of a mechanical tissue grinder and allowed to steep for a minimum of 2 h, but not to exceed 24 h, to ensure thorough extraction of the chlorophyll *a*. The filter slurry is centrifuged at 675 g for 15 min (or at 1000 g for 5 min) to clarify the solution. An aliquot of the supernatant is transferred to a glass cuvette and fluorescence is measured before and after acidification. Sensitivity calibration factors, which have been previously determined on solutions of pure chlorophyll *a* of known concentration, are used to calculate the concentration of chlorophyll *a* and phaeophytin *a* in the sample extract. The concentration in the natural water sample is reported in µg/L.

Full details are available in the complete method text.

Reference

Arar, E.J. and G.B. Collins, 1997. *In Vitro* Determination of Chlorophyll a and Phaeophytin a in Marine and Freshwater Algae by Fluorescence. *In* Methods for the Determination of Chemical Substances in Marine and Estuarine Environmental Samples. USEPA Method 445.0-1.
http://www.epa.gov/nerlcwww/m445_0.pdf

Quality Control		
Reporting	Name	Chl-a Pheo-a
	Units	µg l^{-1} µg l^{-1}
Database Elements	NA	

PHYTOPLANKTON METHODS SUMMARY

Group Name	Phytoplankton	
Parameter	Phytoplankton: Algal Chlorophyll-a and Phaeophytin	
Method	Spectrophotometric determination of chlorophyll (phaeophytin corrected)	
Method #	106-a	

Method Description

The concentration of photosynthetic pigments is used frequently used to estimate phytoplankton biomass. The presence of absence of these pigments is used to distinguish major algal groups. One of the important chlorophyll degradation products is phaeophytin. In the presence of pheopigments, significant error in chlorophyll a values can occur. Chlorophyll and associated pheopigments are measured by spectrophotometry.

Samples are collected in opaque bottles and concentrated by filtration with a glass fiber filter (GF-F) as soon as possible after collection. Held samples should be stored at 4ºC and protected from exposure to light. Filtered samples can be frozen for up to three weeks prior to analysis.

Filtered samples are extracted with acetone and processed with a tissue grinder. Extracted samples are analyzed with a spectrophotometer. The addition of acid, once the cholorphyll a value has been determined, converts the chlorophyll a to phaeophytin a. An absorption peak ratio is used in correcting the chlorophyll a concentration for phaeophytin a.

Full details are available in the complete method text.

Reference

Eaton, A.D., L.S. Clesceri and A.E. Greenberg (Eds.) 1995. Standard Methods for the Examination of Water and Wastewater. 19th ed. American Public Health Association, Washington, D.C. Standard Methods for the Examination of Water and Wastewater (20th ed.) Section 10200H.

Additional Reference

Hotzel, G. and R. Croome. 1999. A Phytoplankton Methods Manual for Australian Freshwaters. LWRRDC Occasional Paper 22/99.

Quality Control		
Reporting	Name	Chl-a Chl-a phaeophytin corrected
	Units	$\mu g\ l^{-1}$ $\mu g\ l^{-1}$
Database Elements	NA	

PHYTOPLANKTON METHODS SUMMARY

Group Name	Phytoplankton	
Parameter	Phytoplankton: Algal Chlorophyll-a and Phaeophytin	
Method	Fluorometric determination of chlorophyll (phaeophytin corrected) (*This method is normally not applicable to freshwater samples.*)	
Method #	106-b	

Method Description

The concentration of photosynthetic pigments is used frequently used to estimate phytoplankton biomass. The presence of absence of these pigments is used to distinguish major algal groups. One of the important chlorophyll degradation products is phaeophytin. In the presence of pheopigments, significant error in chlorophyll a values can occur. Chlorophyll and associated pheopigments are measured by flourometry. The flourometric method for chlorophyll a is more sensitive than the spectrophotometric method and smaller samples can be used.

Samples are collected in opaque bottles and concentrated by filtration with a glass fiber filter (GF-F) as soon as possible after collection. Held samples should be stored at 4ºC and protected from exposure to light. Filtered samples can be frozen for up to three weeks prior to analysis.

Filtered samples are extracted with acetone and processed with a tissue grinder. Chlorophyll Extracted samples are first analyzed with a spectrophotometer (with and without the addition of acid) and then a calibrated fluorometer. A series of calculations with formulas are used to determine the corrected chlorophyll a and phaeophytin a concentrations. This method is normally not applicable to freshwater samples.

Full details are available in the complete method text.

Reference

Eaton, A.D., L.S. Clesceri and A.E. Greenberg (Eds.) 1995. Standard Methods for the Examination of Water and Wastewater. 19th ed. American Public Health Association, Washington, D.C. Standard Methods for the Examination of Water and Wastewater (20th ed.) Section 10200H.

Additional Reference

Hotzel, G. and R. Croome. 1999. A Phytoplankton Methods Manual for Australian Freshwaters. LWRRDC Occasional Paper 22/99.

Quality Control		
Reporting	Name	Chl-a Pheo-a
	Units	µg l^{-1} µg l^{-1}
Database Elements	NA	

PHYTOPLANKTON METHODS SUMMARY

Group Number	Group I	
Group Name	Phytoplankton, Periphyton and Bacteria	
Parameter	Phytoplankton: Algal Chlorophyll-a	
Method	HPLC (high performance liquid chromatography) determination of chlorophylls and their degradation products	
Method #	106-c	

Method Description

The concentration of photosynthetic pigments is frequently used to estimate phytoplankton biomass. The presence of absence of these pigments is used to distinguish major algal groups. HPLC is a useful method for quantifying photosynthetic pigments, such as chlorophyll a, accessory pigments and degradation products. Pigment distribution is useful for quantitative assessment of phytoplankton community composition.

Samples are collected in opaque bottles and concentrated by filtration with a glass fiber filter (GF-F) as soon as possible after collection. Held samples should be stored at 4ºC and protected from exposure to light. Filtered samples can be frozen for up to three weeks prior to analysis.

Filtered samples are extracted with acetone and processed with a tissue grinder. HPLC system is set up, equilibrated and calibrated with working standards prepared from primary standards. Add ion-pairing solution to extracted samples and inject into instrument for analysis. Quantification is accomplished using the response factor from a fluorescence detector and comparing known standards to sample results. See Eaton (1995) for elution order and approximate retention times for chlorophyll pigments and their degradation products.

Full details are available in the complete method text.

Reference

Eaton, A.D., L.S. Clesceri and A.E. Greenberg (Eds.) 1995. Standard Methods for the Examination of Water and Wastewater. 19th ed. American Public Health Association, Washington, D.C. Standard Methods for the Examination of Water and Wastewater (20th ed.) Section 10200H.

Additional Reference

Hotzel, G. and R. Croome. 1999. A Phytoplankton Methods Manual for Australian Freshwaters. LWRRDC Occasional Paper 22/99.

Quality Control		
Reporting	Name	Chl-a
	Units	µg l^{-1}
Database Elements	NA	

PHYTOPLANKTON METHODS SUMMARY

Group Number	Group I	
Group Name	Phytoplankton, Periphyton and Bacteria	
Parameter	Phytoplankton: (a) density and (b) indirect density estimate by light transmission	
Method	a) Phytoplankton Collection and Enumeration (Method 102) and b) Secchi depth	
Method #	107	
Method Description		
Over a 1-year period, twenty controlled experiments were performed using small mesocosms (20-l clear plastic carboys) and plankton communities collected from four sites in shallow, subtropical Lake Okeechobee, Florida. In replicated treatments, macro zooplankton grazers were excluded by size fractionation (115 mu m), and/or nutrients (N and P) were added, and impacts on phytoplankton biomass and productivity were measured after 3-day incubations. Full details are available in the complete method text.		
Reference		
Havens, K. E., East, T.L., Beaver, J. R. 1996. Experimental studies of zooplankton-phytoplankton interactions in a large subtropical lake (Lake Okeechobee, Florida, USA). Freshwater Biology 36: 579-597.		
Quality Control		
Reporting	Name	a) cell density (see Method 102) b) Secchi depth
	Units	a) cells ml^{-1} b) cm
Database Elements		

PHYTOPLANKTON METHODS SUMMARY

Group Number	Group I	
Group Name	Phytoplankton, Periphyton and Bacteria	
Parameter	Phytoplankton: (a) productivity and (b) biomass	
Method	a) Light and dark bottle and b) Filtration	
Method #	108	
Method Description		
Over 1-yr period, twenty controlled experiments were performed using small mesocosms (20-l clear plastic carboys) and plankton communities collected from four sites in Lake Okeechobee. In replicated treatments, macrozooplankton grazers were excluded by size fractionation, and/or nutrients (N and P) were added, and impacts on phytoplankton biomass and productivity were measured after 3-day incubations. Full details are available in the complete method text.		
Reference		
Havens, K. E., East, T.L., Beaver, J. R. 1996. Experimental studies of zooplankton-phytoplankton interactions in a large subtropical lake (Lake Okeechobee, Florida, USA). Freshwater Biology 36: 579-597.		
Quality Control		
Reporting	Name	a) productivity b) biomass
	Units	a) cells ml^{-1} b) mg (dry weight) l^{-1}
Database Elements		