

## CHAPTER 4

Causal and Response Variables  
Field and Laboratory Methods  
Nutrient Enrichment and Ammonia Toxicity

### **Variables and Measurement Methods To Assess and Monitor Estuarine/Marine Eutrophic Conditions**

#### **4.1 INTRODUCTION**

This chapter provides an overview of several measurable trophic state variables that can be used to establish nutrient criteria for estuaries and nearshore coastal waters. Trophic state variables are those variables that can be used to evaluate or predict the trophic status or degree of nutrient enrichment of estuaries and nearshore coastal waters, especially when compared with reference conditions. The primary variables include two causal variables (TN and TP) and two response variables including a measure of algal biomass (e.g., chlorophyll *a* for phytoplankton or macroalgal biomass (AFDW) and water clarity, e.g., Secchi depth or electronic photometer), and the addition of dissolved oxygen, as appropriate. These variables are relevant at the national scale to practically all estuaries and are potentially relevant to nearshore coastal waters.

Several variables are important indicators of nutrient overenrichment for a large number of estuaries, but in many cases the data and supporting science are inadequate for most systems (e.g., algal species composition). Important secondary variables include seagrass and estuarine submerged aquatic vegetation (SAV) distribution and abundance, macroinfaunal community structure, phytoplankton species composition, and organic carbon concentrations, respectively. Seagrasses and SAV typically provide important shallow water habitat information, and hypoxia/anoxia are measures of loss of bottom habitat often associated with deeper waters. Organic carbon (total, particulate, and dissolved) is also included as a secondary variable because this variable is consistent with Nixon's (1995) definition of eutrophication. Changes in benthic macroinfaunal community structure often correlate with organic carbon enrichment and degree of hypoxia and anoxia (Diaz and Rosenberg 1995). The importance of algal species composition has implications for food webs (Roelke 2000). These variables are discussed in Chapter 2.

As indicated in Chapter 2, the concentration of the primary nutrient variables may not correlate well with one or more response variables in estuaries, especially hypoxia or anoxia and measures of phytoplankton biomass. In this case, predictive relationships should be attempted with nutrient loads using first empirical regression models or other statistical approaches if necessary to account for ecosystem-based nonlinearities. Application of mechanistic computer models is another approach (see Chapter 9).

Interpretation of nutrient enrichment indicators, especially for estuaries, is complicated by the interaction with measures of mixing and flushing as discussed in Chapters 2 and 3. Salinity gradients are associated with flushing but also play an important role in the type of biological communities exposed to nutrient

enrichment. These physical considerations must always play a part in nutrient enrichment predictions including establishment of reference conditions as discussed in Chapters 2 and 6.

## 4.2 CAUSAL AND RESPONSE INDICATOR VARIABLES

### Nutrients as Causal Variables

#### *Nitrogen*

Nitrogen is one of the most important limiting nutrients of autotrophic assemblages (e.g., phytoplankton and periphyton) incorporated into estuarine and nearshore coastal marine bioassessments. In those estuaries where N has been demonstrated to limit algal biomass production, it typically does so at higher salinities along the salinity gradient (Chapter 2). Most research has focused on the role of inorganic-N as a stimulant to algal biomass production (Stepanauskas et al. 1999). However, about 70% of the dissolved N transported by rivers worldwide ( $10^{12}$  g yr<sup>-1</sup>) is dissolved organic N (DON) (Meybeck 1982). In contrast to P, control of N sources is more difficult because diffuse gaseous sources of N (N<sub>2</sub>) can be assimilated directly from the atmosphere by N fixation, a process conducted by a variety of bacteria and cyanobacteria (blue-green algae). Also, dissolved inorganic N forms, especially nitrite and nitrate, are highly soluble and do not precipitate easily or sediment out when freshwater enters the brackish zone of estuaries as inorganic P is likely to do.

Total N measured as a water quality indicator consists of organic and inorganic forms. Although some dissolved organic N may be used for algal growth, especially if remineralized by bacterioplankton (Carlson and Graneli 1993; Seitzinger and Sanders 1999), it and particulate organic forms participate in algal biomass production through recycling processes (Chapter 2). In systems with hypoxic or anoxic conditions, the rate of decomposition is reduced. Although still an open question, apparently relatively little of the DON is directly utilized by phytoplankton, except for urea and free amino acids (Antia et al. 1991; Paerl et al. 1999). Dissolved organic N in rainwater (synthetic addition of urea and other constituents in bioassays) was shown experimentally to stimulate bacterioplankton and phytoplankton growth; however, the DON resulted in the dominance of diatoms and dinoflagellates whereas ammonium-N stimulated production more of small monads (Seitzinger and Sanders 1999). Further work is required to test whether this response is widely applicable. Thus, the source of DON can influence the degree of DON utilization by the microbial community. Inorganic N consists of ammonia, nitrite, and nitrate N. Ammonia N is a primary product of microbial degradation of organic N, and, if not used directly by autotrophic algae and vascular macrophytes and microbial heterotrophs for growth, it may be oxidized through nitrification to nitrite and nitrate. Varying proportions of organic N may be relatively refractive and contribute very little to N overenrichment problems. However, the readily recyclable component may contribute to N enrichment problems locally and further seaward. Some experimental or model analysis (e.g., box model) of the utilization of DON and in some cases particulate organic N for each coastal system is usually warranted.

In estuaries, N concentrations, especially the inorganic forms, typically vary widely seasonally, interannually, and along salinity gradients. In temperate river-dominated estuaries, nitrate concentrations may reach very high concentrations (e.g., >100 μM) in tidal fresh to brackish reaches (see Appendix G;

Neilson and Cronin 1981) due to wash-off associated with various land use activities including point and nonpoint sources (e.g., agricultural cropland). By late spring to early summer, the nitrate concentration may be below analytical detection limits. Nitrite concentrations seldom reach high levels in surface waters due to plant utilization and conversion to nitrate through nitrification. The principal bacteria genera that mediate nitrification include *Nitrosomonas*, but species of *Nitrosococcus*, *Nitrobacter*, and *Nitrospina* are also important (Sharma and Ahlert 1977, Watson et al. 1981). If dissolved oxygen is limiting nitrification, then nitrite may accumulate (Helder and de Vries 1983). Ammonia concentrations in open estuarine and nearshore coastal waters located away from point sources typically vary from below detection limits to approximately 1.0 to 5  $\mu\text{M}$ , depending on growing season and rates of organic N decomposition. Much higher values may occur for relatively short periods. The ionized form of ammonia/ammonium is the most abundant reduced form and represents approximately 97% of the total (Sillen and Martell 1964). The equilibration between the ionized and un-ionized fractions is controlled by temperature, salinity, and pH, resulting in a range of un-ionized ammonia of 1% to 5% of the total at typical salinities, pH, and temperature (Emerson et al. 1975). Ammonia may be toxic to marine larvae, not just a stimulus to algal growth. Unionized ammonia concentrations in the range of 1.0  $\mu\text{M}$  approximate those that are known to be toxic to marine larvae, especially molluscs (U.S. EPA 1989). Denitrification may remove from a few to approximately 50% of the TN load entering temperate estuaries annually (Seitzinger 1988, Cornwell et al. 1999) depending largely on residence time of the water, sediment biogeochemical conditions (macroinfauna present to maintain irrigation, oxic conditions in the overlying bottom water), and water column depth. This process helps to modulate extreme DIN concentrations (Chapter 2). Typical values for dissolved inorganic N (DIN) and a few TN concentrations in estuaries and coastal nearshore waters are presented in Appendix G as a basis to help establish expectations for various coastal systems. It should be noted that N concentrations vary widely in space and time and the values in Appendix G are only intended to be rough guides. Specifics of analytical techniques to measure the various forms of N are included at the end of this chapter (Field Sampling and Laboratory Analytical Methods).

In open coastal waters of the North Atlantic Ocean at temperate latitudes, there is a typical seasonal progression in DIN and DIP concentrations associated with phytoplankton blooms. The spring bloom reduces these inorganic forms while phytoplankton biomass accumulates. This progression begins at lower latitudes and moves to higher latitudes. The spring bloom typically crashes in late spring, and summer biomass levels often are nutrient limited. Often a small bloom occurs in the fall following the fall thermocline breakdown that allows mixing and replenishment of nutrients from deeper waters into the upper surface layers, where a short burst of production occurs before light becomes limiting. Accumulation of deepwater nutrients during the winter has been used to assess the potential for spring-summer overenrichment in coastal seas based on trends in “salinity-nutrient mixing diagrams” (European Union Northern Marine Eutrophication Criteria Program, Ulrich Claussen, Germany, personal communication). Seasonal nutrient patterns in estuaries are quite variable. In some estuarine systems, a winter buildup of N and P has been observed (e.g., Patuxent River Estuary), especially when freshwater flows remained low and point sources dominated the nutrient supply (e.g., Flemer et al. 1970). Mixing diagrams also help interpret nutrient behavior in estuaries; however, some precautions are important to recognize (e.g., see Sharp et al. 1986).

At the interface between fresh and marine waters, a process occurs that results in an apparent increase in the ionized ammonia concentration. This process is apparently driven by the increased electrolyte solution of the salts, which has a significant impact on the production and nitrification process, thus yielding higher ionized ammonia levels (Rysgaard et al. 1999). Ionized ammonia adsorption to particles was decreased, especially in the 0 to 10% salinity range, as were the nitrification and denitrification processes. Further evaluation showed that the reduction in nitrification and denitrification processes was due not only to the displacement of bacteria and ionized ammonia from particles, but also to decreased bacterial activity. The projections from these studies were that ionized ammonia would be produced at a rate of 1  $\mu\text{M/g}$  of sediment in the water. The changes in N dynamics that affect adsorption of suspended solids may need to be included when considering acceptable levels in fresh water sources to estuaries.

### ***Phosphorus***

Phosphorus is an important plant nutrient that may limit algal biomass production in tidal fresh to brackish zones of estuaries and some subtemperate to tropical marine coastal systems (Chapter 2). There are no common stable gaseous forms of phosphorus, so the phosphorus cycle is endogenic, without an atmospheric component (Manahan 1997). The main natural reservoirs of phosphorus are poorly soluble minerals (e.g., hydroxyapatite) in the geosphere. Erosion of these materials from terrestrial sources and their transport to the sea are important sources of new phosphorus in seawater. The phosphorus entering the sea is mostly orthophosphate,  $\text{PO}_4^{3-}$  (Kennish 1989). In previous decades, prior to widespread phosphate bans in detergents, estuaries received a considerable portion of P from detergents. The ban resulted for many estuarine systems in an elevated DIN:DIP ratio. In estuaries and nearshore coastal waters, phosphorus is present in dissolved inorganic form as well as dissolved and particulate organic form. Some fraction of P may be strongly embedded in a mineral matrix, and this renders that fraction relatively inert to biological utilization. For this reason, often measures of TP may represent some component that is not biologically available and managers should consider this in developing P criteria. Plants directly take up the phosphates as essential nutrients during photosynthesis. Some algae have the capability to break down dissolved organic P (DOP) with alkaline phosphatase (algal and free phosphatases) and utilize the phosphate as inorganic phosphate (Huang and Hong 1999). Alkaline phosphatase apparently is located on phytoplankton cell membranes, which makes it difficult to determine whether the uptake is direct for DOP or the DOP undergoes enzymatic hydrolysis on the cell membrane. Malone et al. (1996) suggested by inference that Chesapeake Bay phytoplankton may utilize organic sources of P, in part, because the DIN:DIP thresholds approach 160, which is considerably greater than the N:P ratio reported by Redfield et al. (1963). Orthophosphates are typically preferred by autotrophic phytoplankton, although some assimilation of organic phosphorus may occur, especially during periods of P deficiencies (Boney 1975). When plants die, or are eaten, the organic phosphorus is rapidly converted to orthophosphates through the action of phosphorylases within fecal material, phosphatases in the plant cells, and finally by bacteria (Riley and Chester 1971).

To summarize, phosphorus occurs in natural waters and in wastewaters almost solely as phosphates. These are classified as orthophosphates, condensed phosphates, and organically bound phosphates (common analytes are total phosphorus [TP] and dissolved or particulate organic phosphorus [DOP, POP]). These compounds may be soluble, in particulates or detritus, or incorporated as organic P in

organisms. Phosphorus is essential to the growth of organisms and can limit phytoplankton biomass production, which is most commonly observed in freshwater systems (Hecky and Kilham 1988) and some estuaries and coastal marine systems (Chapter 2). In instances where phosphate is limiting, the discharge of raw or untreated wastewater, agricultural drainage, or certain industrial wastes may stimulate the growth of algae. Appendix G provides examples of P concentrations in several forms.

### *Silica*

Silica, as an important algal nutrient, has received much less attention in estuarine nutrient overenrichment studies than N and P based on the limited volume of literature citations (e.g., see Malone et al. 1996) and recent reviews of estuarine eutrophication (Chapter 1). Silica limitation of diatom production, a major algal group that requires Si (and silicoflagellates), often is a measure of N or P overenrichment (D'Elia et al. 1983; Conley and Malone 1992). Dissolved Si is a product of weathering and erosion of rocks on land with subsequent transport to the sea (Conley and Malone 1992). Because Si has essentially no human sources, except possibly from erodible soils under human influence, it is not a strong candidate for regulation. In some parts of the ocean, organisms (such as diatoms and radiolarians) abound that have produced skeletons of a noncrystalline form of hydrated silica-opal. As these skeletons settle to the sea floor they slowly dissolve, releasing silica. Officer and Ryther (1980) predicted that increases in N and P to estuaries and coastal waters from human activities, coupled with the reduction in silicates to the sea from construction of artificial lakes, would alter the N:Si and P:Si ratios. These alterations were postulated to alter phytoplankton populations to reduce the relative abundance of diatoms and enhance the relative abundance of flagellates. Egge and Aksnes (1992) showed that diatoms always numerically dominated the phytoplankton community when concentrations of silica were in excess of 2.2  $\mu\text{M}$ . Dominance by diatoms ceased or became more variable when concentrations of Si were less than this value.

Ryther and Officer (1981) reinterpreted the relationship of N pollution in Long Island Inlets during the 1950s. Nitrogen may have limited the nuisance *Nannochloris* blooms but they hypothesized that the bloom persisted because diatoms had been eliminated by Si depletion. Also, the degree of Si limitation of spring diatom blooms in Chesapeake Bay that fuel summer anoxia has direct ecological implications (Conley and Malone 1992, Malone et al. 1996). Freshwater sources of Si dominate estuarine supplies (Fisher et al. 1988). Typically, Si limitation can be potentially deduced from ambient ratios relative to the nutrient-sufficient N:Si:P biomass ratios of 16:16:1 (Redfield et al. 1963; Conley et al. 1993). In Chesapeake Bay, the dissolved Si:DIP ratio often approximates 100-300 (Malone et al. 1996), suggesting strong Si limitation. Significant increases in Mississippi River N and P concentrations and loading and decreases in silicate have occurred during the 20th century (Rabalais et al. 1996). The increased P loading and associated increased diatom production and eventual burial in river sediments, as predicted by Officer and Ryther (1980), has resulted in a reduced Si supply to the coastal environment. The consequence is that diatom production, generally a preferred phytoplankton group to support higher trophic levels, is now more Si limited than in previous decades. The N:P:Si ratios on coastal Louisiana and Texas now suggest the possibility of a joint nutrient limitation of phytoplankton production.

Silica concentrations for the Coastal Texas/Louisiana coast averaged approximately 5.3  $\mu\text{M}$  in the late 1980s but averaged about 9.0  $\mu\text{M}$  during the early 1960s. Silicate concentrations in the Chesapeake and Delaware Bays and the Hudson River Estuary ranged from about 90 to near detection levels, 30 to near detection limits, and 30 to 3  $\mu\text{M}$ , respectively (Fisher et al. 1988). Eyre and Balls (1999) reported that Si was less likely to limit diatom production in tropical estuaries than in temperate ones because concentrations tend to be much higher in tropical estuaries.

The role of silica may be more important to diatom species composition and food quality as future research may document. More attention in the future should be given to the measurement and assessment of the role of Si in estuarine and nearshore coastal primary productivity and food web dynamics and as a basis for controlling co-limiting N and/or P.

## **Response Variables**

### ***Chlorophyll *a* and Macroalgal Biomass***

Chlorophyll *a* is the molecule mediating photosynthesis in most all green plants (except prochlorophytes, which contain divinyl chlorophyll), including phytoplankton; it is relatively easy to measure either spectrophotometrically or by fluorescence and is commonly used to indicate phytoplankton biomass. However, the amount of chlorophyll per cell can vary widely. Conversion factors from weight of chlorophyll to weight of carbon (a desired biomass unit) can vary by a factor of 10. Adaptation to light levels is the primary reason for observed variability; photoadaptation can cause the chlorophyll per cell to vary widely. The technology for measuring chlorophyll has greatly improved over the decades. The Welschmeyer (1994) fluorometric analysis reduces the interference due to chlorophyll *b* and phaeopigments. The HPLC procedure is capable of detecting and quantifying various pigments characteristic of different algal groups (e.g., diatoms, cyanophyta, chlorophyta, and dinoflagellates) (Jeffery et al. 1997).

Rapid proliferation or blooming of phytoplankton, as reflected in chlorophyll *a* measurements, occurs throughout the ocean but is most often associated with temperate coastal and estuarine waters and at higher latitudes. In winter months, growth of phytoplankton populations is generally minimal because of insufficient light and also because a turbulent and unstable upper water column carries the phytoplankton cells below the euphotic zone (where light is not sufficient) before they can divide.

Chlorophyll *a* concentrations vary widely as a function of nutrient supply, water column stability, euphotic zone depth (light availability), sinking, grazing, disease organisms (e.g., viruses), and flushing/mixing (Chapter 2). Values in excess of 12 to 15  $\mu\text{g/L}$  are likely to cause severe shading of seagrasses (Kelley in press). Concentrations in estuaries during summer optimum growing conditions may exceed 50 to 80  $\mu\text{g/L}$  when nutrient loading is high (Monbet 1992). Summer values in the range of 20 to 40  $\mu\text{g/L}$  are frequently observed in enriched estuaries. In contrast, concentrations in overenriched temperate U.S. estuaries during the winter may decrease to 1 to 5  $\mu\text{g/L}$ . Nearshore coastal areas removed from high nutrient loads may experience chlorophyll concentrations in the range of approximately 1 to 3  $\mu\text{g/L}$  (Appendix G). Very high values may occur during the summer under conditions of high levels of

nutrient enrichment (e.g., the Mississippi River Plume on the Texas/Louisiana Shelf [Rabalais et al. 1996]).

Macroalgal biomass, especially benthic unattached forms (i.e., *Ulva* spp.), often becomes abundant in relatively shallow estuaries that experience nutrient overenrichment. In estuaries that receive most of their nutrient load from groundwater (e.g., Waquoit Bay, Cape Cod, MA; see Chapter 2) benthic macroalgae may shade out seagrasses. Continued enrichment typically leads to reduction of macroalgae as phytoplankton predominate in the water column. Macroalgae are difficult to adequately sample for chlorophyll *a*, and thick mats often contain sheets of algal material that has begun to degrade. The most common method to sample benthic macroalgae is to collect samples and express the biomass on a dry weight basis.

## **Measures of Water Clarity**

### ***Light Attenuation Coefficient***

The Secchi disc has been a mainstay as a tool in estimating water clarity; however, this simple and inexpensive tool does not provide all of the information required to distinguish the light attenuation effects of living phytoplankton pigments (i.e., traditionally estimated by chlorophyll *a*) from other factors (e.g., inorganic suspended sediments, organic nonchlorophyll-based detritus, and humic-like materials) that reduce water clarity. EPA's Chesapeake Bay Program (Chapter 2) has developed an analytical approach that partitions the effect of chlorophyll *a* from total suspended solids that contribute to reduction in water clarity. This approach has been used successfully in estimating the combined factor contribution to light attenuation over submerged aquatic vegetation beds (Dennison et al. 1993). In turbid coastal waters, the analyst should be aware of lower values for the constant 1.7 to estimate the light attenuation coefficient (see Giesen et al. 1990 and references in Chapter 2). More precise estimates of the light attenuation coefficient can be made with electronic submersible light meters including PAR meters (photosynthetic active radiation) and submersible spectral radiometers. These meters are now in widespread use, and their use should be encouraged because they give a direct measure of light attenuation, especially in shallow water where depth may limit use of the Secchi disc.

Attenuation of light in the sea in nonalgal bloom areas is determined principally by the amount of suspended matter present, but in estuaries and nearshore coastal waters, color from humic-like materials may significantly compete with particulate material in light attenuation. In moderately turbid coastal waters, 1% of the surface visible light energy may penetrate to a depth of only 10 to 20 m, but in shallow estuaries depths often are from 10 cm to 3 m or so. There typically is a strong seasonal variability in water clarity in temperate estuaries between the active growing season and the winter, and in subtemperate to tropical estuaries water clarity often is a function of the wet season. In the Atlantic temperate open coastal areas with the coming of spring, the depth of the euphotic zone often increases and the depth of the mixed layer decreases because of the development of the seasonal thermocline. This allows a spring bloom to develop. The thermocline tends to confine the algal cells to the euphotic zone, which becomes rich with nutrients as a result of winter mixing. In estuaries, the pycnocline may also have this effect. In partially mixed estuaries where light is adequate at depth, diatoms may grow below the pycnocline (Malone et al. 1996). If the necessary growth-promoting factors are also present,

conditions are optimal for proliferation of phytoplankton from seed stock, which may be either the plankton cells themselves or their resting stages (Riley and Chester 1971).

### ***Secchi Depth***

The Secchi disc is a useful tool to estimate water clarity (Holmes 1970). Secchi disc measurements often have a longer historical record than electronic measurements, which facilitates assessment of trends in water clarity. Secchi depth measurements are obtained with a 40 cm plastic or metal Secchi disk that is either white or is divided into black and white quadrants on a nonstretchable line that is calibrated in decimeters. The disc should be weighted to maintain a level position, especially under strong current conditions. The disk is lowered into the water until it disappears from view and the depth is recorded. The disk is then slowly raised to the point where it reappears and the depth is recorded again. The mean of these two measurements is the Secchi depth. Observations are made from the shady side of the vessel to reduce problems of glare; however, when a small boat is used for field work a “viewing tube” allows readings under full sunlight conditions. Measurement should be made without sunglasses.

### **Dissolved Oxygen**

Dissolved oxygen (DO) is an integrative measure of ecosystem health and habitat function. As a first-order estimate, the percent saturation of surface and bottom waters is an index of the production/respiration ratio. Dissolved oxygen in bottom waters serves as a measure of habitat availability for benthic animals and pelagic animals that feed on the bottom. EPA has developed saltwater DO criteria for coastal waters between Cape Cod and Cape Hatteras (see [www.epa.gov/ost/standards/dissolved](http://www.epa.gov/ost/standards/dissolved)). Profiles of DO are indicative of oxygen depletion conditions such as hypoxia and anoxia. Lack of oxygen in bottom waters causes sediment to release dissolved nutrients including orthophosphorus, ammonia, and in addition, toxic hydrogen sulfide.

### ***Carbon Compounds***

Organic matter content is typically measured as total organic carbon (TOC) and dissolved organic carbon and is an essential component of the carbon cycle. The rate of organic carbon production and decomposition and the resulting microbial biomass are at the heart of the eutrophication problem. Evaluation of the carbon-containing compounds in an aquatic ecosystem can indicate its organic character. The larger the carbon or organic content, the greater the growth of microorganisms that can contribute to the depletion of oxygen supplies. TOC is a more convenient and direct expression of organic carbon content than are the biochemical oxygen demand (BOD), assimilable organic carbon (AOC), or chemical oxygen demand (COD) methods. TOC is independent of the oxidation state of the organic matter and does not measure other organically bound elements, such as N and hydrogen, or inorganics that can contribute to the oxygen demand measured by BOD and COD. In spite of its versatility, TOC does not provide the same kind of information as BOD, AOC, or COD, and should not be used to replace these methods.

At the surface of the sea, the concentrations of particulate and dissolved organic carbon range up to 12.5  $\mu\text{M}$  and between 75 and 150  $\mu\text{M}$ , respectively. In coastal environments, concentrations of dissolved and particulate organic carbon are greater by factors of ~7-fold. Concentrations of dissolved and particulate

organic carbon in surface waters are equivalent to 150 to 1,800  $\mu\text{g C/L}$  (Millero 1996). Organic carbon represents approximately 50% of the dissolved and particulate organic material in seawater (Millero 1996). However, the major form of carbon in seawater is associated with inorganic carbonate systems.

### **Benthic Macroinfauna**

Benthic macroinfauna are an important biological component of estuarine and nearshore coastal marine ecosystems. These communities contribute to benthic food webs, contribute to nutrient cycling and system productivity through benthic-pelagic coupling of nutrient recycling, help stabilize bottom habitats, and contribute to marine biodiversity. Benthic infaunal communities are quite diverse within an estuary or coastal region. Diversity is a function of salinity, with higher diversities associated with higher salinities (Carriker 1967). Sediment irrigation provided by benthic infauna enhances denitrification by increasing the flux of ammonium into oxic microenvironments where nitrification can occur and the flux of nitrite and nitrate into the anoxic sediment zone where denitrification becomes possible (Chapter 2).

## **4.3 FIELD SAMPLING AND LABORATORY ANALYTICAL METHODS**

The following sections provide additional information on field sampling and laboratory methods for selected variables. A list of suggested methodologies for analysis of biochemical parameters is provided in Table 4-1. These methods have been summarized from nationally or regionally recognized reference compendiums (APHA 1998, ASTM 1976, U.S. EPA 1979, Spotte 1992) and provide acceptable methods for determining the concentrations of nutrients as well as acceptable methods for measuring the effects of those nutrients in estuarine and marine waters.

### **Field Sampling Methods**

#### ***Nutrients, Hydrography, and Sediments***

Physiochemical profiles should be recorded for each field sampling station. Important parameters to be measured include water temperature, pH, dissolved oxygen, salinity, light attenuation, surface radiation, and total depth. Generally, a multiparameter water quality instrument CTD is used. Sampling depth will vary depending on specific objectives; however, enough vertical depth reading should be taken to characterize the physical structure of the water column. For example, CDT measurements might be taken at frequent intervals in the vicinity of the pycnocline (e.g., every 0.1 m in highly stratified estuaries). Overall current dynamics can be mapped with oceanographic tools such as current meters, drift cards, and acoustic Doppler sounders.

Field sampling of discrete water samples for laboratory analysis can be performed using standard nonmetallic plastic water bottles. Samples are drawn into pre-labeled bottles and fixatives are applied as appropriate to the subsequent analysis. Nutrient and organics samples are stored on ice until reaching a shoreside sample handling location. Nutrient samples are filtered using graduated syringes and then frozen. Samples for total TN and TP are filtered or unfiltered as appropriate, and 20 mL of sample is frozen for analysis. See Chapter 5 for additional sampling protocols.

**Table 4-1. Suggested methods for analyses and monitoring of eutrophic conditions of coastal and marine environments (\* = primary EPA preferred causal and response variables)**

<b>Eutrophication indicators</b>	<b>Suggested methods</b>	<b>Detection limit or range</b>	<b>Comments</b>	<b>References</b>
<b>Field</b>				
*Water clarity	Secchi depth	0.1 m	—	EPA 903-R-96-006
pH	CTD probe	0.01 pH	—	—
Dissolved oxygen	CTD probe	0.02 mg DO/L	or Winkler Azide Mod.	—
Salinity	Salinometer	0.1 psu	—	—
Light attenuation	Sensor	0.05% @ 100% light	e.g., LI-COR-LI-192S A sensor	—
Temperature	CTD probe	0.1 °C	—	—
<b>Laboratory analyses</b>				
*Total phosphorus	SM 4500P-E	0.3 µM	Ascorbic acid method	APHA 1998
(including orthophosphate, POP, and DOP)	SM 4500P-E	0.32 µM	Auto. persulfate method	APHA 1998
	EPA 365.2	—	—	EPA 600/4-79-020
	CBP IV.D.2	0.03 µM	Auto. persulfate method	EPA 903-R-96-006
Dissolved orthophosphate	CBP IV.D.3	0.02 µM	Ascorbic acid method	EPA 903-R-96-006
Particulate phosphorus	CBP IV.D.4	0.04 µM	Ascorbic acid method	EPA 903-R-96-006
*Total N, incl. DON, DIN, and PON <sup>a</sup>	SM 4500N-C	0.36 µM	Persulfate method	APHA 1998
	ASTM D3867	0.7-143 µM	Persulfate method	ASTM 1976
	EPA	—	Persulfate method	EPA 903-R-96-006
	EPA-AERP18	—	—	EPA 600/4-87-026
	CBP IV.D.8	1.9 µM	Auto. persulfate method	EPA 903-R-96-006
Total Kjeldahl N	SM 4500org-C with	—	Semi-micro-Kjeldahl method	APHA 1998
	SM 4500NH3-H	1.4-1429 µM	Auto. phenate method	APHA 1998
	EPA 351.3/.1 (mod.)	—	Colorimetric/titration	EPA 600/4-79-020
Ammonia/ammonium	SM 4500NH3-B/H	1.4-1429 µM	Auto. phenate method	APHA 1998
	EPA 350.1	0.7-1429 µM	Colorimetric phenate	EPA 600/4-79-020
	CBP IV.D.7	0.3 µM	Auto. phenate method	EPA 903-R-96-006
Nitrate	SM 4500NO3-F	35.7-714 µM	Auto. cadmium reduction	APHA 1998
	EPA 353.2	—	—	EPA 600/4-79-020
Nitrite	SM 4500NO3-F	35.7-714 µM	Auto. cadmium reduction	APHA 1998
	EPA 353.2	—	—	EPA 600/4-79-020
	SM 4500NO2-B	0.7-71 35.7-714 µM	Colorimetric method	APHA 1998
	EPA 354.1	—	—	EPA 600/4-79-020

**Table 4-1. Suggested methods for analyses and monitoring of eutrophic conditions of coastal and marine environments (\* = primary EPA preferred causal and response variables) (continued)**

Eutrophication indicators	Suggested methods	Detection limit or range	Comments	References
Nitrate + nitrite	CBP IV.D.5	0.01 µM	Auto. colorimetric method	EPA 903-R-96-006
	SM 4500NO3-F	35.7-714 µM	Auto. cadmium reduction	APHA 1998
	EPA 353.2	—	—	EPA 600/4-79-020
	EPA 4.1.4	0.7-143 µM	Technicon autoanalyzer	EPA 503/2-89/001
Particulate N	CBP IV.D.6	0.01 µM	Auto. colorimetric method	EPA 903-R-96-006
	CBP IV.D.8.10	1.36 µM	Filtration/combustion	EPA 903-R-96-006
Total organic carbon	SM 5310TOC-D	>0.1 mg C/L	Wet oxidation method	APHA 1998
	SM 5310TOC-C	>0.01 mg TOC/L	Persulfate method	APHA 1998
	EPA 415.1	—	—	EPA 600/4-79-020
Dissolved organic carbon	SM 5310TOC-C	>0.01 mg TOC/L	Persulfate method	APHA,1998
	EPA 415.1	—	—	EPA 600/4-79-020
	ASTM D2574-79	—	—	ASTM 1976
Particulate carbon	CBP IV.D.10	0.5 mg/L	Catalytic combustion	EPA 903-R-96-006
	CBP.IV.D.9	0.097 mg/L	Filtration/combustion	EPA 903-R-96-006
Total silicates	SM 4500SiO2-D	0.33-0.83 µM	Heteropoly blue method	APHA 1998
	ASTM D859-68	—	—	ASTM 1976
	CBP-IV-15	0.17-23.3 µM	—	EPA 903-R-96-006
Total suspended solids	EPA 370.1	—	—	EPA 600/4-79-020
	CBP IV.D.15	0.22 µM	Molybdosilicate method	EPA 903-R-96-006
	SM 2540-D	2-20,000 mg/L	Dried at 103-105°C	APHA 1998
	CBC IV.D.13	2.0 mg/L	Filtration/heat	EPA 903-R-96-006
Total volatile solids	SM 2540-E	—	—	APHA 1998
	Estuarine	—	—	EPA 430/9-86-004
BOD	SM 5210-B	—	5-day method	APHA 1998
	EPA 405.1	—	—	EPA 600/4-79-020
COD	CBP IV.D.11	—	5-day method	EPA 903-R-96-006
	SM 5220-D	—	—	APHA 1998
	EPA 410.4	—	—	EPA 600/4-79-020
<b>Biological measures</b>				
Phytoplankton biomass	—	—	—	—
Zooplankton biomass	—	—	—	—
Chlorophyll <i>a</i> <sup>b</sup>	SM 10200-H	0.01 mg/M <sup>3</sup>	Fluorometric, HPLC, Spectro.	APHA 1998

**Table 4-1. Suggested methods for analyses and monitoring of eutrophic conditions of coastal and marine environments (\* = primary EPA preferred causal and response variables) (continued)**

Eutrophication indicators	Suggested methods	Detection limit or range	Comments	References
Phaeophytin	EPA AERP12	—	—	EPA 600/4-87-026
	ASTM D3731-79		Spectrophotometer	ASTM 1976
	CBP IV.D.12	1.0 µg/L	Spectrophotometer	EPA 903-R-96-006
	SM 10200-H	0.01 mg/M <sup>3</sup>	Fluorometric, HPLC, Spectro.	APHA 1998
	EPA AERP12	—	—	EPA 600/4-87-026
	ASTM D3731-79	—	Spectrophotometer	ASTM 1976
	CBP IV.D.12	1.0 µg/L	Spectrophotometer	EPA 903-R-96-006
Dinoflagellate density	—	—	—	—
Diatom density	—	—	—	—
Dinoflagellate/diatom	—	—	—	—
Perennial plant density	—	—	—	—
Ephemeral plant density	—	—	—	—
Epiphytic growth	—	—	—	—
Phytoplankton blooms	—	—	—	—
Fish kills	—	—	—	—

<sup>a</sup> DON, dissolved organic N; DIN, dissolved inorganic N; PON, particulate organic N.

<sup>b</sup> Phytoplankton segments: The HPLC procedure is capable of detecting and quantifying various pigments characteristic of different algal groups (e.g., diatoms, cyanophyta, chlorophyta, and dinoflagellates) (Jeffrey et al. 1997).

## Laboratory Analytical Methods

Detailed methods and references are given in Table 4-1. Some general considerations are presented in the following sections.

### Water Column Nutrients

#### *Nitrogen Compounds*

Several methods have been used to determine the concentration of N species in the marine environment. Methods presented in this document are relatively easy to use, do not require extensive instrumentation, provide detection limits below those expected in marine environments, and are in general use by many investigators. The most common forms of N in eutrophication evaluation in order of decreasing oxidation state are nitrate, nitrite, ammonia, and organic N. The sum of these is expressed as TN and is not to be confused with total Kjeldahl N (TKN), which is the sum of organic N and ammonia. Total N can be determined through oxidative digestion of all digestible N forms to nitrate, followed by quantitation of the nitrate. Nitrite is an intermediate oxidation state of N, both in the oxidation of ammonia to nitrate and in the reduction of nitrate. Such oxidation and reduction may occur in wastewater treatment plants, water distribution systems, or natural waters. Ammonia is produced largely by deamination of organic N-containing compounds and by hydrolysis of urea. The two major factors that influence selection of the method to determine ammonia are concentration and presence/absence of interferences (e.g., high concentrations of colored organic substances such as humic-like materials or paper mill effluents).

Total N is measured by the persulfate method, which digests all organic and inorganic – containing compounds. All N-containing materials (except nitrogen gas) are measured after sample digestion has occurred. Various organizations have adjusted sample volume or automated the process and produced different ranges of detection. The lowest detectable concentration is ~ 0.7  $\mu\text{M}$  of TN. This is in the range of the measured available N (0.7 to 5.0  $\mu\text{M}$  TN) for studies performed off the continental shelf in the North Atlantic from 1956 to 1958 (Kennish 1989). Kjeldahl N minus the ammonia concentration is the surrogate measurement for all organic N-containing compounds.

**Ammonia/ammonium** is measured by the indophenol blue (= phenate) or specific ion electrode methods after conversion of ammonia and ammonium to ammonia. This is done by raising the pH of the sample above 11. This method has some essential features (e.g., minimal interference from waters highly stained with humic materials and paper mill effluents); however, the level of detection is relatively high (e.g., 2.0  $\mu\text{M}$   $\text{NH}_3\text{-N}$ ) but adequate for ammonia-rich waters (Flemer et al. 1998). Ammonia electrodes do not work directly in seawater. In the spectrophotometric methods, the ammonia is reduced to monochloramine and then reacted with phenol to form a blue color. In the specific ion electrode method, the ammonium is converted to ammonia using a strong basic solution and partial pressure of ammonia gas (i.e., free ammonia) in solution, which is related to the dissolved ammonia concentrations by Henry's Law.

**Nitrates and nitrites** are measured in combination using the cadmium reduction procedure of Wood et al. (1967). This colorimetric method determines the concentration of these two materials after reaction

of nitrites to produce an azo dye, the color of which is proportional to the concentration of the combined nitrates and nitrites. Total nitrate is determined by subtracting the concentration of nitrite from the combination of the two. The process for measurement of nitrite produces the same azo dye as the combined measure, but without the Cd reduction. The difference in these two measures is the nitrate concentration.

### ***Phosphorus***

The target detection limit for measurement of P in seawater is ~ 0.3 µM. The procedures for the measurement of total particulate and dissolved P as well as orthophosphate in seawater provide detection limits that are less than this value (U.S. EPA 1996). These procedures convert the phosphorus-containing compounds to orthophosphate through the digestion of the sample with alkaline persulfate. This treatment is then reacted with ammonium molybdate and antimony potassium tartrate in acidic solution to produce an intense blue complex with ascorbic acid. Interferences with elevated concentrations of Si can be avoided by maintaining an acid concentration in the reagents and analyzing the material at elevated temperatures of ~37°C. The resulting phosphomolybdic acid reduction produces a purple-blue complex that is measured at 885 nm on a spectrophotometer. This method of measuring reactive silicate is recommended in Millero (1996).

### ***Silica***

The target detection limit for measurement of Si in seawater is ~0.7 µM. Producing pigmented silicomolybdate complex by procedures contained in U.S. EPA (1996) provides adequate sensitivity after the samples are filtered (0.45 µm GF/F filter) to remove interfering particles and turbidity, and after the interferences of phosphates and arsenates are removed with oxalic acid. The resultant filtrate is treated with a solution containing metol-sulfate (p-methyl-amino-phenol sulfate) to produce a blue color that is evaluated more efficiently than the yellow color recommended for evaluation in U.S. EPA (1996), with a spectrophotometer at 812 nm (Strickland and Parsons 1968). This method of measuring reactive silicate is also recommended in Millero (1996).

### ***Carbon***

Total carbon consists of inorganic and organic forms that are in particulate and dissolved size classes. The distinction between total and organic carbon is based on acidifying samples to remove the inorganic forms and filtering through 0.45 µm GF/F filters to remove the particulate forms. Total carbon is measured by burning the sample to release the particles contained on the glass fiber filter. This converts the carbon to CO<sub>2</sub>, which is then transported to a thermal conductivity detector for measurement. The carbon left behind in the filtrate is catalytically combusted using a platinum catalyst at ~680°C that is then transported to a nondispersive infrared detector. The EPA methods (U.S. EPA 1996) will provide adequate detection of both dissolved and particulate carbon in the total and organic phases. The difference in total carbon and organic carbon represents the inorganic fractions that are primarily CaCO<sub>3</sub> shells.

## **Sediment Analyses**

### ***Bulk Sediment***

Cores are collected from field sites to help determine the historical record and sedimentation rate. Short cores, the upper 30 cm of the substrate, can be obtained with a HAPPS core, designed to collect a relatively undisturbed core of surficial sediment (Kannerworff and Nicolaisen 1973) and used to profile sedimentary particulate organic carbon and N. Carbon-N analyses follow the method of Hedges and Stern (1984); samples for dissolved constituents in pore water are extracted either by whole-core squeezing or by centrifugation (Devol et al. 1997, Brandes and Devol 1995, Lambourn et al. 1991). Deep coring devices are used to collect continuous sediment core samples 2 to 3 m into the sediment bed. These deeper cores are used for analysis of  $^{210}\text{Pb}$ , carbon and N, sulfide, and biogenic silica in order to determine burial rates of  $^{210}\text{Pb}$  and  $^{210}\text{Ra}$ .

The sedimentation rate is estimated based on the change in activity of naturally occurring  $^{210}\text{Pb}$  radionuclide produced at a constant rate from the decay of  $^{210}\text{Ra}$ , using the excess  $^{210}\text{Pb}$  inventory method of Anderson et al. (1987). Excess  $^{210}\text{Pb}$  is determined from the difference between total  $^{210}\text{Pb}$  activity in the sediment and the activity of the background  $^{210}\text{Pb}$  being produced from  $^{210}\text{Ra}$ . To collect samples for measurement of  $^{210}\text{Pb}$  and  $^{210}\text{Ra}$  activity at depth with the sediment, cores are sectioned and each section is then homogenized and placed in a precleaned 16 oz jar, with a small subsample removed and placed into a glass vial for particulate C and N analysis (Evans-Hamilton, Inc. 1998).

The excess  $^{210}\text{Pb}$  inventory method yields accumulation rates ( $\text{g}/(\text{cm}^2/\text{yr})$ ), which are converted to a sedimentation rate ( $\text{cm}/\text{yr}$ ) using the bulk sediment density  $\text{g}/\text{cm}^3$ . For evaluation of seasonal trends, the upper cm is subsampled at 0.25 cm intervals, and in 1 cm intervals below the first cm, following the assumption that any seasonal storage of N or carbon would manifest almost entirely at the surface of the sediment.

### ***Pore Water Profiles***

Pore water profiles of manganese, iron, nitrate, and oxygen demonstrate that oxidation of iron and magnesium yields less energy than does oxidation of carbon by oxygen or nitrate. Consequently, concentration peaks of these species are located below the depletion depths of oxygen and nitrate. In anaerobic environments, after the supplies of oxygen, nitrate, manganese, and iron are exhausted, sulfate reduction is the dominant mode of organic matter oxidation and nutrient remineralization.

Sulfate reduction rate can be measured with the radiotracer method of Christensen et al. (1987). A significant fraction of the oxygen flux may be consumed by the reoxidation of sulfide produced during sulfate reduction (Canfield 1993).

Sediment traps are used to measure the quantity and composition of the flux of materials settling through the water column to the sediment. There are four materials of interest: chlorophyll as an indicator of planktonic algal remains, pheopigments as an indicator of degraded plankton that has been consumed by zooplankton, particulate organic carbon (POC), and particulate organic N (PON). Total sedimentation rate is corrected for resuspension materials in order to derive the net flux to sediment. Samples are

collected by in situ benthic flux chambers, and measurements of oxygen, silicate, nitrate, ammonium, phosphate, and N gas are made (Evans-Hamilton, Inc. 1998).

### **Determination of Primary Productivity**

Primary productivity refers to the growth rate of the phytoplankton community and is commonly measured using trace amounts of radioactive carbon (as bicarbonate) that label the photosynthetic reaction. Additional variables are measured to support these data: biomass (as estimated by chlorophyll *a*), incoming solar radiation, and nutrient concentrations at depth. Primary productivity, *P*, is defined as

$$P = \mu \times B$$

where  $\mu$  is the specific growth rate (growth normalized per cell) and *B* is the biomass of the phytoplankton population (amount of cells). These variables are > 'compound' = as they in turn depend on other variables. Growth rate depends on light (solar radiation), dissolved nutrients in the water column, and water temperature. The phytoplankton biomass is determined by the net result of growth and loss (grazing, mixing, sinking) processes and reflects enrichment conditions.

To estimate primary productivity, samples are collected at varying depths corresponding to predetermined light levels. Fresh samples at each light level are collected for analysis of chlorophyll *a*, nutrients, and primary productivity in two sets of two clear bottles and one dark bottle; each set is filled for ambient treatment and nutrient spike treatment. Nutrient spiking consists of adding an initial concentration of 10  $\mu\text{M}$  N ( $\text{NH}_4\text{Cl}$ ) and 1  $\mu\text{M}$  phosphorus ( $\text{KH}_2\text{PO}_4$ ) to seawater. Nutrients are monitored from additional samples collected and tested for nitrate, nitrite, ammonium, orthophosphate, and silicate. Samples are inoculated with  $^{14}\text{C}$ -labeled sodium bicarbonate and, if appropriate, the nutrient spike, and placed in a screened bag to simulate the light level from which they were collected. Samples are incubated at in situ conditions for 24 hours and then transported to the laboratory for filtration using glass fiber filter paper (Whatman GF/F, nominal pore size 0.7  $\mu\text{m}$  or smaller pore size). The filters are placed into vials containing EcoLume scintillation cocktail. The specific activity of the filtered particulates is measured in a scintillation counter. Primary production is calculated as  $\text{mg C}/(\text{m}^3/\text{day})$  using the basic equations found in Parsons et al. (1984) (Evans-Hamilton, Inc. 1998).

In productive coastal waters, measurements using the light and dark bottle technique with changes in dissolved oxygen often can be used in place of the  $^{14}\text{C}$  method (Strickland and Parsons 1968). In some cases, free water gas-based (e.g., DO) methods are possible to measure ecosystem metabolism (Odum 1956; Odum et al. 1959; Kemp and Boynton 1980).

### **Phytoplankton Species Composition**

Samples collected from the field are analyzed to identify and enumerate autotrophic phytoplankton, as well as heterotrophic dinoflagellates and microzooplankton species. From 20 to 50 mL aliquots of samples are settled in separable counting chambers for at least 24 hours before examination under phase-contrast optics with an inverted microscope following the classic Utermöhl technique (Lund et al. 1958). A single transect across the center of the chamber is counted at 390 $\times$  magnification for

flagellates; 150× magnification is used for other organisms. From 25% to 100% of the chamber bottom is examined, depending on cell concentrations in the sample. Appropriate multipliers are used to convert all counts to common units of cells/L (Sournia 1978). Organisms are identified to the lowest taxonomic category possible. Even quite small changes in the physical and chemical parameters and availability of micronutrients can have a significant effect on the growth constants of algae. A difference in doubling time of 25% between two fast-growing organisms can lead to one outnumbering the other by 15 to 1 in a week and quickly lead to alterations in species assemblages (Riley and Chester 1971).

There are numerous algal species in estuarine and open coastal waters that are considered to be harmful (e.g., see Dortch et al. 1998, Anderson and Garrison 1997, Anderson 2000). This is a rapidly changing area of marine ecology and experts should be consulted for specific taxonomic identifications.

### **Macrobenthos, Macroalgae, and Seagrasses and SAV**

Macroinfauna are typically sampled with coring devices or bottom grab samplers and wet-sieved through 0.5 µM mesh sieves to separate the animals from very fine sediments. Stacked sieves can be used to remove larger shell fragments and sand particles. A relaxant (e.g., 0.3% propylene phenoxylol) is applied prior to addition of formalin. Samples are usually preserved in 10% buffered formalin for several weeks and then transferred to 60%-70% isopropanol (Diaz and Rosenberg 1995).

Macroalgae are typically sampled by collecting algal material by hand from a known surface area of the habitat. Various devices may be used (e.g., 0.5 m stainless or plastic hoop).

Both above- and below-ground seagrass and SAV biomass can be collected from a known area of the bed. Various techniques have been used. An often-used method is to shove metal strips along the sediment surface in a square meter pattern and anchor the strips at all four corners by pushing a sharp spike through holes drilled at each end of the strips. Then, the plant material separated to species can be clear-cut with sharp shears and taken to the laboratory and dried in a heated cabinet at 60°C to constant dry weight. A sharp spade is required to collect below-ground roots and rhizomes. This material should be identified and dried to constant weight.