Sampling Coastal Dinoflagellate Blooms: Equipment, Strategies and Data Processing

PETER J.S. FRANKS
DONALD M. ANDERSON
Biology Department
Woods Hole Oceanographic Institution
Woods Hole, MA, USA 02543

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Abstract

Brief descriptions of a variety of low-cost systems for sampling dinoflagellate populations are given, together with sampling schemes for the field, and the factors which will affect the sampling program. Methods of data collection and reduction are discussed, and a brief introduction to methods of data interpretation is made.

Introduction

Dinoflagellates are important constituents of the coastal phytoplankton community throughout the world. Under conditions which are still poorly understood, certain species can dominate the phytoplankton community, creating a visible coloration of the water: a "red tide". The ability of these organisms to swim may contribute to the formation of such dense blooms by allowing the dinoflagellates access to deep reservoirs of nutrient-rich water. However, swimming creates particular problems in sampling dinoflagellates and in interpreting their vertical and horizontal distributions.

It is becoming increasingly apparent that much of the large-scale variability in dinoflagellate distributions can be explained by the advection and diffusion of blooms by physical processes. These processes include wind-driven, buoyancy-driven and tidally-generated motions of the water column, coupled with seasonal cycles of temperature and freshwater runoff. At a minimum, dense CTD (Conductivity, Temperature and Depth) coverage in time and space is necessary to understand the forcings and resultant motions. These samples should be coincident with biological sampling to provide maximum insight into the couplings of the physical and biological systems.

Many questions arise: what is sufficient coverage, what types of samples should be taken, and when should they be collected? Here we offer some insights based on our experience in sampling coastal dinoflagellate populations in the Gulf of Maine, USA. First we describe sampling methodologies: the choice and construction of sampling gear, the types of samples to take, and the interfacing of different types of instruments. We then describe sampling strategies: the selection of stations in both time and space, and data reduction and interpretation. We hope this information will be useful to those designing field programs in coastal areas, and that our guidelines will lead to sufficient and interpretable data sets that can be compared to similar data sets from other regions.

Sampling Methodologies

Dinoflagellate populations normally show a great deal of vertical structure in natural waters. In many situations this structure is intimately linked to the hydrography, the cells being found within the seasonal pycnocline or zone of maximum vertical density change. In other cases, the cells may accumulate quite independently of the hydrography due to their ability to swim. Sampling gear must be capable of sufficient vertical resolution to distinguish these possibilities. This often requires biological sample spacing of 5m or less in the vertical. Here we will describe a variety of sampling systems which are capable of dense vertical resolution for a range of field situations.

Pumping Systems

In shallow (<5m) embayments, a simple integrating tube sampler may be the most appropriate sampling device. These samplers consist of a length of 6.25-cm internal diameter (i.d.) PVC

pipe, fitted with a cork attached to a line threaded through the pipe to a handle at the top (Fig. 1). A short foot at the bottom keeps the sampler from being pushed into the benthos. The sampler is slid vertically into the water until the foot rests on the bottom. Once the depth to the bottom is known, the tube is raised and moved to a new location nearby, and lowered until the foot is just off the bottom. This procedure prevents the water column samples from being contaminated with resuspended material. The line is then pulled to seal the tube with the cork. The tube is emptied by pouring from the top, since pouring from the bottom causes spillage around the cork. The integrated vertical sample can be poured into a carboy or bucket for further subsampling (see below). The advantages of this type of sampler are: 1) low cost; 2) ease of construction; 3) one sample integrates any vertical heterogeneity of the organism; and 4) ease of deployment. The disadvantages include: 1) no vertical resolution; 2) relatively small volume; and 3) no real-time vertical information.

In shallow areas, and relatively calm seas, the tube sampler described by Lindahl (unpub. ms.) may be the most appropriate sampling device. This low-cost sampler consists of lengths of 2-cm i.d. PVC pipe or garden hose, linked with valves and easily-separated connectors (Fig. 2). The length of a section determines the vertical

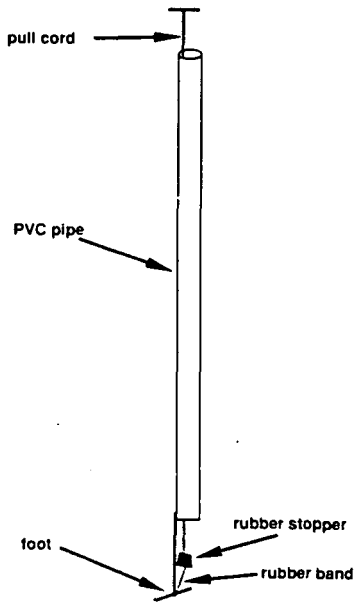

Fig. 1. The tube sampler. When the cord is pulled at the top, the cork seals the bottom of the tube. The rubber band keeps the cork oriented properly until the cord is pulled.

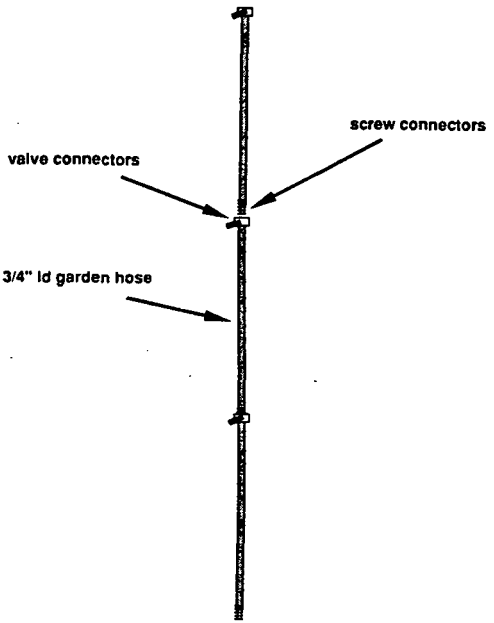

Fig. 2. The hose sampler. Lengths of hose are joined by valves and connectors. All valves must be open during deployment, and the surface valve closed upon recovery. A valve must be closed before the section of hose above it is removed, or the sample will be lost.

resolution of sampling. The sampler is slowly lowered with all valves open until the hose is filled. The top valve is then closed, and the hose raised until the next valve down can be closed. The upper section can now be removed, and its water drained. This procedure can be repeated until the whole length of hose has been raised. Hydrostatic forces within the hose will hold the water within the hose while it is being raised. Thus a small-volume vertical profile of the water column is obtained. The advantages of this sampler are similar to those of the tube sampler described above, although vertical resolution is obtained. The disadvantages are: 1) small volume; 2) no real-time vertical information; and 3) smearing of vertical structure within the pipe due to its narrow diameter.

Most vertical areas are dynamically complicated, requiring detailed coverage of both the biology and the hydrography. The samplers described above can be useful, but are nevertheless somewhat limiting due to their small volume and inability to provide continuous vertical profiles. We suggest the use of a pump profiling system. These systems have the advantages of: 1) high volume, permitting sampling of a number of variables; 2) continuous vertical profiles; and 3) the possibility of real-time observations of vertical features. The disadvantages are: 1) relatively high cost (generally < US$200); 2) they are more difficult to deploy than the sampler described above; and 3) they require an AC or DC power source for the pump and associated instruments.

The pump profiling system at its simplest is a pump and a length of hose. Aspects of the system design which must be evaluated include the position of the pump (at surface or at depth), the insertion of various flow regulators and a bubble trap, and the inclusion of subsidiary sampling devices such as fluorometers, autoanalyzers, etc. Here we describe the configuration used in our laboratory, with justification for each of the features. This configuration is certainly not exclusive of other arrangements; we hope that readers will use the information here in designing pump profiling systems specific to their own needs.

The central feature of the pump profiler is the pump. We use a "Lil' Giant" submersible pool pump (available from swimming pool supply companies, and scientific supply houses). This pump requires AC power, as would another alternative, the submersible well pump (available from dealers who drill wells for drinking water). Another feasible pump is a boat bilge pump which can run on DC power (available from marinas and boat supply companies).

The pump need not be submersible, but it should be able to withstand being soaked in salt water. If a deck pump is used, it should be self priming: priming the pump is the most difficult aspect

of the deployment of the Lil' Giant system. The pump can be suspended either just below the surface or at the bottom of the hose. The consideration here is the formation of bubbles due to cavitation within the pump. The surface pump is likely to cavitate if the hose below is too long. We use 40 m of 2-cm i.d. garden hose, the maximum length useable with this pump. Placing the pump at the base of the hose solves the cavitation problem, but generates a new problem with the length of electrical wire over the side of the vessel. Kinks in the hose and the tangling of wires are the most serious deployment problems after the flow has started.

Since the Lil' Giant pump is not self-priming, the hose must be completely filled with water before the pump is plugged in. After much trial and error, we found that putting the hose over the side of the vessel until it is fully submerged is the best way to fill it: this means that all our vertical profiles are taken as the hose is raised. The hose must be deployed carefully, as it has a tendency to twist and kink. Kinks are fatal to a profile. The most common location of kinking is where the hose attaches to the wire. To ease the strain here, we use a dual thickness of radiator hose, clamped to the wire with hose clamps, with a screw connector for the garden hose at the other end (Fig. 3). This is a particularly convenient arrangement, since the hose may be unscrewed at any time if the wire is required

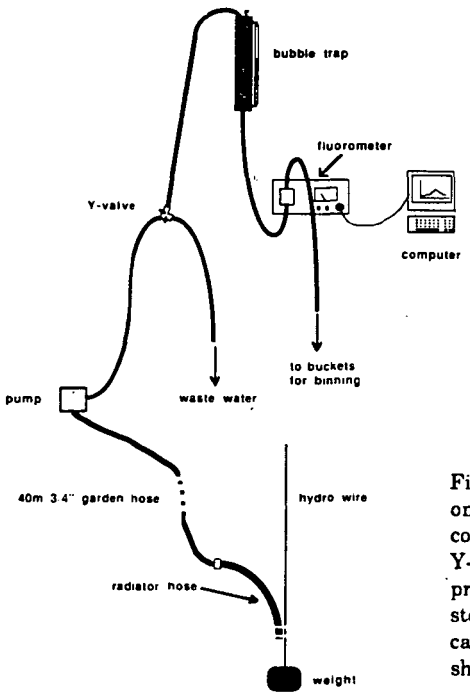

Fig. 3. The pump profiling system. This is only one of a variety of possible configurations. The flow is regulated at the Y-valve. The radiator hose at the wire prevents the hose from kinking and stopping the flow. A variety of instruments can be included in-line; a fluorometer is shown here as an example.

for something else, without having to remove the radiator hose and hose clamps. Any reasonably stiff length of hose can be substituted for radiator hose, so long as it will not kink. It is also useful to have duct tape handy: it can be used as a hose wrap should the hose kink or fail. Note also that a heavy weight is required to keep the hose as vertical as possible. We use a 30 kg weight attached to the wire (never to the hose itself).

The hose leading out of the pump brings the water on deck. A Y-valve is necessary for dividing the flow into the portion to be sampled, and the waste, which is pumped overboard. This Y-valve is critical to maintaining a steady flow to the instruments. Steady flow is achieved by adjusting the amount of waste water, not by adjusting the flow of water to the instruments. The valve to the instruments should always be wide open to avoid bubble formation.

Bubbles within the hose can be a serious problem with certain instruments such as fluorometers and autoanalyzers. To mitigate the problem, we include a bubble trap between the Y-valve and the instruments (Fig. 4). The bubble trap consists of a 1-m length of 10-cm i.d. acrylic pipe, fitted with stoppers and hose connectors at the top and bottom. A small chimney tube at the top allows air to escape. A length of clear tubing joined to the top and bottom with right-angled connectors allows visualization of the water level within the pipe. A 20-cm length of hose extends from the top hose connector into the bubble trap. The level of water within the bubble trap should

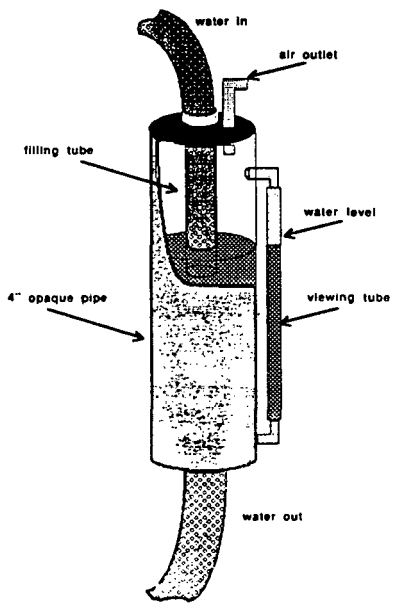

Fig. 4. Cut-away view of the bubble trap. This should be made of opaque material so that the phytoplankton are not over irradiated. The water level should be kept above the end of the inlet hose so that no new bubbles are created. The level of water is shown by the clear tube on the side of the bubble trap.

always be kept above the level of this hose so that no more bubbles are created. The bubble trap itself should be located at the highest point of the pumping system, so that all bubbles within the system may escape. The height of the bubble trap will largely be determined by the head of the pump and the geometry of the ship.

From the bubble trap, the flow feeds by gravity into the attached instruments, and back to the deck. The water may be collected in carboys to obtain samples integrated over any desired depth interval as the hose is raised or lowered. For certain analyses (e.g., productivity or chlorophyll) the sample containers should be opaque or acid-washed, although a seawater rinse is sufficient for species counts.

The protocol for sampling with the pump profiling system is complicated by the residence time of the water within the hose. Thus the water which is being pumped on deck was obtained from the depth the hose inlet occupied some time ago. The method we use to correct for this requires measurement of the residence time. This was done using a flow-through fluorometer to detect a spike of chlorophyll introduced at the hose inlet. Simple coloured dye would work, as long as it is visible to the eye or to a spectrophotometer after passage through the hose. The calculated transit time should be at the first appearance of the dye at the end of the hose: smearing within the hose will cause an initial spike to be spread over a considerable distance within the hose.

Once the transit time within the hose is known, the procedure for performing a vertical profile is relatively easy. The variables which must be decided before the profile is begun (and preferably before the cruise begins) are: 1) the rate of rise of the hose; and 2) the flow rate. The former will depend mostly on the winch being used. We have found that a rate of 2 m/min. gives reasonably dense vertical coverage. However, most winches will not raise that slowly. Our protocol is thus to raise the hose 1m every 30 seconds, and pause at that depth. The hose inlet usually takes 5 or 6 seconds to rise 1 m. Thus the profile is taken in a series of steps. Water from 5-m intervals is collected in individual buckets for subsampling. Clearly, it is important to time the raising of the hose and the bucket collection carefully so that a representative sample is collected that integrates over the 5-m interval.

In order to know the depth from which the water that arrives on deck was pumped, the winch operator and the person handling the hose must work independently. At the start of the profile, both persons should start their stop watches. The winch operator keeps his going, raising the hose 1 m every 30 seconds, beginning 30 seconds after the start signal. The person handling the hose waits an amount

of time equal to the transit time of the water in the hose, at which time the water being pumped on deck is water for the first interval. At that time, the hose handler should restart his stopwatch, to time the filling of the bucket. For example, to integrate over 5 m, the hose handler would place the hose outlet into a bucket for 2.5 min. (if the hose is being raised at 2 m/min.). Continuing this process will give samples binned into 5-m intervals. The winch operator must pause at the surface so that the hose handler can "catch up". The hose inlet must not break the surface, or the pump's prime will be lost.

Various strategies for binning or integrating of samples will be described below. Subsampling from the bins depends on the information needed. We sieve one liter from each bin through 20-mm Nitex mesh (epoxied onto a cylinder of 8-cm PVC pipe) and preserve it in 5% formalin for cell counts. An additional 500 l is filtered through GF/A filters. The filters are used for chlorophyll analyses and the filtrate is frozen for nutrient analyses. The flow rate should be adjusted at the Y-valve so that sufficient water is obtained for all these analyses, as well as for the auxiliary, on-line instruments.

Auxiliary Instruments

A variety of auxiliary instrumentation is available for interfacing with the pump profiling system, depending on the needs of the project and the available budget. Many of the instruments are relatively costly (> US$5000), and so may not be available to smaller laboratories.

Our particular pumping system includes an in-line fluorometer (Turner Designs Model 10-00R with flow-through cell and chlorophyll *a* filter set). This fluorometer is interfaced to a portable personal computer (NEC APC IV Powermate Portable) through an A/D (analog/digital) board (Metrabyte Dash-8; Metrabyte Corp., 440 Myles Standish Blvd., Taunton MA, 02780 USA) following the instructions in the fluorometer manual. The excellent software supplied with the A/D board was modified to plot fluorescence on the computer screen in real time, and to store the data to disk. The screen plot allows visual location of the fluorescence maximum, and can be used in real time to modify sampling and "binning" procedures. We have found the vertical fluorescence profiles to be an invaluable tool in our phytoplankton field studies, even though the dinoflagellates of interest are seldom the dominant source of fluorescence.

Alternative in-line devices could include an autoanalyzer (e.g., Technicon AutoAnalyzer II, available through scientific supply companies) to obtain detailed nutrient profiles. A transmissometer

could be included in-line to obtain turbidity data. Some sort of flow chamber must be designed for the transmissometer which is especially sensitive to bubbles. We suggest using a submersible transmissometer, located below the hose inlet.

The A/D board may have additional available channels. These can be used to digitize the signal from a light meter or pressure sensor attached near the hose inlet. Once again, these instruments require additional wires, which can cause tangling and kinking of the hose.

As mentioned above, we consider it essential to obtain coincident hydrographic and biological information. It is somewhat unfortunate that most dinoflagellate blooms are associated with strong signals in salinity: salinity is much harder to measure than temperature. Temperature is sometimes a useable surrogate for salinity, but not reliably. In dynamically simple areas such as shallow embayments, salinity can be measured adequately with a hand-held, relatively inexpensive refractometer. This device has an accuracy of about 0.2 ppt, which is reasonable for most coastal situations. If no more sophisticated instruments are available, temperature readings, measured with a thermometer, and salinity values, measured with a refractometer, should be taken at least at 0.5 m intervals. These measurements can be made on water exiting the hose.

Inexpensive battery-operated temperature/conductivity probes, such as those manufactured by InterOcean Systems Inc. (3540 Aero Ct., San Diego, CA 92123 USA) can be lowered to learn details of water column structure, but accuracy is limited. A more sophisticated system might include an in-line thermosalinograph (available through InterOcean Systems, Inc.). This instrument may also be interfaced to the computer, allowing rapid data aquisition and storage. The quality and durability of such instruments is of paramount importance: nothing is more frustrating than trying to collect data with unreliable instruments. Always try to test an instrument in the field before purchase, and talk to others who have used the instrument.

The instrument we recommend is a CTD (Conductivity, Temperature, Depth) profiler. We use the "Sea Cat Profiler" (Sea Bird Electronics, 1808-136th Pl. NE, Bellevue WA, 98005 USA). This small CTD stores data internally during a cast, thus no extra electrical wires are required over the side of the vessel. The data can be subsequently transferred to a personal computer using the various programs supplied with the CTD. The instrument itself is practically indestructible and foolproof. We generally mount our CTD below the hose inlet, with the sensors pointing upward in order to take data as the instrument is raised through the water.

Station Selection: Spatial

The decision of when and where to locate stations can be overwhelming. Coastal areas have notoriously complicated physical systems, requiring relatively dense spatial coverage. The main forcing over much of the year is the wind, which varies on time scales of hours and days. What is the best strategy for dealing with these problems, without spending your whole life at sea?

The first suggestion we make is to plan as dense CTD coverage as possible. Hydrographic data are an absolute necessity for interpretation of any data concerning biological distributions in the ocean. One criterion for deciding on CTD station spacing is the internal Rossby radius of deformation, Ri. This length scale is the natural length scale for most physical features in the ocean: the width of frontal zones, the size of eddies, the width of river plumes, etc. (Franks, 1990, in press). It is calculated from the thickness of the surface layer, h, the densities of the upper and lower layers, r and r', the acceleration due to gravity, g, and the Coriolis frequency, f:

$$R_i = \frac{gh\,(\rho' - \rho)}{f}$$

This length scale is typically < 5 km in most coastal regions. Hydrographic station spacing of this order or less will allow resolution of features such as fronts and river plumes. Since most dinoflagellate blooms are associated with such features, it is important to be able to resolve them with some confidence.

One of the main factors contributing to the complicated dynamics of coastal regions is the physical barrier formed by the coast. This feature will tend to align physical systems (e.g., wind-driven upwelling, coastal currents and river plumes) parallel to the coast. This means that hydrographic variables will tend to show greater changes across shore than along shore. For this reason, it is important to obtain good cross-shore coverage. We suggest sampling to at least 5Ri from the coast, in order that coastal features be adequately sampled.

Most physical systems in coastal areas tend to follow bathymetric contours. At areas where the curvature of the coast is very sharp, a physical feature such as a coastal current will separate from the coast and move into open water. The criterion for this separation is roughly given by the Rossby number, R_o:

One of the main reasons for recommending a CTD profiler is that it may be used without the hose pumping system. A vertical profile

with the pumping system may take half an hour, whereas a CTD cast need only take a few minutes. Thus dense CTD coverage may be obtained in an area with more sparse biological profiles. The variations in hydrography can be used to explain details of the biological distributions and dense CTD coverage will always make data interpretation easier. An additional advantage of many CTD profilers is that they may be expanded to include *in situ* fluorometers, transmissometers, light meters, O2 sensors, etc. These instrument packages are easily deployed even in fairly rough seas, when deployment of a pumping system may be impossible. They also allow dense sampling of a variety of fields.

One final word on sampling equipment: always plan for the worst. Bring spares of all pieces of equipment: hose, pumps, valves, connectors, radiator hose, hose clamps, etc. A supply of duct tape is a necessity. Always make contingency plans if any aspect of the sampling program should fail at sea, e.g., if the pump loses its prime, the computer fails, or the fluorometer breaks. Planning ahead for such emergencies will help to make the best out of a bad situation, and may prevent the waste of a lot of time and money.

Sampling Strategies

Here we describe various factors to consider when designing a sampling program. We examine certain physical parameters which merit consideration, and suggest some biological features which may influence timing and location of stations. Finally we describe some standard techniques of data reduction and interpretation.

$$R_o = \frac{u}{fr_c}$$

Here u is the velocity of the current, f is the Coriolis frequency for that latitude, and rc is the radius of curvature of the coast (or the bathymetry). If this number can be calculated, and it is greater than 1, the coastal current is likely to separate from the coast and move offshore. The sampling scheme should be adjusted accordingly, and more offshore samples taken. The orientation of the transects may also be adjusted: they should, in general, be oriented perpendicular to the feature being sampled. Near a sharp bend in the coast, the transects may be oriented almost parallel to the coast in order to obtain good hydrographic coverage of a coastal current (see Fig. 5).

If multiple transects are to be run, it is preferable that they be oriented parallel to each other. This will give even data coverage,

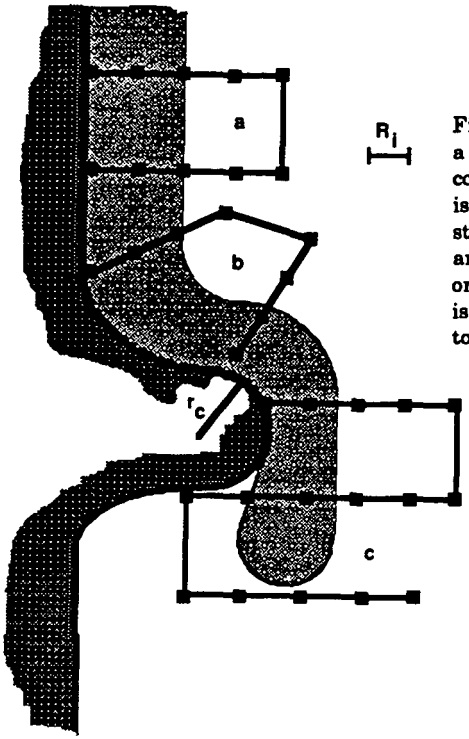

Fig. 5. An example of a sampling scheme for a coastal current which separates from the coast at a cape. In section a, where the coast is straight, the legs are parallel, and the stations evenly spaced. In section b, the legs are oriented perpendicular to the coastline or the bathymetry. More offshore coverage is made in areas where the feature is likely to separate from the coast such as section c.

with no poorly sampled areas. It may be more efficient, in terms of the ship, to orient the transects into a Z shape. However, relatively large gaps in coverage occur at the top and bottom of the Z. A more even coverage is obtained with an E-shaped cruise pattern. This will be found to be important when trying to contour the data and make surface maps of features.

In the vertical, dense coverage is always preferable. Some workers advocate binning of samples into three categories: below the thermocline, within the thermocline, and above the thermocline. We do not suggest using this procedure for several reasons: 1) the thermocline may not correlate with the dinoflagellate peak or the nutricline; 2) the sample resolution is low; 3) the variable depth of the thermocline will make data reduction and interpretation difficult; and 4) unexpected features may be missed. Rather, we recommend obtaining as many evenly-spaced samples as is feasible given sample processing time. The even spacing allows for quick and easy plotting of data, with good resolution of most vertical features. We use a binning interval of 5 m, and recognize that this spreads the cell concentrations and nutrients out vertically. Thus, a feature which is

1-m thick in the *in situ* fluorescence becomes 5-m thick in the cell counts.

We also stress obtaining coincident CTD data. These data will help to resolve binning problems by giving a detailed picture of the vertical structure of the water column. The more different types of data obtained in vertical profiles, the easier will be the interpretation of the data. For example, we have found transmittance to be a good inverse tracer for *in situ* fluorescence. Since they are obtained by very different methods (transmittance via submerged instrument, fluorescence via the hose), we are confident that the strong correlation between the fields is real. Thus the transmittance gives an independent check on the hose profiling system.

Station Selection: Temporal

Considerations for the timing of sampling are numerous. Physical factors such as wind, rain, tides and sunlight have all been shown to affect dinoflagellate distributions. Seasonal considerations are also important, as dinoflagellates often show fairly restricted periods of extensive growth.

The linkage of dinoflagellate distributions with hydrographic features is strong in both space and time. Forcing such as the wind and tides will have effects on both the hydrography and the dinoflagellate distributions and should be taken into account when planning cruises. However, for most sampling programs the size of the ship is the main consideration in timing cruises: a small vessel cannot be used in heavy seas and deployment of a pump profiling system may be impossible in even moderate swell. This causes aliasing with respect to the wind: cruises are only made in light seas. Our solution to this has been to make multiple cruises, as often as possible, and to interpret the CTD data with reference to continuous wind data obtained nearby.

Tides are more predictable, the semi-diurnal and fortnightly tides being the most prominent. A good knowledge of the local tides is important in deciding whether tidal advection or mixing will be a serious problem. In small embayments, the tides may be the predominant mode of forcing, while in shallow coastal areas, strong tides may create fronts which accumulate phytoplankon. If possible, samples should be taken at the same phase of the tide each time. For large-scale features, the tides may be relatively unimportant. At the very least, the phase of the semi-diurnal tide should be recorded at each station, in order that any aliasing be taken into account during data reduction.

Tides are also important when sampling continuously at a single station, for example when performing vertical migration studies. For this reason, samples should be taken at an interval of 3 hours or less, in order that all phases of the semi-diurnal tide be resolved. This may lead to a large number of samples to process, but the data will be less subject to misinterpretation.

Over a season the frequency of cruises depends largely on the organism being sampled and its suspected distribution. In general, though, at least one cruise should be taken before the particular dinoflagellate blooms, in order that the initial conditions of the water masses are known. When studying the distribution of a particular dinoflagellate, it is best to concentrate cruises early in the bloom. This will allow good resolution of the physical systems mediating bloom distribution as the bloom develops. In particular, numerous cruises can help distinguish between in situ growth, and alongshore advection, a particularly difficult problem in much dinoflagellate research.

If the dinoflagellate being studied is toxic, the local shellfish or fish monitoring programs can provide valuable information for planning the location and timing of cruises. We rely heavily on the state-run shellfish toxicity monitoring program for planning cruises: the timing of toxicity indicates how often cruises should be taken, while the spread of toxicity determines the extent and location of a given cruise. As the bloom spreads, we alternate between cruises along one transect, gathering detailed vertical information, with cruises having extensive alongshore coverage (in the E pattern), but less vertical resolution. In the latter instance, we are able to sample 25 stations (continuous CTD profiles, 2 bottle casts for cell counts at each station) from a fast (18 kt) 10-m vessel in 8 hours. Our single transect with more detailed data at each station covers only 5 stations in the same time from a 15-m ship.

In the absence of a monitoring program, there are some environmental cues which correlate well with dinoflagellate blooms. Most dinoflagellate blooms are found in the pycnocline of a well-stratified water column. This stratification can be caused either by salinity differences or by heating. Thus strong rains or several sunny days in a row can be important in bloom formation. Keeping a close eye on the weather is important when sampling for dinoflagellates.

Data Reduction: Smoothing, Plotting and Interpretation

The detailed vertical coverage obtained by a pump profiling system with a CTD is both a boon and a bane: the large amount of

data provides an excellent picture of the vertical distributions, but data processing can be tedious and time-consuming.

One of the main problems in using a variety of instruments is the diverse nature of the data sets generated. Merging these data sets into a single visualizable data set can be a difficult task. The techniques of data smoothing and interpolation become indispensable at this stage. Data smoothing is generally necessary to remove instrument spikes and noise. A variety of tools exists for smoothing data; we recommend the use of cubic splines. An excellent book, "Numerical Recipes" (Press et al. 1986) describes the use of a variety of splines, and gives computer codes for their implementation. A useful offshoot of the smoothing process is that the smoothed data are easily interpolated. Thus vertical profiles obtained with a variety of instruments can be interpolated onto the same vertical grid, and merged into a single data file.

Data gathered via the pumping system (e.g., fluorescence) will generally be stored as a time series, whereas CTD data are plotted versus depth. To merge a time series with a depth profile, some simple manipulations of both data sets may be necessary. First, the CTD data and the time series data must be converted to the same sampling rate. Thus, if the CTD samples at 0.5-second intervals but the fluorometer samples at 2-second intervals, every four CTD data points should be averaged. The unwanted portions of each data set should then be removed (i.e., the CTD up- or downcast data and the fluorescence data up to one hose-transit-time from the start of the profile). The two data sets can then be merged, line for line, creating a single data set. This data set can then be smoothed and interpolated onto a vertical grid for plotting depth profiles.

Discrete data such as cell counts, chlorophyll samples, or nutrient data can be smoothed and interpolated in the same manner as the CTD data. However, the low vertical resolution makes this an unreliable method of data manipulation. We prefer plotting such data as histograms in a vertical profile (Fig. 6). This method leaves no ambiguity as to sample location and lets the readers draw their own conclusions as to adequacy of sampling. Fig. 6 demonstrates another useful property of cubic splines: they can be used for calculating gradients of quantities. Once the coefficients for the spline are known, it is easy to differentiate them to find the slope of a property. This process is described in Press et al. (1986) and computer codes are given for implementation. In the case of Fig. 6, we have calculated the Brünt-Väisälä, frequency, or vertical gradient of density, N^2:

$$N^2 = \frac{g}{\rho} \frac{r\,\partial\rho(z)}{\partial z}$$

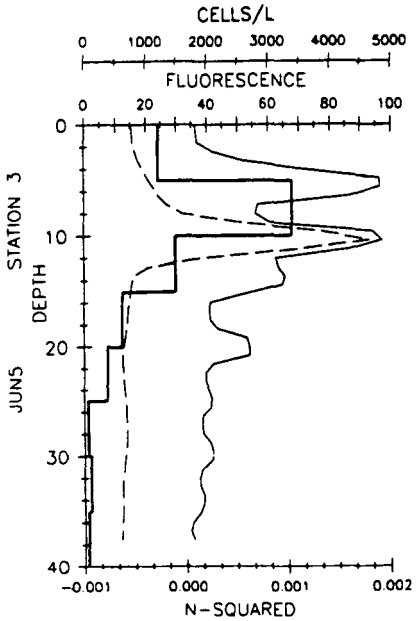

Fig. 6. Vertical profiles of cell concentration (*Ceratium longipes*, cells/l, heavy solid line), *in situ* fluorescence (dashed line), and N^2 (light solid line). As described in the text, maxima in N^2 correspond to maximum gradients in density (pycnoclines). The cell counts, binned into 5-m intervals, show a maximum coincident with the peak of *in situ* fluorescence, and the deeper pycnocline.

Here g is the acceleration due to gravity, r(z) the vertical density profile, and z the vertical coordinate. This quantity will show a maximum where the density gradient is strongest (i.e., the pycnocline). Thus it is very useful for assessing correlations between the density and other fields. As can be seen in Fig. 6, the maximum cell concentrations are found at the maximum gradient in density.

Plotting data is an art in itself. Numerous plotting packages exist for personal computers which allow quick and easy visualization of data. However, many of these packages will not allow plotting in the standard oceanographic format: a decreasing vertical axis. One way around this is to manipulate the data so that the depth is negative, with the surface being zero. This is not a very elegant solution, but cannot be avoided in many cases.

Plotting the vertical profiles of several properties on a single set of axes is a very useful means of visualizing the vertical correlations of various fields. Such a plot is shown in Fig. 7, where the raw and smoothed data sets are shown for comparison. The strong correlation between the phytoplankton fluorescence and the water density becomes obvious. This plot also shows some of the problems associated with smoothing data. These data were smoothed using an objective mapping routine after Levy and Brown (1986). The main problems associated with smoothing appear at the boundaries of the data set, in this case the surface and the bottom. In Fig. 7 it can be

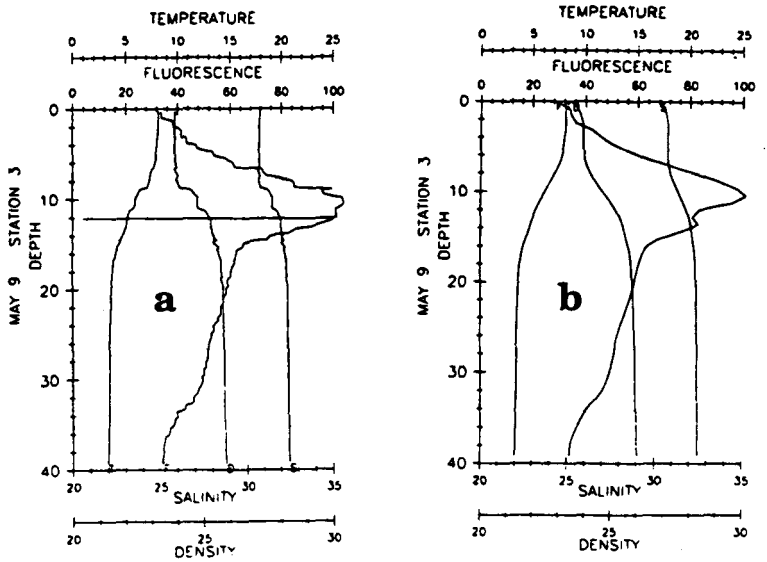

Fig. 7. Plots of the vertical profiles of temperature (°C), salinity (ppt), density (σT), and in situ fluorescence (relative units). Panel a shows the raw data (approximately 500 points in each profile), while panel b shows the smoothed profiles (50 points per curve). The small letter at the top or bottom of the profile indicates the property being plotted (T,S,D or F). Note that the smoothed salinity and density profiles tail off at the surface, while the raw data are vertical. This is an artifact of the smoothing program used.

seen that the smoothed data set shows a gradient at the surface, whereas the raw data show no such slope. This occurs because of the low number of data points near boundaries. Cubic splines can overcome some of these problems, but it is important to check the smoothed data against the raw data before conclusions are drawn. The second point where the smoothing program fails is in removing the instrument spike in the fluorescence at 12 m. Since we know that this spike was due to the instrument changing scales, and was not a real feature, we could manually remove the spike from the raw data before smoothing.

If many vertical profiles have been generated, it may prove fruitful to examine horizontal variations in properties. In this case we recommend contouring of data. Contouring of vertical profiles is a surprisingly difficult and frustrating task. If the stations were not evenly spaced, some interpolation scheme must be available for mapping onto a regular grid. If the depths of profiles vary because of bathymetry, some method should be available for masking out the bottom. Without this, the contouring program will generally create its own data in this region.

Given these caveats, contouring is still an extremely powerful tool in oceanographic research. A series of contour plots of some of our own data are shown in Fig. 8. The strong correlations of the diverse fields are immediately apparent from this type of plot, and the vertical variability is easily visualized.

The highlighting of vertical variations between stations is the most important aspect of contour plots. Most of this variability is caused by physical forcings; different types of forcings will show characteristic patterns in the cross-shore hydrography (Franks, in press). With experience, recognition of the particular physical system becomes more routine. Fig. 8 shows a river plume front, with an associated population of *Alexandrium tamarense*. The salinity gradient indicates the presence of the river plume, and the slope of the pycnocline suggests that it is moving towards the reader. The

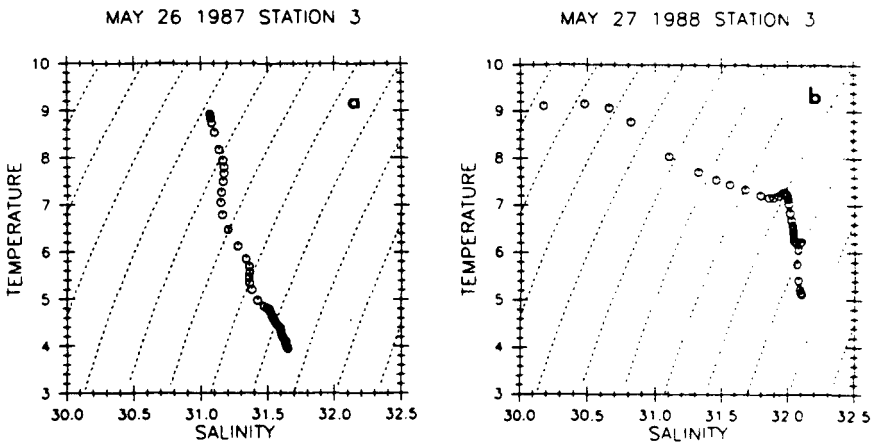

Fig. 8. Temperature/salinity plots from the same location, one year apart. The dashed lines are curves of constant density. Panel a shows a profile with a thermocline, while panel b shows a profile with a halocline. Note that the temperature range in the two panels is the same, but the salinity range is much less in a than b. The profile in b covers a much wider range of density.

magnitude of the slope allows an estimate of the current speed: ~10 cm/second. An introductory physical oceanography text will explain how these calculations are made.

Much information about dinoflagellate populations can be obtained by identification of water masses. This is most easily done using temperature/salinity (T/S) plots. The convention in oceanography is to make temperature the vertical axis, and salinity

the horizontal axis. Plotting a vertical profile on such axes makes individual water masses readily apparent: deep water masses are cold and salty, thermoclines and haloclines will point at right angles to each other, and the surface water will be warm, fresh or both. Thus water below the pycnocline will cluster toward the lower right of the plot, while surface water will be toward the top or the left, depending on whether the pycnocline is due to temperature or salinity, respectively. In Fig. 9, two T/S plots are shown from the same location a year apart. Fig. 9a shows a typical profile with a thermocline, while Fig. 9b shows a profile with a halocline. The differences between these are obvious and demonstrate the variability possible in the hydrography of coastal regions. Such plots

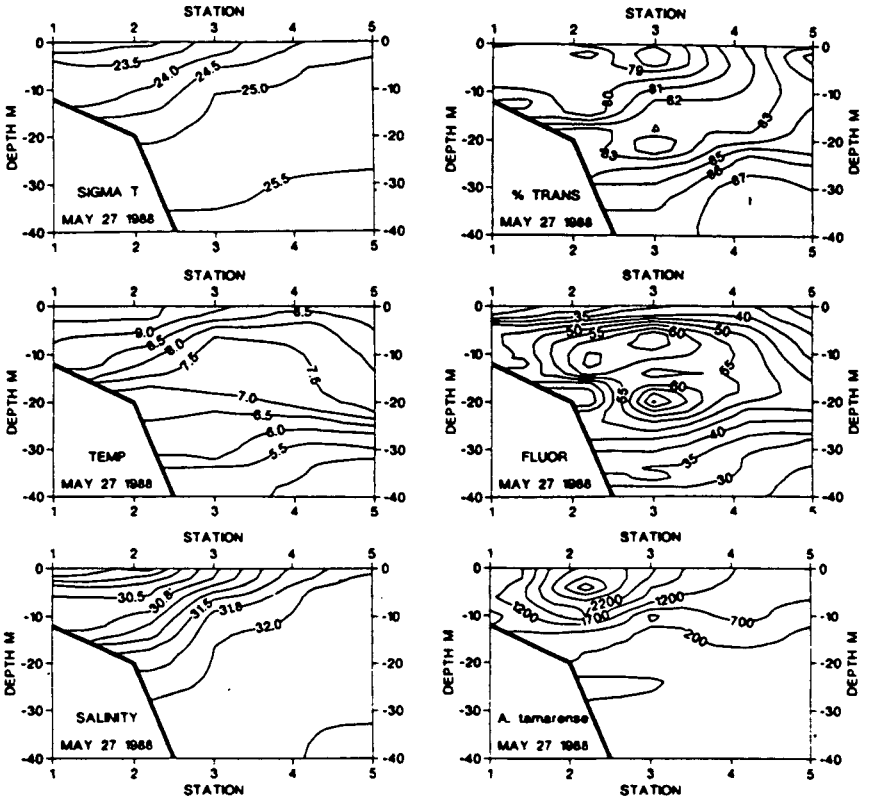

Fig. 9. A series of contour plots of data gathered in the Gulf of Maine. Clockwise from the upper left: density (σT), % transmittance, in situ fluorescence, cell concentration (Alexandrium tamarense, cells/l), salinity (ppt), and temperature (°C). The thick line at the bottom left of each panel is the bottom. The maximum depth is 40 m, the length of our hose. The stations are ~7 km apart; station 5 is 30 km offshore. Note the strong, sloping pycnocline created by the salinity gradient. The cells are found mainly within the fresher water and the pycnocline.

also bear strongly on the distributions of cells: in Fig. 9a, the pycnocline is formed by heating, so we might expect the cells to be horizontally uniform. In Fig. 9b the pycnocline is formed by a salinity gradient, indicating the presence of a river plume. We might expect considerable horizontal variability in cell concentrations due to their association with the river plume front.

Numerous other tools exist for visualizing data. Plotting a profile versus density rather than depth can be a useful way to assess correlations with the hydrography. Three-dimensional plots of density surfaces can lead to insights into the local dynamics. An introductory physical oceanography text, taking physical oceanography courses, or obtaining the help of a local expert for interpreting complicated hydrographic data are useful steps.

Of all the points made above, none can be stressed more than the importance of obtaining coincident hydrographic and biological samples. It is only with a good knowledge of the local physical dynamics that confident interpretations of biological patterns can be made.

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During the Brunei Darussalam workshop, a field trip enabled participants to see some field equipment in action. Photos show: 1. F.J.R. (Max) Taylor demonstrating his compact 20 μm plankton net. 2. Yasukatsu Oshima has a similar net. 3. Sherwood Hall uses a 5-m long net to harvest cells for toxin analysis. 4. Fernando Rosales-Loessener inspects a plankton sample in a capillary tube using a hand lens. 5. Tomotoshi Okaichi demonstrates a hand microscope.

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Using a sediment corer during the field trip. 6. Kelly Rironro about to release the corer, watched by Ranjith De Silva (left). 7. A participant from Brunei Darussalam extracts the tube containing the sediment sample, under the watchful eye of Yasuwo Fukuyo (right). 8. Webber Booth holds a core sample inspected by Ranjith De Silva (left) and Willy Pastor (right). Yasuwo Fukuyo is reassembling the corer. 9. Webber Booth and Yasuwo Fukuyo storing and labelling sediment samples.