Bioacoustical oceanography at high frequencies

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High-frequency acoustical technology is used to measure the spatial distributions of small zooplankton with meter-scale resolution in the vertical, resolutions of hundreds of meters or better in the horizontal, and with temporal resolutions of minutes. Both size and abundance measurements can be made by using sensors in a cast mode from a ship, from undulating towed bodies and by placing instruments on moorings. This presentation includes an overview of some of the technical concepts that are involved, examples of the use of the sensors and systems that have been developed and brief discussions related to the algorithms used to convert acoustical measures to biologically relevant estimates for small zooplankton.
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Introduction

The research was originally stimulated by a recognition that the conventional sampling technologies of the day (e.g. nets and pumps) were limiting progress in biological oceanography, especially in the resolution of questions on zooplankton ecology. Additional stimulus and interest was generated by the paucity of quantitative volume-scattering data at frequencies above about 30 kHz. Since the early 1970s, there has been considerable success in applying modern acoustical technology to the problem of sampling small zooplankton. Much of the acoustic volume-scattering at very high and ultra-high acoustic frequencies (100 kHz to 10 MHz) in the sea can be explained by the distribution of small zoo-plankton and micronekton. The understanding of acoustic scattering has been advanced, based on the morphology and physical properties of the sound-scattering organisms. This technique is sufficiently robust that it is possible to obtain useful estimates of the abundances, including size, of small zooplankton using acoustic technology. These estimates can be made on meter scales in the vertical, on scales of hundreds of meters or better in the horizontal, and on temporal scales of minutes at a fixed location. Placed in the context of ancillary measurements of the physical oceanography and estimates of phytoplankton biomass (fluorescence or optical attenuation), the acoustic estimates can reveal both coherences and the lack thereof in different oceanic and coastal marine environments, between zooplankton, phytoplankton, and physical fields.

While there are challenging technological problems that remain untreated, or that have only been partially addressed in zooplankton acoustics, data from these new acoustic tools have already revealed a complex, patchy, dynamic ocean environment, and a variety of biological responses to the heterogeneity of local ocean physics in both time and space. A few examples of the data collected over several years in different ocean areas are illustrated here, but the reader is referred to the literature cited in the references and bibliography for many more examples of data, as well as more detailed descriptions of the various kinds of sensors, systems, and modes of deployment than can be included here.

Methods and approach

Background

Underwater sound has been used for several decades to monitor stocks of pelagic fish. A good description of the acoustic methodology which has evolved for that purpose resulted from discussions in the Working Group on Fisheries Acoustics Science and Technology (FAST) of the International Council for the Exploration of the Sea (ICES), and can be found in Simmonds et al. (1992).

One of the more serious constraints in fisheries acoustics is that innovation has been suppressed in favor of "standardization". Changes to implement new technology are not easily introduced into the "quasi-political", international fisheries assessment community, even when

new information or technological change might result in a better absolute biomass assessment. Reasons for this vary from country to country but, in general, they are related to maintaining comparable methods and data when comparing inter-annual or multi-platform assessments which are used to establish fishing quotas. Such changes could lead to costly and time-consuming challenges in legal systems which have difficulty dealing with technical issues.

Basic research into the ecology of zooplankton and micronekton deals principally with science rather than with management, political, and economic issues. Plankton acoustics researchers have therefore had the freedom to operate without some of the essential limitations that have constrained the older field of fisheries acoustics. As a consequence of this freedom to explore unique ways of approaching the problem, several important distinctions have evolved and now stand out as critical differences between the approach taken by our research group and conventional approaches traditionally used by fisheries scientists.

Early in our work a decision was made to describe the basic physics of the scattering process and to use that information to create and validate mathematical models to describe scattering from the animals that we wished to study. These models would, hopefully, include enough measurable parameters so that one could extract more information from measuring the scattering than would be possible with the more traditional model that is based on empirical regression techniques. Our initial approach was to propose mathematical models to describe the acoustic scattering expected from animals, based on scatterer shape, size, and the acoustic contrasts due to density and compressibility differences with sea water. The predicted scattering was compared with measurements of scattering from live animals in order to "validate" the mathematical models. This process is generally called "the forward problem" in zooplankton acoustics. The "inverse problem", more interesting and also more challenging, is to measure acoustic scattering and to extract information about the biophysical characteristics (e.g. size, presence of a gas bubble, etc.) of the organisms from those measurements (Holliday, 1977a; Greenlaw, 1979).

In acoustics, there is a heavy reliance on quantifying parameters in relation to widely accepted standards and units. Those standards allow investigators to compare results (inter-annually and across systems), manufacturers of equipment, and different platforms. The standards used in acoustics are internationally accepted measures, such as meters, pascals, and seconds. There are also less codified, but equally precise, standard definitions and terms. For example, "calibration" in acoustics takes on a special meaning. It means the determination of the transfer function between pressure and voltage or current in the internationally recognized units of volts,

amperes, meters, kilograms, and seconds. The use of widely accepted "standard units" and "standard definitions" (e.g. volume scattering strength) is also philosophically different from making measurements with "standardized instrumentation". In addition, "intercomparison" should be used when a biologist determines a regression between an acoustic measure and the biomass collected by conventional methods. Our work in zooplankton acoustics emphasizes measuring and reporting quantities in "absolute" rather than relative units. While numerous comparisons have been made between our acoustic estimates and the results from conventional sampling with pumps and nets to evaluate our techniques, we do not rely on these comparisons to "calibrate" our method.

In zooplankton acoustics, history tends to support the idea that standardization of sensors or methods, without a sufficient understanding of the underlying physical principles, would have been a serious mistake in the development of the technology. Had we settled on a specific system, or even a limited number of frequencies, with which to sample zooplankton acoustically, there is little likelihood that there would have been a robust capability today to estimate zooplankton size acoustically. More critically, the underlying physical principles would have guaranteed that single-frequency abundance estimates would often have been suspect. Extrapolation of results collected at one frequency would not necessarily have been useful in understanding results obtained at another. This is due to the inherently non-linear, non-monotonic dependence of target reflectivity on both acoustical frequency and organism size. Understanding this dependence at a basic level led ultimately to the use of the inherent complexities of scattering to allow extraction of both size and abundance information from acoustic scattering measurements.

The distribution of marine zooplankton in the ocean is sufficiently heterogeneous that it would be extremely challenging to create a rigorous, robust empirical regression between acoustic scattering and zooplankton biomass by attempting quantitative sampling with conventional nets and pumps, even if a simple relationship were to exist. In addition to problems of collecting truly comparable samples from the same population with both acoustical and direct sampling tools in a patchy environment, there are serious problems with avoidance and extrusion with nets or pumps. This heterogeneity, which we have observed on spatial scales of meters, limits the degree to which we can test our acoustic techniques. To avoid a dependence on these sampling problems we rely on understanding the basic physical and biological processes that relate acoustical scattering to the size, abundance and morphology of the animals. We determined that the agreement between the size–abundance structure measured acoustically (multiple frequencies) and the result from sampling
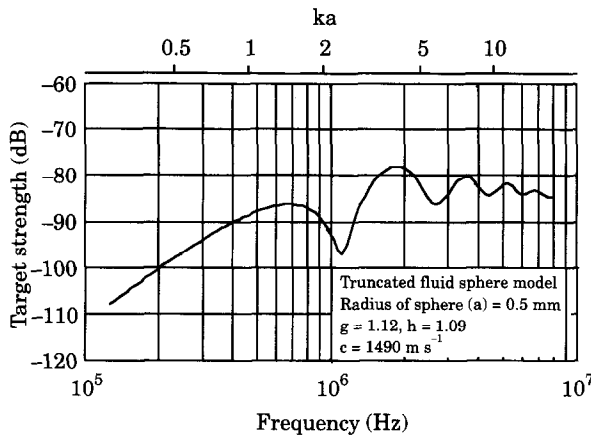
Figure 1. Target strength versus frequency, based on the truncated fluid sphere scattering model. The symbol g is the density contrast of the scatterer and sea water, and h is the compressibility contrast. The speed of sound (c) is representative of sea water at typical salinities and temperatures. The target strength is in decibels (dB). The scatterer equivalent spherical radius (a) is the radius of a sphere with the same actual volume as the scattering organism, which is rarely spherical. This quantity is also frequently designated in the literature by the symbol "esr". A scale for the product of the wave number and the radius (ka) is also shown. For an animal with a shape like a calanoid copepod, multiplying the equivalent spherical radius (a) by between 4 and 5 will approximate the animal's length.

(high-volume plankton pump) is as good as the agreement between two pump samples spaced 1 min apart (Costello *et al.*, 1989).

Target strengths of small zooplankton and micronekton

The scattering from an object depends on the contrasts between various material properties and the same physical properties of the surrounding medium, in our case, sea water. The critical material properties in zooplankton acoustics are the ratio of the sound speed in the scatterer to that in water as well as the ratio of the compressibility of the body of the organism to that of water. Scatterer size is also a critical variable in the target strength (acoustic reflectivity) of any object, including zooplankton. More generally, the ratio of scatterer size and the wavelength of the sound in the surrounding medium determine the dependence of target strength on size and frequency.

The dependence of scattering on size and frequency (Fig. 1) is such that when the product of the wave number for the sound (k=2πf/c, where f is the acoustic frequency used and c is the speed of sound), and the scatterer size (a) is small, i.e., ka ≪ 1, the scattering is considered to be in the "Rayleigh" domain. When ka ≫ 1, the scattering is characterized as being in the

"geometric" region. Near ka = 1, the scattering is in the "transition zone". This characteristic form of the scattering curve is modulated in level by the sound speed and compressibility ratios mentioned earlier. Fixing k, by specifying frequency and making k a constant, shows the dependence of scattering on variations in the size, a. Similarly, if the scattering is from a target of a single size, a, then fixing "a" shows what would happen at a variety of frequencies. The curve illustrated in Figure 1 is based on a simple model for small zooplankton. The truncated sphere model (Holliday, 1992) has served relatively well for predicting and interpreting results when the ecosystem was dominated by copepods and other small crustaceans. Other models may be more appropriate for different species and different shapes. For example, euphausiids, being relatively elongate compared with calanoid copepods, are better described by a model based on a cylinder, rather than a sphere (Stanton, 1988, 1989a). At a basic level, however, the rough shape of the dependence of target strength (TS) on ka is as shown in Figure 1, with variations in the precise position of the transition zone and the peaks and quasi-nulls located in the geometric regime.

Using single frequencies

The approach in fisheries acoustics

Single-frequency acoustical estimates are currently widely accepted for fisheries assessment (Simmonds *et al.*, 1992). There are good reasons for believing that the single-frequency approach routinely provides at least internally consistent relative abundance estimates for some fish stocks with a precision that is accurate enough for stock management purposes. The sound wavelengths typically used in the assessment of nekton are quite large relative to the size of a fish. This, and the complexity of fish as acoustic targets, work to one's advantage in fisheries acoustics. Zooplankton acousticians, however, typically use wavelengths that are on the order of the size of the animals in order to maximize the reflections obtained from relatively small, weak zooplankton scatterers.

Limitations due to non-monotonicity of target reflectivity with frequency and size

Because of the wavelengths used in zooplankton acoustics, when measuring backscattering at only one frequency, increases in scatterer size can lead to an increase in acoustic scattering levels, to a decrease, or to no change at all (Fig. 1). Likewise, changes in the acoustic frequency can lead to either increases or decreases in scattering levels. This results from the non-monotonic form of the relationship between target strength, size,

and frequency. This general form varies somewhat with scatterer shape, aspect angle, etc., but most realistic models will exhibit resonances and interferences with varying degrees of non-monotonicity (e.g. Anderson, 1950; Pieper and Holliday, 1984; Stanton, 1989b). While the curve in Figure 1 is for individual scatterers, the same phenomena apply to aggregations of scatterers.

It is critical to remember that the non-monotonic character of the scattering curves means that there is no way to distinguish uniquely a change in size or a change in abundance when using a single acoustic frequency. One could, in principle, have more larger size animals present, and observe lower acoustic scattering levels than with fewer, smaller animals.

Assumptions for single frequency work

If one must work with a single frequency, one should ensure that the following assumptions are valid: (1) A single size organism dominates the acoustical scattering; (2) the organism size is known; (3) there is a tractable, validated model for the organism that relates its size and the acoustical frequency in use to its reflectivity (target strength) in quantitative terms; (4) multiple scattering effects are negligible; (5) shadowing effects are negligible; (6) any dependence of the reflectivity on any other parameter, e.g. depth or temperature is known; (7) the measurement platform, environment, and distribution of organisms are sufficiently stationary (wide sense) that one can estimate the power in the echoes.

Although these conditions are restrictive, ecosystems and environments can be identified within which single-frequency work may be quite valuable. In general, however, one must examine each of these constraints individually. A further caution is that one must always consider all of the organisms present in the study area from which sound may be scattered, not just the species of interest to the investigator.

Algorithm

If the conditions listed above are met, the relation between the abundance of organisms and distance from the transducer face (z), e.g. depth, for a down-looking system, N(z), is given by:

$$N(z) = <\sigma_v(f_0,z)>/\sigma(f_0,a_0).$$

$<\sigma_v(f_0,z)>$ is the ensemble average of single-ping measurements of volume-scattering strength, corrected for propagation losses and system constants such as source level, beam directivity effects, receiving sensitivity, absorption, and sound-spreading. The ensemble average is necessary since each echo realization is random because of the random distribution of scatterers and changes from ping to ping. Statistically, in a temporally

and spatially stationary situation, the quantity $<\sigma_v(f_0,z)>$ is the expected value of the ensemble of echoes. The quantity $\sigma(f_0,a_0)$ is the acoustical scattering cross-section for an individual of known size (a_0) at frequency (f_0). The target strength, TS, and the scattering cross-section, σ, are related by: $TS(dB) = 10 \log_{10}[\sigma(f_0,a_0)/4\pi]$.

Implementation

Single-frequency echo-sounders (vertical beam orientation) and sonars (horizontal beam) are common at frequencies that are appropriate for fisheries research. There are also a few systems that are utilized to collect upward-looking profiles. A few such systems are also commercially available at frequencies appropriate for micronekton, but they are not optimal for small zooplankton. To achieve a good signal-to-noise ratio one needs a frequency that would optimize both the effects of absorption of sound in the water, system and ambient noise in the sea, and the target reflectivity at or near the organism size. For small zooplankton this means a frequency in the transition zone, e.g. about ka=0.8 to 1.8 (hundreds of kHz to tens of MHz) where the absorption of sound becomes a limiting factor. Consequently, one must either accept limited ranges for the performance of a sensor or use a system that is designed to be deployed down the water column.

An example

The following example from 15 km south of Los Angeles is as much a warning about using a single frequency to assess zooplankton as it is an example of the use of single-frequency acoustics. The dependence of volume-scattering strength on depth is illustrated at four frequencies (165 kHz, 420 kHz, 1100 kHz, and 3000 kHz). The system used was a four frequency Tracor Acoustic Profiling System (TAPS). Depth and temperatures were also measured and logged internally by this system. The acoustical data were collected rapidly on interleaved ping cycles that ensonified overlapping volumes of nearly the same size (<0.1 m³). Thus, the population of zooplankton examined at each frequency was as close to the same assemblage of individuals as it is currently possible to achieve at sea from a ship. Data have been averaged into 2 m bins (Fig. 2).

Note that the depth-dependence of volume-scattering strength depends strongly on the frequency. With the data from any single frequency, discrete vertical structure, e.g. scattering layers, some of which appear at other frequencies and others of which do not, can easily be identified. With only a single-frequency profile and no additional information, it is not possible to relate these curves to the vertical distribution of zooplankton biomass because the ecosystem was not dominated by a single-size organism.

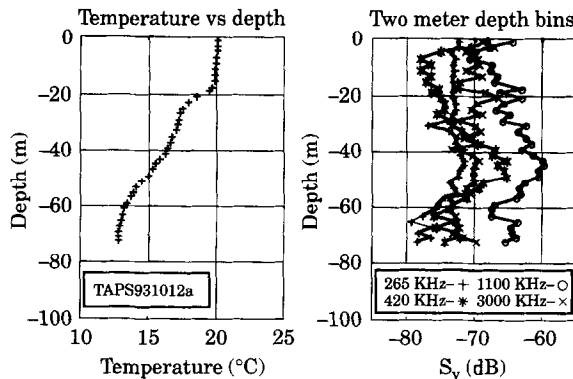Temperature vs depth

Two meter depth bins

Figure 2. Temperature and four-volume-scattering strength profiles collected during daylight hours at the shelf slope break on the northern edge of the San Pedro Basin, south of Los Angeles, USA on 12 October 1993.

A detailed examination of the profiles reveals that there is not a simple relationship between any of the four volume-scattering strength curves and any other. The scattering levels also do not increase monotonically with increasing frequency. For example, between 40 and 50 m the scattering at 420 kHz exceeds that at both 256 kHz and 1100 kHz. These observations, at first glance, are somewhat discouraging, since a simple interpretation that increased scattering means a higher abundance or more biomass is clearly suspect. However, it was similar data that first led us to a detailed examination of the basic physics of sound-scattering by zooplankton.

Adding frequency as an independent variable in zooplankton bioacoustics

The motivation for using more than one frequency

Having recognized the possibility that dependence of target strength and volume reverberation on frequency may give information on the size–abundance structure of a zooplankton community, quantifying that dependence was the next step. For the zooplankton ecologist, the size–abundance structure of a population can be an indicator of the processes that drive the community. The combination of rapid, quasi-synoptic acoustic surveys over large areas, with rapid processing of the acoustic data to reveal the size and abundance structure of the zooplankton with depth, and in relation to ocean physics is an appealing prospect. In carefully chosen marine and limnological ecosystems, the size–abundance structure might even be sufficient to allow identification at a species level based on measurements of size alone.

Mathematical scattering models

The complex dependence of scattering on frequency is what makes it possible to extract the embedded size–

abundance information from multifrequency acoustic data. Physically, this complexity arises from constructive and destructive interferences in acoustically induced vibrational modes of the zooplankter. When extracting size–abundance structure from multifrequency data, i.e. when solving the "inverse" problem, the accuracy of the biomass estimate and the resolution of the size structure depend strongly on having made measurements at frequencies that are both sufficient in number and appropriately placed to accurately define detail in the target strength structure. This applies both to the scattering from individuals and the aggregate. An overview of the "inverse" problem and its dependence on the models one uses can be found in Greenlaw and Johnson (1983).

Several mathematical models were selected which seemed appropriate to describe acoustic scattering from small zooplankton. Anderson's (1950) fluid sphere model and Johnson's (1977) high-pass approximation to the full fluid sphere model were among those examined. Volume-scattering strengths were measured for natural assemblages of zooplankton in the sea at four frequencies that were selected to test these models (Pieper and Holliday, 1984). Comparison of the acoustic measurements and estimates of what the volume-scattering strengths should have been if the theoretical models were accurate revealed that the Anderson fluid sphere model was much better than the Johnson high-pass model. The monotonic model did not predict the "inversions" in scattering level such as those pointed out above. While to a first order, the fluid sphere model replicated the measured scattering when we used it and the results of conventional, direct, high volume pump sampling to work the forward problem, there were significant deviations. The full Anderson model is quite sensitive to the scatterer's physical shape. In particular, it assumes a degree of symmetry that is unrealistic for most zooplankton. From a physical and mathematics view, we hypothesized that removal of these symmetries would suppress all but two of the motions of a plankter in an ensonifying pressure field. Consequently, we removed all of the higher-order terms in the fluid sphere model that depended on symmetry except the first radial mode term. We also left the dipole motion term, which describes the translational motion of the particle in the sound pressure field, intact. We refer to this modification of the fluid sphere model as the truncated fluid sphere model. The mathematics can be found in Holliday (1992).

Calculations based on the truncated fluid sphere model were compared with measurements of scattering from individuals. Having established that the target strengths obtained from preserved, freshly dead or frozen animals were not representative of live ones (Greenlaw, 1982), multiple measurements were made from about 40 species of live marine zooplankton in a shipboard tank at sea. The measurements of the

individual target strengths were made over a very wide frequency range from 100 kHz to 10 MHz using six discrete transducers and an impulse technique developed for use at lower frequencies (Holliday, 1972). The results confirmed that the truncated fluid sphere model was a slightly better representation of the scattering from small zooplankton than was the full fluid sphere model (Pieper and Holliday, 1984).

There are important ongoing efforts to improve the models available to describe the scattering of sound from zooplankton, micronekton, and fish. For example, Stanton has modeled and measured scattering from infinite cylinders, finite length cylinders, bent cylinders, rough cylinders, and several combinations of these variations (e.g. Stanton, 1988, 1989a, b; Gurley and Stanton, 1993; Stanton et al., 1993a, b). These models may be particularly appropriate for describing scattering from krill, shrimp, some amphipods, and other elongate animals. Weston (1967) proposed a model (based on one proposed earlier by Andreeva (1964)), which until recently has been the best available for fish with swimbladders. Clay (1991, 1993) has recently published a new model for fish with swimbladder that compares well with available experimental data and should be subjected to additional testing and validation. These models show promise for extending results on zooplankton to more complex organisms with different shapes, allowing perhaps the identification of biophysical details in addition to size (e.g. shape, internal structure, presence of a gas bubble, rigid exoskeleton, or shell). More importantly, however, good models could eventually lead to automation of a process for acoustically classifying and segregating organisms by their biophysical characteristics. For example, with the rapid increase in computing power of the last decade and accurate scattering models for fish, fish larvae, euphausiids, and copepods, one could potentially implement and test untried features of the original basic algorithm for the inverse problem in zooplankton acoustics. In the long term, one could foresee rapid automatic classification of animals into groups, with simultaneous estimation of the size spectra of each group and estimates of the abundances at each size (see Holliday, 1977a).

Having confirmed that there was a reasonably adequate model for scattering from small zooplankton, we proceeded to build a Multifrequency Acoustical Profiling System (MAPS) with which we could make measurements of volume-scattering (reverberation) in the sea by profiling in a cast mode or operating in an undulating towed mode.

A multifrequency method

Assumptions

When more than two frequencies are available to the investigator, one should ensure that the following assumptions are valid: (1) There is a tractable, validated model for the organism that relates its size and the acoustical frequencies to its reflectivity (target strength) in quantitative terms; (2) multiple scattering effects are negligible (can be slightly relaxed if only size is required. In practice, this is rarely a problem with zooplankton); (3) shadowing effects are negligible (can be slightly relaxed if only size is required. In practice, this is rarely a problem with zooplankton); (4) any dependence of the reflectivity on any other parameter, e.g., depth or temperature is known; (5) the measurement platform, environment and distribution of organisms are sufficiently stationary (wide sense) that one can estimate the power in the echoes from an ensemble of scatterers; and (6) the frequencies used must span the transition from Rayleigh to geometric scattering for all of the organisms contributing to the acoustical scattering (it is mandatory that this condition be met for *all* organisms present rather than just those of interest in the science that the acoustic sampling is supporting!).

Algorithm

The basic algorithm used to "invert" multifrequency data (Holliday, 1977a) has been successfully used to study small zooplankton (e.g. Kleppel et al., 1988; Holliday et al., 1989; Pieper et al., 1990; Smith et al., 1992; Napp et al., 1993), krill (Greenlaw, 1979), mesopelagic fishes (Kalish, 1986), and epipelagic fishes (Holliday, 1977b, 1985).

There are several mathematical approaches by which the algorithm can be implemented. The method we have used most often is the non-linear least-squares (NNLS) method (Lawson and Hansen, 1974). We determined experimentally that the NNLS method will usually allow a solution for cases that are under-determined by factors of between 1.5 and 2. This has generally been the case for the distributions of small zooplankton we have encountered from a wide selection of geographic areas and ecosystems, e.g. the California Current, the Gulf Stream core, the slope water of the Northwest Atlantic, the coastal transition zone off northern California and the Irish Sea. Nevertheless, the astute investigator will carefully examine the data and the convergence of the inverse calculation for each case when making use of the ability to find under-determined solutions. The unexpected presence of scatterers for which the scattering models implemented in the NNLS code are not appropriate can potentially introduce data-dependent errors if the method is used blindly.

Implementation

MAPS is described elsewhere (e.g. Holliday et al., 1989) and only a brief overview will be given here. It consists of 21 narrow beam sonars, operating at discrete

frequencies between 100 kHz and 10 MHz. Because of the high acoustic absorption at these frequencies and the relatively low reflectivity of the small organisms, MAPS must be used in either an undulating, towed mode or a cast mode in order to position the instrument near the organisms to be studied.

The sonar beams ensonify small overlapping volumes (ca. 0.01 m^3) horizontally displaced from the MAPS instrument in rapid sequence (0.7 s). A very small volume was intentionally chosen to discriminate against larger organisms with relatively small numerical abundances, e.g. adult fish, fish larvae, or euphausiids, in favor of those organisms that occur in abundances of several per cubic meter or higher. Fish or larger micronekton can often be identified and even enumerated by their target strength, the history of occurrence during multiple echo-ranging cycles and the envelope statistics of the echoes. When the echoes are received from the scatterers in the ensonified volume, echo-integration is performed on the square-law detected signal within a programmable range gate. In much of our work, we have found that, in practice, the range gate is usually set to begin at about 2 m and to end at about 3 m horizontal distance from the transducers. This trade-off involves such considerations as maximizing the reverberation levels, minimizing the effects of transducer ringing, minimizing absorption effects and having a small enough sampling volume to discriminate against being dominated by scattering from numerically rarer, larger organisms. Ensemble averages from multiple-echo ranging cycles (e.g. 16 pings) are computed and the estimate of the acoustic power in the echoes is made with corrections for such variables as absorption, spreading, and a variety of system parameters (e.g. source level, receiving sensitivity, transmit and receive directivity). Volume-scattering strengths can be, and usually have been, further averaged over programmable depth intervals. Depth bins of 2 m in the vertical were found to be reasonable for most of the vertical structure we encountered, but finer vertical structure was often observed in the presence of strong physical and fluorescence gradients.

A variety of abiotic measurements are made following each echo-ranging cycle, including fluorescence, temperature (8 ms time constant, 0.05°C), pressure, and conductivity. Derived products, computed in near real time were salinity, and sigma t. Chlorophyll *a* estimates were derived for a real time display and were then corrected to reflect the comparison of fluorescence levels and filtered phytoplankton. When it was desirable to obtain conventional samples of the organism, we used a high-volume plankton pump and several varieties of plankton nets. The conventional, direct samples were used for comparison with the acoustic data as "ground truth" and, after we gained sufficient experience with the system performance, to obtain data on species, sex, developmental stage, and gut contents.

System calibration for MAPS was performed at various times by using several broadly accepted acoustic methods, including the use of standard hydrophones and a surface self-reciprocity method (Bobber, 1970). Standard targets, such as are typically used at the lower frequencies in fisheries acoustics, have not been used because of the complexity of the resonances of spheres, the effects of attaching a tether with which to handle the target, and the large levels of scattering anticipated for sphere sizes that can be reliably handled. The standard target technique depends on operating on a relatively smooth part of the target reflectivity curve. In practice, this usually means the Rayleigh slope or at least the lower frequency side of the first resonance peak in the reflectivity. For frequencies as high as 10 MHz, the radius of an appropriate standard sphere would be 25 µ or less.

Example of MAPS technology

A set of volume-scattering strength profiles collected at the shelf slope break in Santa Monica Bay during 7 October 1982 is used here to illustrate the kind of data the MAPS produces (Fig. 3). These data are one of several casts made along a transect in the area. The context, temperature, chlorophyll *a* and σ_t distributions with depth and distance along the transect can be found in Pieper *et al.* (1990). This cast is located at ca. 20 km offshore in Figure 4 of that article.

The volume-scattering strength profile for this station is typical in the sense that the lowest values of S_v are deep and at low frequencies and the highest values tend to occur near the surface, often at intermediate or higher frequencies. The range of scattering strengths exceeds 30 dB or a factor of 1000. There is no monotonicity with either depth or frequency. Peak values of volume scattering (-40 dB) occur at about 5 m and 1.6 MHz. Evidence of a "scattering layer" can be observed between ca. 20 m and 50 m.

The data of Figure 3 were transformed via the NNLS algorithm and the truncated fluid sphere model for scattering from small zooplankton and are displayed (Fig. 4) as $\log_{10}$ (biovolume in mm^3 m^{-3}) versus size (a in mm). If the scatterers have the shape of a calanoid copepod, multiplying the equivalent spherical radius (a) by 4 or 5 will provide an approximate length for the organisms.

There are several clear peaks in the distribution of plankton biomass in near-surface waters. While entrained bubbles are a possibility, their very high target strengths relative to zooplankters can usually be used as a discriminant when one suspects that they are present. In practice, we have seldom found entrained bubbles to limit performance, partly because rough weather is seldom a serious factor off southern California. Nonetheless, we have worked before, during, and after
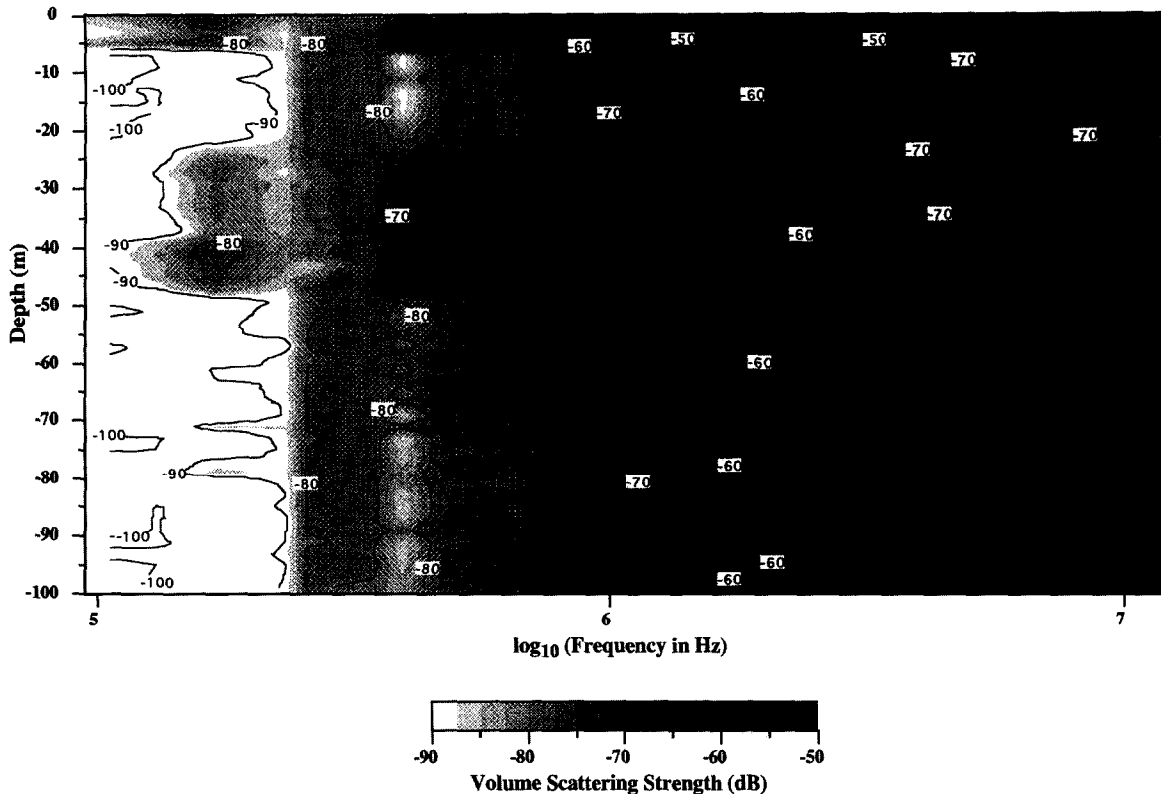
Figure 3. Volume-scattering strength data collected with the MAPS during daylight hours at the shelf slope break in Santa Monica Bay, near Los Angeles, CA, USA on 7 October 1982.

hurricanes in the Atlantic and have rarely found bubbles to limit our performance, usually only above about 5 m for brief periods. With MAPS, very small bubbles have resonances that vary with the bubble depth, but which cause the lowest frequencies to indicate very high scattering levels, thereby identifying the source of the scattering by observation of the scattering spectrum with frequency. Usually, even moderate-size free bubbles entrained into the water column by breaking waves rise rapidly to the surface and disappear on time scales of tens of minutes.

On one occasion, exceptionally high volume-scattering strengths were observed near Catalina Island, about 20 miles from the southern California coast after a brief, but intense period of high winds and breaking waves. The scattering was very patchy in space and persisted for hours after the storm. Bubbles were suspected as the source of the scattering, but pump samples were collected in case an unanticipated biological source might be responsible. The pump samples revealed significant quantities of the mucous web of *Oikopleura*. We suspected, but were unable to confirm by direct observation, that very small bubbles (a few microns in size) had been trapped by the mucous webs and were prevented from rising to the surface, finally being absorbed *in situ*.

In Figure 4, the highest scattering levels are consistent with the presence of zooplankton, larval fish, or possibly micronekton (e.g. juvenile euphausiids). A peak that might be associated with these latter organisms can be seen at a depth of about 5 m and an equivalent spherical radius (a) of about 3.7 mm. The biovolume at that size and depth was ca. $32 \text{ mm}^3 \text{ m}^{-3}$ of water. Smaller organisms were also located at approximately the same depth. It is unlikely that organisms with equivalent spherical radii over ca. 1 mm equivalent spherical radius would have been quantitatively sampled by our plankton pump due to avoidance. The biovolume at an equivalent spherical radius of about 250 μ (0.25 mm) and 5 m depth was $382 \text{ mm}^3 \text{ m}^{-3}$.

A layer of organisms was also present at depths between about 20 m and 50 m. Copepod nauplii and immature calanoid copepods such as *Clausocalanus*, immature and adult females and *Oikopleura* are likely candidates for the biomass indicated between 0.1 mm equivalent spherical radius and 0.25 mm equivalent spherical radius. Larger adult copepods (e.g. *Calanus*, *Pleuromamma*, *Centropages*, and *Metridia*) fall within the range 0.25 mm to 0.6 mm. Large chaetognaths with equivalent spherical radii between 0.785 mm and 0.825 mm were present in a pump sample collected
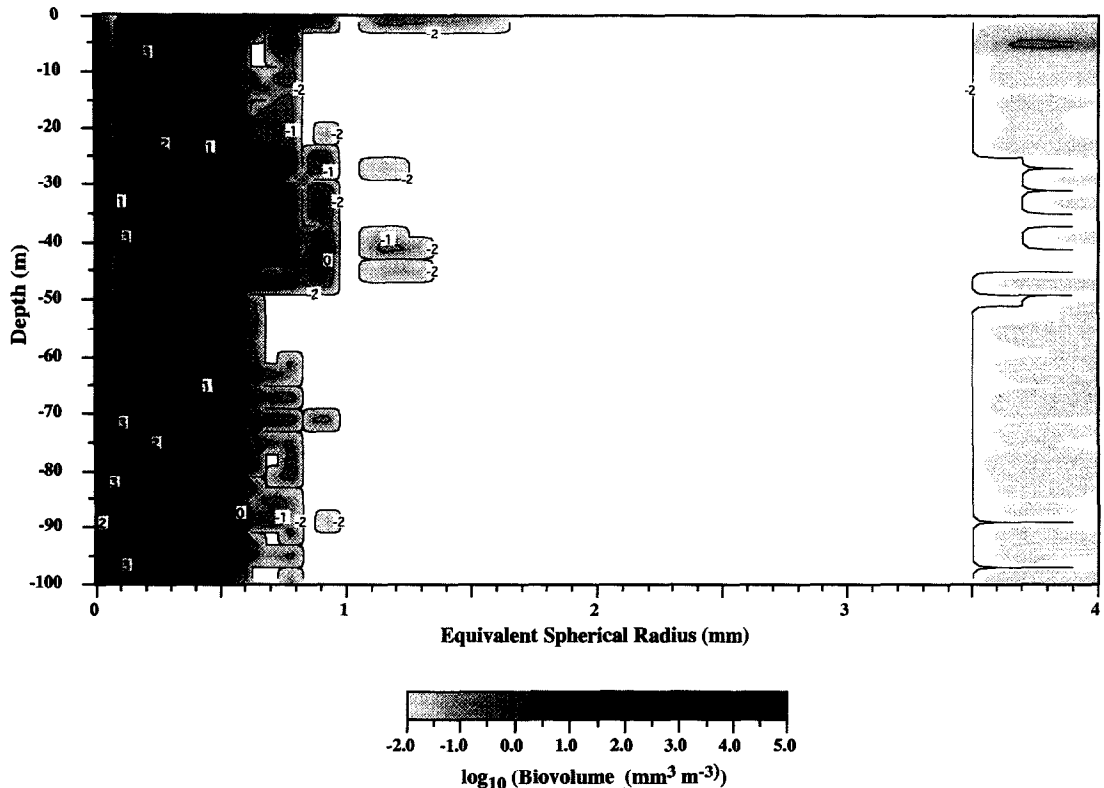
Figure 4. Computed $\log_{10}$ (biovolume)–size spectra profiles based on MAPS data collected during daylight hours at the shelf slope break in Santa Monica Bay, near Los Angeles, CA, USA on 7 October 1982. These calculations were derived from the volume-scattering data illustrated in Figure 3 using the NNLS method.

about 9 km to the north-west of this cast on the previous day.

One of the more productive uses of the MAPS has been to define zooplankton patterns by size with respect to the physical and phytoplankton fields in vertical cross-sections. This is accomplished either by using the MAPS in an undulating, towed mode, at speeds up about 6 knots or by making vertical casts at discrete stations along a transect. As mentioned earlier, Figure 4 of Pieper *et al.* (1990) illustrates one of many patterns that we have collected that indicate some degree of coherence between the biovolume, physical and phytoplankton fields at kilometer scales in the horizontal and meter scales in the vertical. At lesser spatial scales, the coherence often appears to be somewhat weaker.

A two-frequency method

Rationale for research

After substantial experience with the MAPS system, it was decided to test a less complex and less expensive system. While there would be less information available from a 2, 4, or 6 frequency system than from a 21 frequency one, there were probably appropriate eco-systems and interesting problems that would benefit from applications of acoustical technology with a lower size resolution than MAPS.

A number of research ships are currently equipped with at least two echo-sounders using difference frequencies. There are also applications in which moored instruments are needed to determine long-term temporal changes and where power consumption is critical. Greenlaw (1979) described an algorithm for processing volume-scattering strengths at two frequencies. The result was an estimate of the size and abundance of plankton when the assemblage was dominated by a single size, which did not have to be known beforehand. Thus the strict constraint required to use a single frequency is relaxed for two frequencies, in that the size does not have to be known before abundance can be calculated. Greenlaw's immediate application was directed at euphausiids, but with the availability of an appropriate model the theory is applicable to other species.

Assumptions

When working with only two frequencies, one should ensure that the following assumptions are valid: (1) A
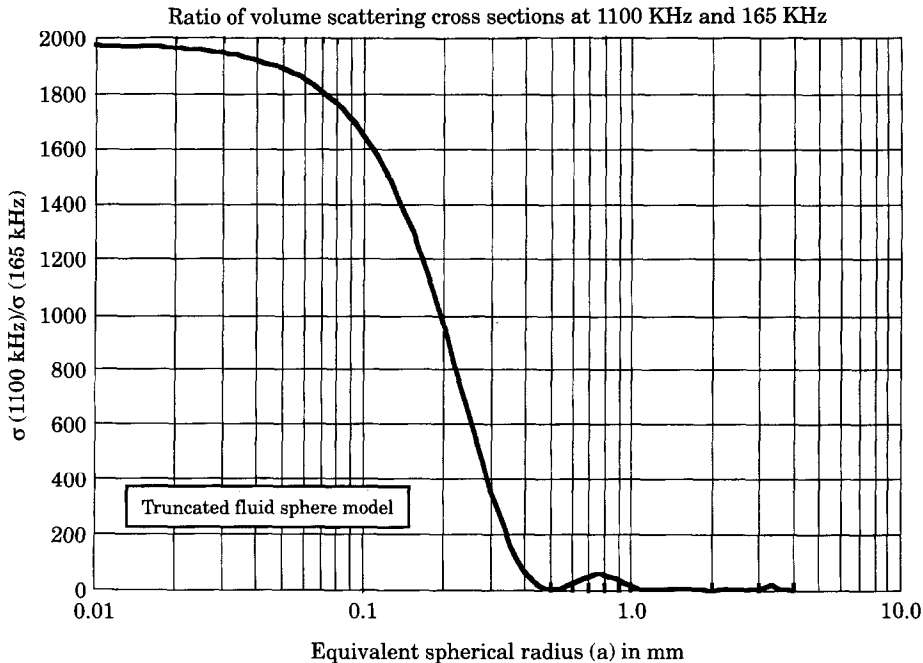
Ratio of volume scattering cross sections at 1100 KHz and 165 KHz

Figure 5. The relationship between the ratio of acoustical scattering cross-sections expressed as $(s_v(f_{hi})/s_v(f_{lo}))$ and the size (a) of a spherical scatterer that would result in that ratio. Note that the solution has multiple roots below some value of the ratio which depends on the frequencies selected. This calculation is for $f_{hi}=1100$ kHz and $f_{lo}=165$ kHz and uses the truncated fluid sphere model.

single size organism dominates the acoustical scattering at both frequencies for any given ensemble average (e.g. a set of echoes or a single depth bin); (2) there is a tractable, validated model for the organism that relates its size and the acoustical frequency in use to its reflectivity (target strength) in quantitative terms; (3) multiple scattering effects are negligible (can be slightly relaxed if only size is required. In practice, this is rarely a problem with zooplankton); (4) shadowing effects are negligible (can be slightly relaxed if only size is required. In practice, this is rarely a problem with zooplankton); (5) any dependence of the reflectivity on any other parameter, e.g. depth or temperature is known; (6) the measurement platform, environment, and distribution of organisms are sufficiently stationary (wide sense) that one can estimate the power in the echoes.

Algorithm

Following Greenlaw (1979), if one makes acoustical measurements at two frequencies f_{lo} and f_{hi}, and if $\sigma_v(f_{lo})$ and $\sigma_v(f_{hi})$ are the respective acoustic volume-scattering cross-sections, where the relationship between the representation of volume-scattering strength in decibels (dB) and the cross-sections σ_v are given by $\sigma_v=10^{(S_v/10)}$, then the ratio of the scattering strengths can be written in terms of a function φ, that depends on f_{lo}, f_{hi}, and a. Symbolically:

$\sigma_v(f_{hi})/\sigma_v(f_{lo})=\varphi(f_{lo}, f_{hi}, a)$.

If one uses the high-pass model, then the solution for the equivalent spherical radius (a) is analytically tractable in closed form and is:

$a^4=(2/3)(r^4 - R)/((2\pi f_{hi}/c)^4(R - 1))$.

Here, c is the speed of sound in sea water at the appropriate temperature, salinity, and depth, and the symbols r and R are defined as:

$r=f_{hi}/f_{lo}$ and $R=\sigma_v(f_{hi})/\sigma_v(f_{lo})$.

Two of the roots of the solution for the equivalent spherical radius are imaginary and one of the real roots is negative. Consequently, the only real, positive root is the size we have set out to compute.

For other models, such as the truncated fluid sphere model, there is usually no simple solution for the size, a, in the expression φ. In the case of most interest for small zooplankton, the truncated fluid sphere model, we have solved the equation empirically and the result of that solution is illustrated in Figure 5. Note that the non-monotonic nature of this and other appropriate scattering models for small zooplankton are reflected in a set of real solutions at small ratios, rather than a single physically meaningful root. With only scattering data at two frequencies, there is insufficient information to select the correct root. Data from another source are
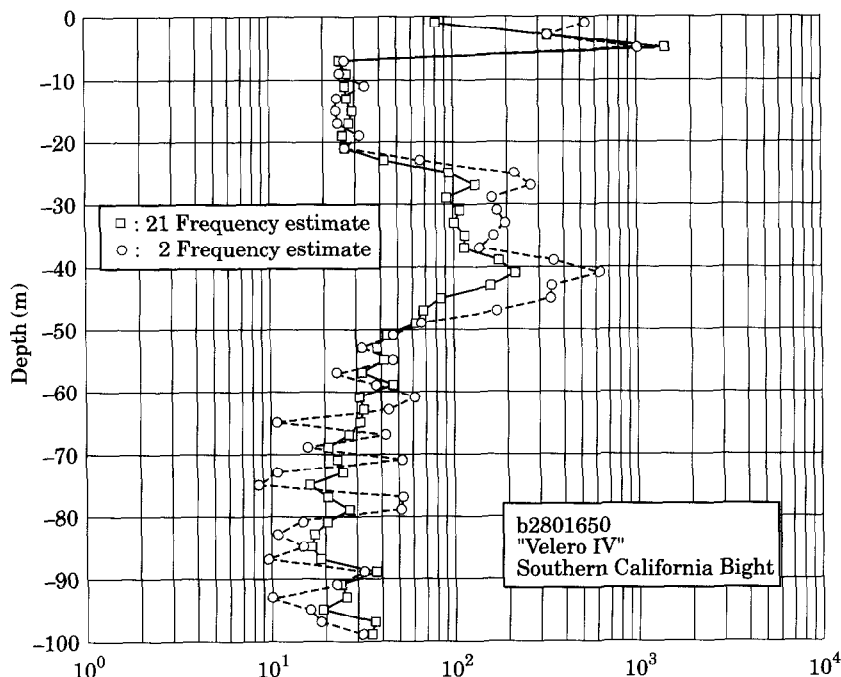
Figure 6. A comparison of two algorithms for converting volume-scattering strengths to estimates of biovolume. The first algorithm is the NNLS algorithm using 21-volume-scattering strength profiles at frequencies between 100 kHz and 10 MHz (*). The second algorithm is the two-frequency algorithm (○). Two scattering profiles were selected from the 21-frequency MAPS cast (165 kHz and 1100 kHz).

necessary to resolve this ambiguity. For the analyses discussed below, we have chosen to select the smallest real root when the magnitude of the ratios of scattering strengths allows multiple root solutions.

When one has determined the radius of the dominant contributor to the scattering, then the relationship:

$$S_v(f) = TS(f,a) + 10 \log_{10}(N)$$

can be solved to reveal

$$N = 10^{((S_v - TS(f,a))/10)}.$$

Here, N is the numerical abundance at size a, TS is the target strength of the acoustically dominant organism (from the scattering model), expressed in dB and either f_{hi} or f_{lo} can be used at the discretion of the researcher. Usually, one would choose the frequency with the best reverberation to electronic system noise ratio.

Example from the MAPS data set

In addition to allowing us to obtain a better understanding of the physics of acoustic scattering and the distribution of the organisms that cause much of the scattering in the sea at high acoustical frequencies, the MAPS system also provided us with a database of volume-scattering strength profiles with which we can evaluate interesting inverse algorithms as they are conceived. One such volume-scattering strength profile from the MAPS database, collected during the afternoon of 7 October 1982 near Los Angeles, was processed with the two-frequency algorithm discussed above. The MAPS data for this cast have been averaged into 2 m depth intervals. The spatial context for this data can be found in Pieper *et al.* (1990), where vertical sections of σ_t, chlorophyll *a*, total biovolume, and two size distributions are presented. The profile discussed below is the third station (at ca. 20 km offshore) in Figure 4 of that paper. Profiles at 165 kHz and 1.1 MHz were selected as the scattering data for a test of the two-frequency algorithm. Within the constraints of the assumptions detailed above, we computed the acoustically dominant size (a non-linear, weighted, average of actual size) for each frequency ratio and the resulting biovolume based on the dominant size estimated. If the ratio allowed multiple roots, the smallest was chosen.

The biovolumes computed from the two-frequency algorithm were then compared with the total biovolume estimated by using all 21 frequencies (Fig. 6). The total biovolume is the sum of the biovolumes at each size (for each depth interval). Considering the disparity of information content when using only 2 versus 21 frequencies, the two-frequency result is a remarkably robust estimator of biovolume. Further, there is a real possibility that
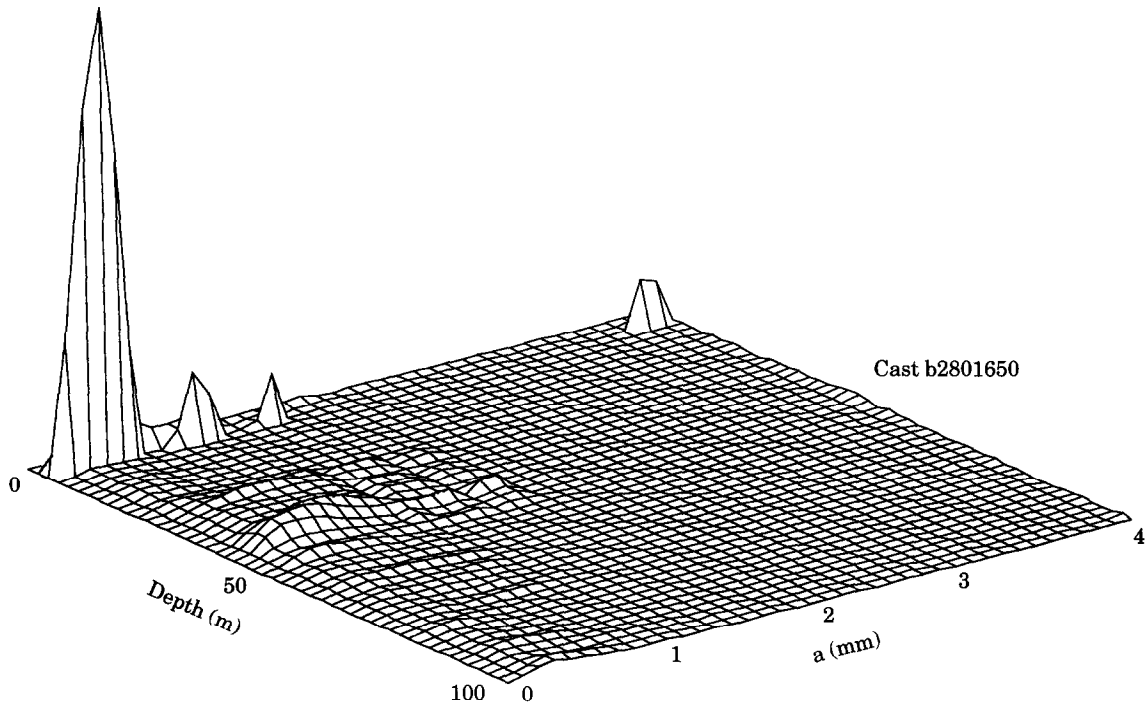
Figure 7. Calculated distribution of biovolume at the sizes estimated with the 21-frequency algorithm. The biovolume at the peak near the surface was ca. 1400 mm^3 m^{-3}. Note the broad distribution of scatterer sizes in the depths between 30 and 60 m. A similar range of sizes is present near the surface with an additional peak near 4 mm equivalent spherical radius. With volume-scattering data at 21-frequencies one can make independent estimates of abundance at 21 sizes. In this case, the underdetermined solution was computed for 40 discrete sizes. The resolution across size was not evenly spaced, with 50 μ resolution under 1 mm, 100 μ bin spacing between 1 mm and 2 mm, and 200 μ resolution at larger sizes, up to 4 mm equivalent spherical radius. The log of this display is contoured in Figure 4.

the smallest root is not always the correct one. In the absence of additional information this can lead to incorrect results. There were no direct samples of the organisms for this particular MAPS cast, but the extensive testing discussed above had given us some confidence in the 21 frequency acoustic inverse (e.g. see Costello *et al.*, 1989).

The two-frequency algorithm requires that only one size scatterer dominate the acoustical reverberation at both of the frequencies used. If the assumption of a "dominant" size is not strictly valid, there will be errors in the size estimate. If the size spectrum for scatterer size is relatively narrow, the estimate will usually degrade relatively gracefully as one violates the underlying assumptions, i.e. the errors may not be large for small deviations from a single line size spectrum. If, however, several sizes are present and those sizes are significantly different, the size computed by the algorithm will be a complex, non-linear, weighted average of the actual sizes present. For the data we are using in this example, a continuum of sizes was present, ranging from ca. 100 μm to over 1 mm equivalent spherical radius at depths where there are significant differences between the two estimates. The size spectra for the biovolume from the

21 frequency inverse (Fig. 7) is displayed for comparison with the two-frequency inverse (Fig. 8). It is clear that since the biomass is distributed over several sizes, the two-frequency algorithm is "selecting" a size that it considers "dominant" and that a sufficient amount of biomass to account for the total scattering observed is being assigned to that size. Thus, the peaks projected by the two-frequency inverse (Fig. 8), particularly those between about 20 m and 50 m depth, are larger than is actually present at that size (Fig. 7). Biovolume at a given size is proportional to the cube of the equivalent spherical radius. A comparison of the two inverse profiles reveals that the product of the numerical abundance times the cube of the dominant size calculated with the two-frequency algorithm was smaller than the sum of biovolumes calculated for each of the 40 sizes between 0.025 mm and 4 mm using the 21 frequency inverse (Fig. 6). This results in two-frequency estimates of biovolume that are higher at some depths than were obtained with the 21 frequency inverse.

Simply put, there is more information embedded in scattering data from 21 frequencies than in two and the estimates are more accurate when more information is available. In general terms, the size estimates from the
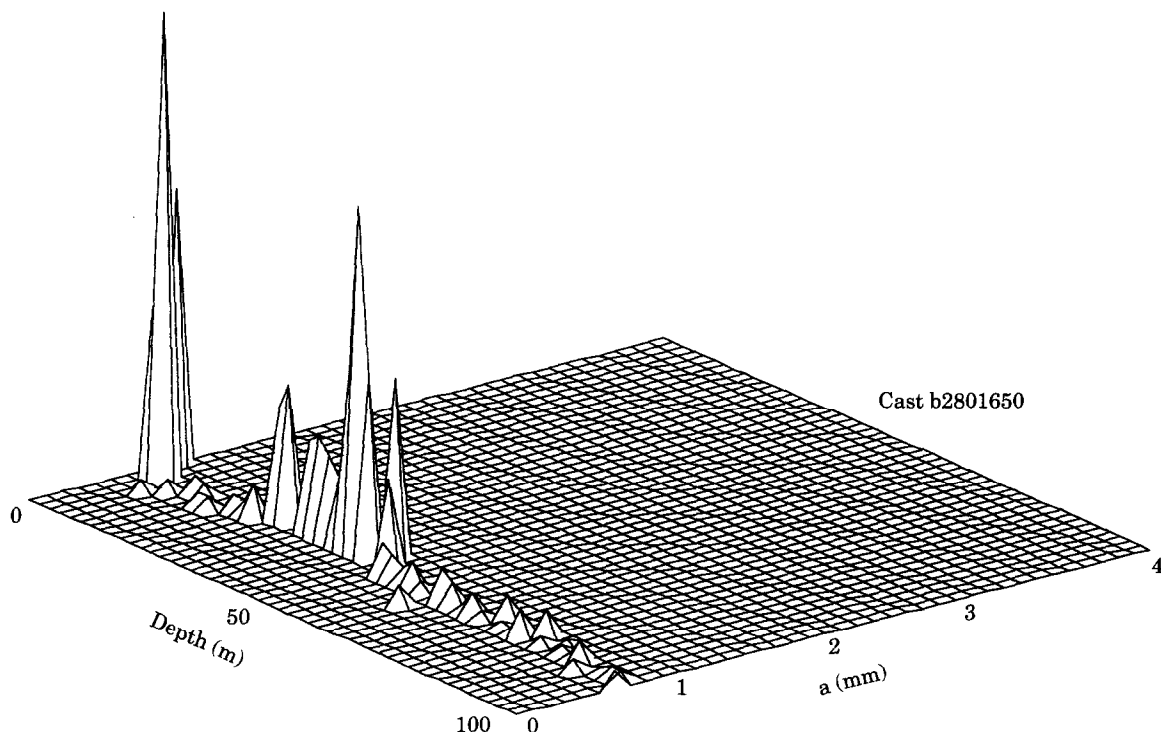
Cast b2801650

Figure 8. Calculated distribution of biovolume at the sizes estimated with the two-frequency algorithm and the truncated fluid sphere scattering model. The biovolume at the peak near the surface was 1380 mm^3 m^{-3}. Note the shift in size towards larger scatterers in the layer between 30 and 60 m.

two-frequency method were larger than the "average" sizes calculated with 21 frequencies. The biomass estimates, however, were remarkably robust considering the differences in the amount of information and the complexity of the two measurement systems.

The BITS bi-frequency sensor

Most of the previous discussion deals with various acoustic sensors to determine spatial distributions, either in the horizontal or the vertical. If one repeats a cast, even a few minutes later, with resolutions of meters in the vertical, it is likely that a slightly different profile will be obtained. This is a natural consequence of heterogeneity in the plankton community biomass distribution and of our inability to examine the same small-scale water mass in the sea twice, even on the downward and upward part of the same cast. At a point in an advective field, this heterogeneity is seen as a component of the variability.

Other components are the natural changes in time due to reproduction, growth, and death. With the exception of a few very carefully selected, well-bounded ecosystems, one will observe a "parade" of an assemblage of animals when measurements are made at a single place, for example, from a mooring. In spite of the difficulties of interpreting such data, neither determining synoptic vertical or horizontal cross-sections of the biomass–size spectra nor measuring at a single geographic location, will answer all of the important questions on zooplankton ecology. Both methods of deployment of acoustic sensors have a place in our acoustic "toolbox" and both should be seriously considered when defining an experimental protocol. Often, they should be used together to get a realistic measurement of both spatial distribution and temporal variability.

To satisfy the requirements of long-term monitoring programs, we designed, built, and deployed a mooring-based remote-sensing system for zooplankton. This system, named after our science program, Biophysical Interdisciplinary Trophic Studies (BITS) also includes a capability for measuring a few critical abiotic parameters. Capability for expansion to other measurements is also provided in the data stream.

The pilot BITS system is currently moored on the northern edge of the San Pedro Basin at the shelf slope break near Los Angeles, California, USA. It was first installed in the fall of 1992 and is used both to acquire data on the acoustic and zooplankton variability at that site and as a test bed for continuing development of this technology. At present the mooring is instrumented

with eight bi-frequency sensors at depths of 10, 20, 25, 30, 35, 40, 50, and 60 m. Additional sensors have been built, and include an eight-frequency zooplankton sensor and a dual-beam/echo statistics sensor for fish larvae and micronekton. The bi-frequency instruments include rudimentary optical sensors to measure downwelling irradiance. Water temperature sensors (thermistors) are also on each bi-frequency sensor. Temperature sensors are also included on an eight-frequency acoustic sensor and on a dual beam/echo statistics instrument, both of which will be added as funding permits. A capability for substantial expansion has been designed into the system to allow inclusion of more sensors of all kinds as the technology advances. At present, there are two key technological barriers, fouling of optical ports and available electrical energy. In one deployment at this location, during a moderate ENSO (El Niño) event, we experienced no detectable degradation in acoustic performance because of fouling over nearly 9 months. While we are still evaluating the data, the optical ports appear to have been partially occluded by growth during the last month or two of the deployment. A relatively high incidence of vandalism has been experienced and continues to be of concern when any system is located in coastal waters.

Data from the BITS sensors are collected on programmable intervals, initially at a 15 min interval and for later data, 30 min. The data are stored in a computer memory on the surface buoy. Wind speed, ambient light, and air temperature are measured on the buoy and included in the data stream. A two-way packet radio communications link (VHF) is used to automatically download the data to a shore station within line-of-sight of the mooring. A variety of other information is also transmitted (e.g. battery voltages). If the automatic download fails, we can manually retrieve the data via a modem from virtually any location with a telephone line. A variety of operating parameters can also be changed over this communication link. HF packet communications is possible in those instances where line-of-sight is not an option. We are increasing the on-board storage from about 6 weeks to over 6 months. In the event of a radio or terminal node controller failure at the buoy, we can "plug in" to the buoy on a sampling or maintenance cruise and retrieve the data *in situ*. The most serious problem with the packet communications link has been the frequency of traffic on our licensed frequency, which must be shared with numerous other users.

Sample of BITS data

Data from the meteorological package on the BITS mooring (Fig. 9) reveal that the fall and winter of 1992 were characterized by falling ambient light levels and air temperatures, both well-understood seasonal effects. A detailed examination of surface irradiance on a diel time scale reveals the influence of the usual coastal fog and early morning clouds at the site. Winds were light and variable, with peak wind speeds rarely exceeding 10 m s^{-1}. The variance of the air temperature increased as winter approached. A fluxgate compass and a wind-direction sensor have been added since these data were collected.
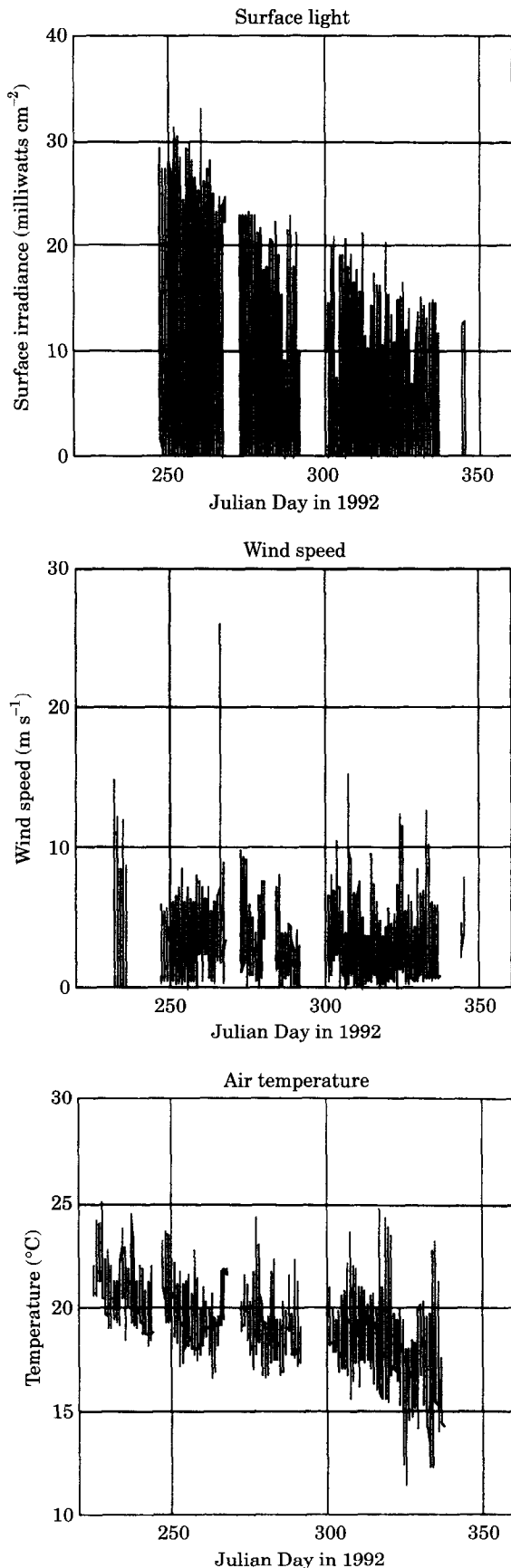
The trend in the downwelling light record at 40 m (Fig. 10, top left panel) reflects variations in the surface light, but is modulated by scattering and absorption above this depth as well. A close examination of the differences between the sub-surface light records at all depths indicates differences in absorption coefficients as a function of depth. We ascribe most of these differences to scattering and absorption by phytoplankton, since there is little turbidity of terrestrial origin off the southern California coast. Our light sensors consist of inexpensive, simple silicon photodiodes and were installed to hold a place in our data stream for the eventual deployment of multi-spectral or PAR sensors. Initial results of our data analysis indicate that there is good correspondence with chlorophyll estimates made from extinction profiles based on these simple sensors and historical values from the area. (See Riley (1956) for the algorithm we use to convert extinction coefficient to estimates of chlorophyll).

The water temperature record (Fig. 10, center left) reveals a maximum range of approximately 6°C. The variance of the temperature increased in early November, at which time water temperatures at 40 m exceeded 14°C.

Volume-scattering strengths at both frequencies (Fig. 10, top right and center right) also indicated generally increasing variability as winter approached, as did the differences between scattering at these two frequencies (Fig. 10, bottom left). Both the 165 kHz scattering and that at 1.1 MHz varied by three orders of magnitude during this observation period. Some of the variance can be attributed to local diel migrations, primarily of euphausiids. Depths of over 900 m occur in the San Pedro Basin within a few kilometers of the mooring. These animals can easily migrate to surface waters over the San Pedro Basin and be advected past the mooring site.

The difference in scattering at the two acoustic frequencies (Fig. 10, bottom left) indicates variability in the size–abundance spectra during the period from early August through mid-December. Recalling that the two-frequency algorithm has proved a relatively good indicator of biomass, even when size cannot be extracted because there is not a single dominant size present, we calculated the biovolume for this period (Fig. 10, bottom right).

Zooplankton biomass at 40 m decreased by about an order of magnitude between August and December. An

Surface light

Julian Day in 1992

Wind speed

Julian Day in 1992

Air temperature

Julian Day in 1992

increase in variability of zooplankton biovolume was associated with the appearance of warmer water beginning in early November. The basic assumptions required to assess the magnitude of the variability in size were not met at this location and time; therefore, we refrain from doing more than indicating that had the size spectra not varied the differences in scattering strengths would have been constant.

It would often be beneficial to obtain additional size discrimination for the organisms present in order to gain more information about the biological system. One solution to the requirement for additional resolution for animal size is to use the BITS data stream to trigger event-driven sampling near the BITS mooring site. Half-kilometer or better horizontal and sub-meter vertical scale acoustic sampling can be accomplished with the TAPS four-frequency sensor. Sampling with the TAPS allows us to place the "parade" of organisms that pass the BITS mooring in the context of a spatial "snapshot". The TAPS allows one to determine the size–abundance structure of the scattering organisms within four to eight size bins, depending on the particular zooplankton/micronekton assemblage and their environment. Limited numbers of net (e.g. MOCNESS) samples provide information on species composition.

In many situations, however, four frequencies still do not provide enough information on size structure, especially if larger organisms are present while one is attempting to quantify the structure of smaller ones. While the 21 frequencies used in MAPS may be more complex than is required for many uses, our next generation instruments will make use of six and eight frequencies to gain better size discrimination than is available with two or four frequencies. Six and eight-frequency systems are presently in use on undulating vehicles and on moorings in the Arabian Sea. We anticipate adding eight-frequency sensors to our southern California BITS mooring and to a Georges Bank mooring in 1995. Recent adaptations of this technology include stand-alone sensors for vertical profiling, bottom-mounted vertically profiling multifrequency sounding in shallow water (e.g. lakes and estuaries), and under-way, profiling for high resolution (limited range) mapping of zooplankton in the water column.

Acoustic technology for zooplankton and micronekton research has reached a state of maturity which now requires parallel efforts. There are sensors and systems which can, and are, being routinely used in research programs. These systems should become widely available in the next decade. The base of technology available

Figure 9. Meteorological data from the BITS mooring in the Southern California Bight. Data are shown for the fall and winter of 1992. This is a pilot site used for engineering development work and the gaps are periods during which modifications and maintenance were being done.
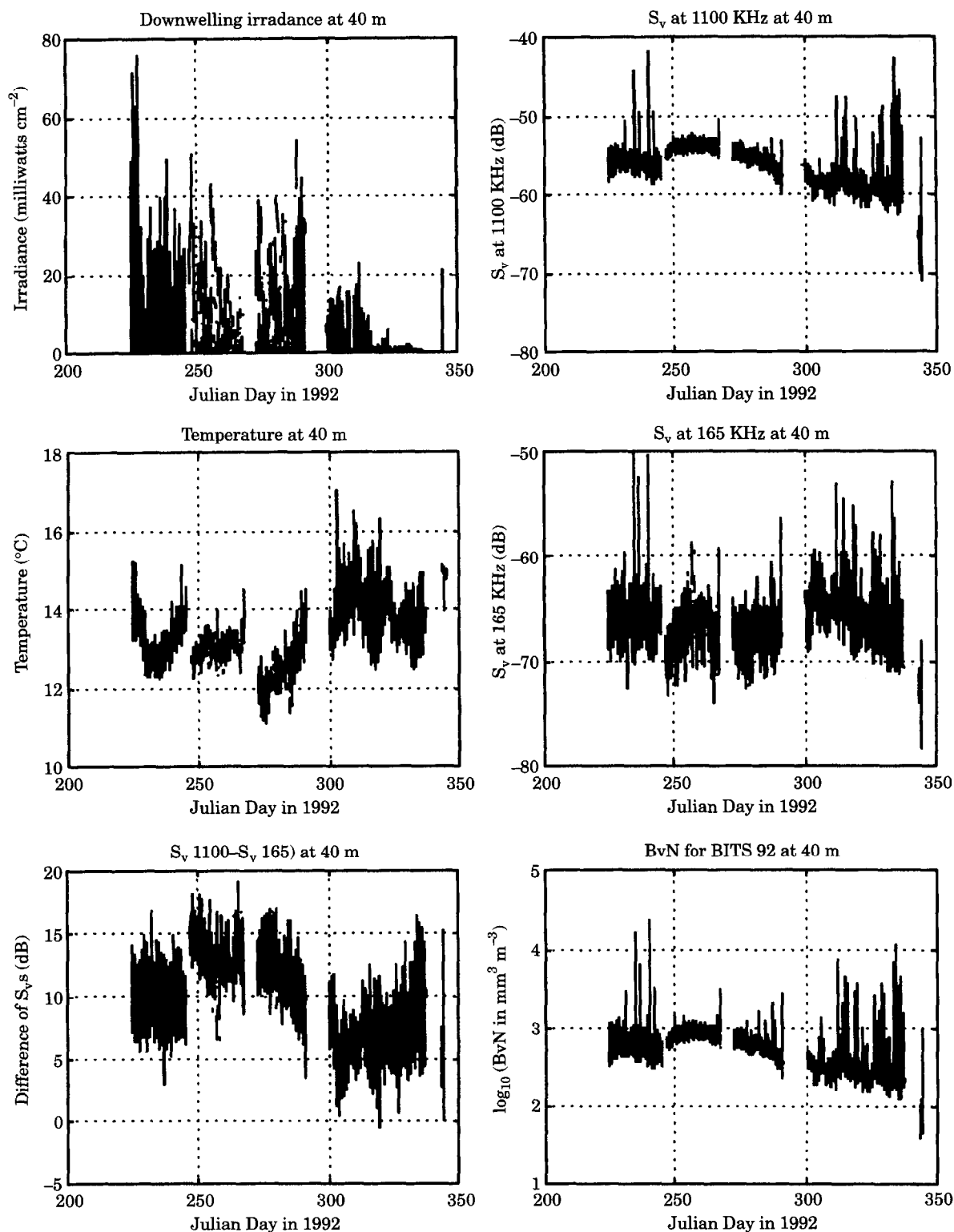
Figure 10. Data collected by the BITS sensor located at 40 m depth, 33°33.9′N, 118°08.4′W at 100 m water depth, on the northern edge of the shelf slope break of the San Pedro Basin near Los Angeles, CA, USA. Downwelling ambient light, water temperature, acoustical volume-scattering strengths, and the calculated zooplankton biovolume are illustrated. Samples were collected on the hour and half-hour.

to apply to the development of new, more capable sensors has barely been touched. It is mandatory that continued efforts be applied to the refinement of old sensors and techniques of deployment, and to the development of new approaches to acoustic sensing of planktonic organisms.

At the moment, one of the most promising avenues of basic research is the potential of synergistic, quantitative combination of acoustic sensors and sensors based on other physical technologies, such as underwater optics. Like acoustics, optics is also a "toolbox" of technologies, e.g. imaging optics, multispectral transmissometers, fluorometers, which are all candidates for joint application to many critical problems in describing how zooplankton live, reproduce and die in the sea.

Acknowledgements

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