Sensing Plankton: Acoustics and Optical Imaging

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1. Introduction

Planktonic communities are ubiquitous and diverse assemblages of organisms, which play a key role in structuring pelagic ecosystems. Photosynthetic phytoplankton, which are discussed in other chapters in this volume, play a key role as they support oceanic food webs and alter the biogeochemical cycling of nutrients and carbon in the ocean. Planktonic animals, or zooplankton, serve as a link between primary production by unicellular phytoplankton and higher trophic levels such as pelagic fish stocks, many of which are commercially exploited. The environmental and biological factors controlling the distribution and population dynamics of pelagic communities is a central goal of biological oceanography. The importance of understanding the abundance, dynamics, and interactions of planktonic organisms with increased spatial and temporal resolution has led to the development and application of improved sensors to observe and quantify them. As documented in this volume, Harmful Algal Blooms (HABs) can exert a dominant (and harmful) influence on pelagic ecosystems via the consumption of nutrients, the production of toxins, and the creation of anoxic conditions. The precise mechanism and how dynamics of these food webs is affected is of great interest. Here, we consider the development of technology to study and understand HABs with the ultimate goal of perhaps mitigated their effects.

In considering the relationship of HABs to zooplankton it will be necessary to understand the effects of both HABs on zooplankton and the effects of zooplankton on HABs. Although we have much to learn, initial efforts in these areas by Turner et al. (2000) and Buskey (2003) have helped to determine the relationship of phytoplankton to zooplankton and leave many questions for future work. Perhaps some of the technologies described in this chapter will be useful in order to unravel interesting aspects of these ecosystems.

The goal of this article is to provide the reader with a contemporary review of acoustic technologies that have been developed for monitoring zooplankton. In addition, a survey of underwater optical imaging of both zooplankton and phytoplankton is presented. As a general outline, first, a review of the basic physics of sound scattering from zooplankton is presented. This is then followed by an introduction to the propagation of light in the sea and its special relevance to underwater optical imaging. The background information is presented so that the reader will have a good idea of the underlying physical and environmental principles that form the basis for the current generation of underwater systems. A survey of these systems, which includes many examples of contemporary underwater acoustical and optical systems, is presented. These systems range from commercially available conventional off-the-shelf technology (COTS) to one-of-a-kind instruments that may someday transition into the standard repertoire of oceanographic facilities.

As will be revealed in the following sections, there is, in fact, no perfect technology for observing zooplankton in the sea. Optical techniques offer the highest possible resolution and present the data in a form that is readily interpretable by humans, due to our extensive experience with visual observation. Acoustics offers the possibilities for longer range imaging of these animals, however suffers from a lack of specificity, a non-intuitive basis for scattering strength, and a lack of knowledge of the multidimensional features of the scattering functions. On the most basic level, the net techniques that have been used for many years, offer the highest observational accuracy in permitting species identification, however suffer from problems associated with avoidance, the inability to non-invasively obtain certain classes of animals, and biases which depend on the size and swimming speeds of the animals.

Taken together, these three techniques are the standard repertoire for observing the animals. Each has significant strengths but also significant weaknesses. Taken together, however the methods can complement each other. In the case of acoustics, due to the ease of creating, recording and processing sound, there has been a proliferation in the use of acoustical techniques for sensing zooplankton. Several excellent surveys of sound scatter by organisms in the sea and the contemporary instruments, which have evolved to sense zooplankton (Griffiths et al., 2002), (Foote and Stanton, 2000), have been written. In addition, there are textbooks that consider the use of acoustics for fisheries assessment (McClennan and Simmonds, 1992). A book on acoustical oceanography (Medwin and Clay, 1998) describes both fisheries and zooplankton acoustics along with the basic physics of sound and sound propagation in the sea.

In the case of optics, the ever rapid development of advanced recording, processing and algorithmic techniques has fostered a recent generation of optical zooplankton observational equipment. A recent book chapter (Foote, 2000) and a review of zooplankton survey techniques (Wiebe and Benfield, 2003) describe many of the contemporary systems that are available. The interested reader is referred to these articles for further information and perhaps, a different view of these topics from those of this author.

2. Physics

In this section the basic physics of the process of sound scattering from organisms will be reviewed with special emphasis on applications to zooplankton. This will be followed by a review of the properties of light propagation and scattering and the principles of underwater imaging.

2.1 Foundations of Acoustical Sensing of Zooplankton

In order to understand the possibilities for using sonar in assessing the distribution, abundance, and behavior of zooplankton, the general principles of active sonar systems should be considered. The general setup, as illustrated in Figure 1 consists of a set of electronics for both transmitting and receiving the sonar signal. These are then connected to a transducer that transforms the electrical signal into a sound wave. This wave then propagates into the medium where it is both attenuated and scattered. Upon scatter from an organism of interest, the sound is the transformed back from a pressure wave into an electrical signal that is then recorded and analyzed.

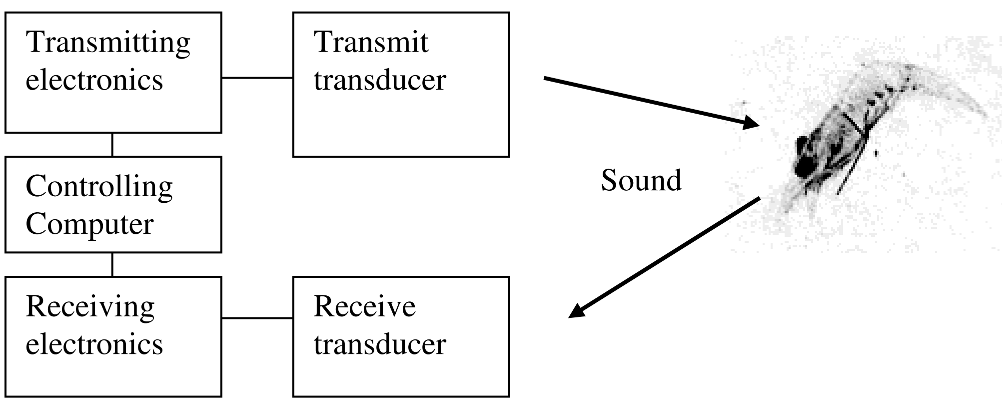

Figure 1: The basic components of a sonar system for recording reflections from zooplankton

In considering both the production and receipt of sound, there are standard texts that discuss the basic principles of sonar systems and their implementation (Urick, 1983), (Kino, 1987). Briefly, the sonar equation is the standard mathematical vehicle by which the delectability of animals can be determined. A general formulation of this equation for a single frequency that relates the intensity (I_{rcvd}) of the received sound (watts-m^{-2}) to the acoustic power which has been transmitted (W_{xmit}) in watts by an omni directional source for a single target at range r from the source as:

$$I_{rcvd} = \frac{W_{xmit} e^{-2ar}}{r^4} \frac{\sigma}{4\pi} \quad . \tag{1}$$

Here, a is the absorption coefficient for the specific frequency of interest (ordinarily considered to be only a function of the state of the water) and σ is referred to as the acoustic cross section (units of m^2). Pressure levels at the receiver can be computed from the relationship between the intensity and pressure. Since pressure is a time varying quantity the root mean square pressure (p_{rms}) is a more meaningful parameter that can be related to the average intensity using

$$< I >= \frac{p^2{}_{rms}}{\rho c} \, . \tag{2}$$

When the pressure is in pascals, the density of the media (water), ρ is in kg-m^{-3}. c is the velocity of sound in the media (m-s^{-1}). Accounting for a non omni-directional source can be accomplished in a straightforward manner.

The equation illustrates the general features of almost every remote sensing system that the radiation that has been produced is both exponentially attenuated (due to absorption) and spherically spread (a consequence of conservation of the energy). Although equation (1) is certainly the most intuitively appealing, most sonar engineers prefer to cast the equation into logarithms and reference the source levels to micropascals. The interested reader is referred to standard texts for the details. For our purpose here the important parameter is the relationship between the Target Strength (TS) and the acoustic cross section. The relationship is

$$\mathrm{TS} = 10 \log \frac{\sigma}{4\pi} \quad . \tag{3}$$

Note that this implies an exponential scale for TS. We also remark that target strength can be related to the fraction of sound scattered by a target as

$$\mathrm{TS} = 10 \log \frac{I_{refl}}{I_{\mathrm{inc}}} \tag{4}$$

where I_{inc} and I_{refl} are the incident and reflected intensities of the sound field. One important, and often overlooked, detail is that the incident sound level is taken at the organism. However, the reflected sound level is usually referred to at a 1 m range. Probably the most intuitive way to think of TS is as the logarithm (to the base 10) of the fraction of energy that is reflected by the target. Target strength of -60 dB therefore implies that 10^{-6} of the energy incident on the target will be reflected. As this is a typical value for a large zooplankter, the characterization of animal reflectivity in-situ can be a challenging endeavor.

In many situations the reflections from individual animals cannot be discerned and the sonar system is operated in a mode that is called echo integration. In this mode the recorded energy is the result of the reflection from the myriad of organisms that are in the sonar beam. The higher the diversity of scattering types (to be defined below), the more difficult the data is to interpret. In the most difficult situations, empirical relationships can be established which relate the integrated energy to animal abundance; however, their validity needs to be determined via ancillary methods such as net tows.

A more general approach considers the scattering function, β , (here we use β in order to be consistent with the optical theory presented below) as a function of the orientation of the target relative to the incident and reflected sound. Figure 2a demonstrates that in the most general formulation, this is a four dimensional function. Since this function can be very complicated (and perhaps rarely measured), some type of simplification is typically made in order to reduce its dimensionality. A common one is that the source and receiver are collocated as shown in Figure 2b. This is referred to as a monostatic apparatus and is by far the most common way to measure backscatter from animals. Assuming that the source and receiver are collocated leaves only two remaining degrees of freedom; the orientation of the animal (θ_i, φ_i).

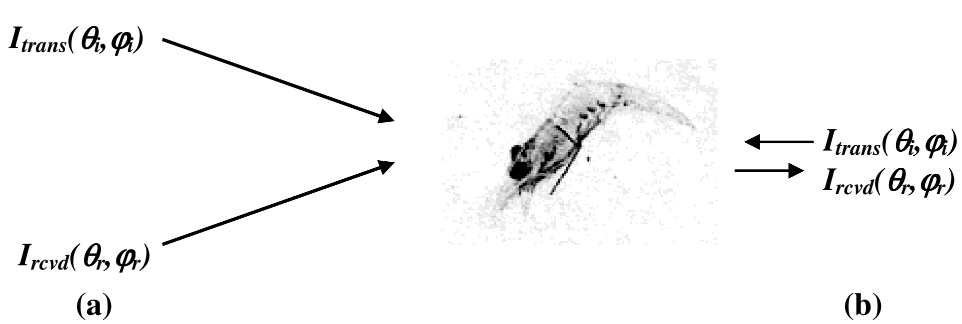

Figure2: (a) An illustration of the geometrical origin of the four dimensional scattering function $\beta(\theta_i,\varphi_i,\theta_r,\varphi_r)$. The angles (θ_i,φ_i) and (θ_r,φ_r) are the relative angles between a coordinate framework fixed to the animal orientation and the angles of incidence and reflection, where θ_i and φ_r are the polar and azimuthal angles. (a) Shows a bistatic configuration and (b) shows a monostatic configuration $(\theta_i=\theta_r,\varphi_i=\varphi_r)$.

One of the earliest theoretical formulations of zooplankton scattering was the fluid filled sphere, treated by Anderson (1950). This spherically symmetric function was computed (by two computers, in this case people) as a function of the product of the equivalent spherical radius, *r,* and the wave number of the incident sound, $k = 2\pi/\lambda$. Since then, increasingly sophisticated models of acoustic backscatter have evolved, taking into consideration the relevant symmetry and acoustical properties of the organisms under consideration. Since zooplankton present a great variety of body morphologies and compositions, measurement of even a representative number of scattering functions presents a great challenge. In addition, the necessity of understanding their angle-dependent scatter, hopefully in-situ, presents an added layer of complication. In some sense, due to these facts, there has been a substantial interest in modeling zooplankton scattering with the hope that such models could "fill in the gaps" between the necessity for measurement and the need to reconcile any given acoustical experiment with an understanding of the composition of the volume under consideration.

One area that has seen much research has been the modeling of scattering from elongated fluid filled bodies. These efforts have been primarily aimed at understanding the scattering from krill. In a series of articles that span over a decade, Stanton and colleagues have formulated both simple (Stanton et al., 1993), (Stanton et al., 1993) and more complicated models (Lavery et al., 2002) in order to understand the scattering from these organisms. A recent review summarizes efforts and outlooks at modeling the scattering from these animals (Stanton and Chu, 2000). As one example, Figure 3 shows a graph of acoustic backscatter target strength versus $k = 2\pi\, r/\lambda$, the ratio of equivalent radius to wavelength multiplied by 2π, for the deformed fluid cylinder

(Stanton et al., 1993b) as a function of animal orientation. The graph was produced using assumptions about the two important parameters of material composition: animal density contrast (ratio of animal density to that of the surrounding water, *g*=1.06) and sound speed contrast (ratio of sound speed in the animal to that of the surrounding water, *h*=1.06) (Demer, 1994). The product of these parameters *gh* is called the acoustic impedance (similar to the refractive index in optics). Greater values of acoustic contrast lead to higher reflectivity (again similar to the refractive index in optics). The assumed values for these parameters were subsequently validated.

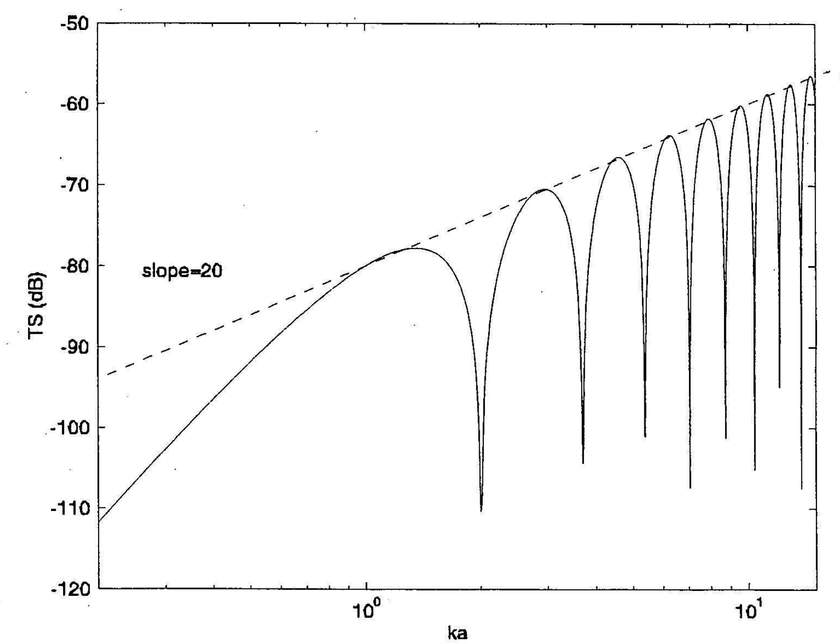

Figure 3: Backscatter Target Strength (TS) versus kr for the bent cylinder model of Stanton (1993b) as computed by Demer (1994) at 420 kHz.

The graph clearly shows a transition from a steeper region (at low *kr*) where a small increase in *kr* will result in a larger increase in scattering (approximately proportional to incident frequency *f* raised to the fourth power) to a more gently increasing region where the slope asymptotes to 20 (due to the dependence on cross sectional area). The former area is called the Rayleigh regime and the larger *kr* region is called the geometrical or geometric scattering regime. The nulls, or deep areas of low backscatter, are due to coherent interference between different parts of the body. Most system designers interested in detecting single species try to "tune" their wavelengths so that *kr* is greater than or equal to one as this leads to an efficient use of power (however see discussion below on multispectral imaging) with a minimum of attenuation due to absorption. As such, the $kr = 1$ point is often a design goal for the systems. So, for example, considering a 1.13 mm copepod of equivalent spherical radius r = 0.2 (McGehee et al. 2003) the $kr = 1$ implies that one should use a wavelength of λ=1.25 mm, or a frequency (assuming $f\lambda$ =1500m/s) of f = 1.2 MHz. The equivalent spherical radius is that value of r in the spherical model which best matches the observed frequency spectrum from the more complex organism. It is because of the low backscatter target strength of animals that are small relative to the wavelength of the sound that low frequency sonars are of little use in detecting small organisms, unless their abundance is extremely high. The interested reader should be aware that many graphs of target strength are really the "reduced target strength" which subtracts a quantity of $10\log L^2$ from the displayed quantity (Lavery et al., 2002). Here L is the "equivalent" length of the animal, a number similar to the equivalent spherical radius, which accounts for the systematic change in target strength as a function of animal length.

Considering the variety of zooplankton, it is clear that a wide number of morphological traits need to be considered. Zooplankton comprise over 30 phyla with diverse body plans. Some of the more important ones are the crustacean zooplankton (euphausiids, copepods), the gelatinous forms (salps, medusae), animals with hard shells (pterapods), and zooplankton with gas inclusions (siphonophores). Certainly, the broad classes of animals can be used to predict backscattering, however, in each case the prediction of sound backscattering will likely be dependent on the details of animal morphology and composition. Animal composition will also change based on physiological condition and lipid reserves, and perhaps other environmental factors. It certainly remains an open issue to determine the applicability of the existing models to the variety of zooplankton that populate the sea.

Griffiths et al. (2002) note that there are several classes of animal sound scattering models which have been considered. Fluid like (euphausiids, copepods), animals with hard shells (pterapods), and animals with gas inclusions (siphonophores). An added complication in the case of the animals with gas inclusions is that there is a resonance frequency for the bubble. However, even far away from this resonance peak, the presence of a gas filled inclusion will produce a large degree of backscattering. Figure 4 summarizes a comparison of the target strengths of a number of animals computed using a variety of scattering models. The graph makes it clear that the simple interpretation that acoustic scattering is proportional to animal abundance or biomass is not possible for diverse assemblages of animals. In one recent example of the difficulties associated with a simplistic approach, it was noted that the scattering from pterapods in a given field trial dominated the acoustic backscattering even though they comprised only 0.1% of the individuals (Griffiths, 2002). These facts are in agreement with modeling and measurements by Stanton et al. where it was shown that the frequency-dependent scattering from a 21.8 mm decapod shrimp was extremely similar in magnitude (excepting fine structure) to a 1.9 mm gastropod. The authors remark that the echo energy per unit quantity of biomass of the 2-mm-long gastropod is about 19,000 times that of the 30-mm-long salp. This, of course, underscores the need for additional sampling schemes such as net tows or independent optical imaging of the animals as discussed below.

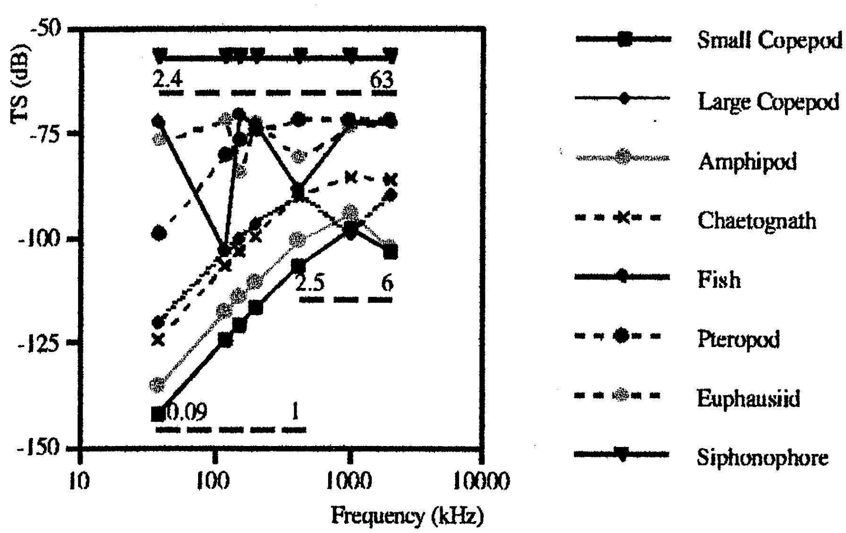

Figure 4: A set of curves that depict the backscatter Target Strength (TS) dependency upon frequency as a function of animal type (Griffith, 2002)

An interesting option, extensively considered by Holliday over several decades (Holliday and Pieper, 1989; 1995), is to use multiple frequencies (narrow band) of interrogation. Since the reflected energy is approximately a linear superposition of the frequency dependent scattering from the animals, providing the individual scattering functions are linearly independent, in principle, an inversion can be performed to compute the relative contributions of each component. As part of this work, it was revealed that the inversion is ill-posed, however by positively constraining the solution, it was demonstrated that it is possible to compute the relative contributions of the different scatterers, even in underdetermined systems. The efficacy of such a technique is a function of the frequency bands that have been chosen and the equivalent spherical radii of the particles. A poor choice of frequencies may render the inversion impossible, while a good choice will promote success. Greenlaw and Johnson (1983) considered the mathematical theory related to the “inverse” problem of computing animal abundances as a function of animal type from the collected multiple frequency backscatter data.

One further complication that should be considered in any attempt to perform a “routine” survey is the effect introduced by animal orientation upon backscattering. Figure 5 shows the result of comparison of a computer model of the backscattering from a euphausiid (source/ receiver at the same location) with recorded data (McGehee et al., 1998) as the animal orientation changes from “head on” to a side view. The figure illustrates that the increase in the effective cross sectional area normal to the beam direction is greatly reduced for the head on view (0º) with respect to broadside at 90º and 270º (~25 dB). Moreover this effect can be large for small changes in tilt angle, resulting in a large change in the amount of reflected energy. Complications due to this effect in the field are particularly severe for elongated animals that do not have gas inclusions (e. g. euphausiids). Recent studies by Warren et al. (2002) have confirmed these observations and also extended them to the study of elastic shelled organisms. Increased knowledge of animal orientation or “behavior” is thus a prerequisite for higher accuracy in echo sounder survey results.

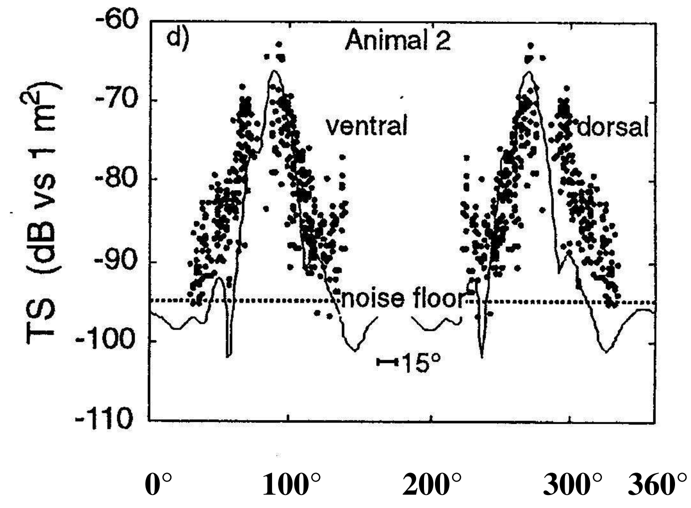

Figure 5: Laboratory measurements of backscattered Target Strength (TS) (dots) and model predictions (solid line) versus animal orientation of Euphausia superba (McGehee, 1998) at 120 kHz.

In considering the common methods that are used for "observing" the animals, we remark that there are two traditional modes for performing sonar studies: Echo integration and echo counting. In the echo integration mode, the volume backscattering is recorded and animal abundance is then estimated using either a single frequency or multiple frequency inversion. In the echo counting mode, the reflections from individual animals are recorded and the number of animals can be inferred from the multiplicity of traces. In practice, problems with the echo counting approach arise when animal densities become high because the multitude of reflections can "saturate" the echo-counting technique and individual traces may no longer distinguishable. In this case, the total energy in the recorded sound is related to the abundance of animals using some type of linear relationship (McClennan and Simmonds, 1992). This relationship forms the basis for much of fisheries abundance estimates and there is a large literature dealing with this methodology and the types of corrections that are needed under various operating conditions.

The user of any acoustic system for estimating zooplankton abundance and/or behavior has many factors to contend with. In environments where there is low species diversity, the interpretation of acoustic backscattering data can be greatly simplified. However, the interpretation of the data still requires the consideration of animal size and orientation. In many "real world" situations, the diversity of animal species can be high and thus, acoustics seems to be most valuable when accompanied by other methods for constraining the interpretation of the reflected energy. Animal identification can accomplished with net tows (with all of the associated issues related to avoidance, etc), or optical imaging methods. The optical methods have their own set of related issues (small interrogation volumes, reactions of the animals to lights, etc.). The following section considers the use of optical imaging and its implementation in observing zooplankton.

2.2 Principles of Underwater Optical Imaging

The basic principles of underwater light propagation and its ramifications for underwater optical imaging have been known for some time now and the use of optical imaging systems for underwater sensing, exploration, and discovery are commonplace to every diver or person who enjoys snorkeling and has marveled at the underwater landscape. Starting with the pioneering work of Siebert Duntley (1963) who was originally at the Massachusetts Institute of Technology and then the director of the Scripps Visibility Lab, a number of researchers have been involved in the advancement of underwater imaging (Mertens, 1970; Smith, 1984; Dolin and Levin, 1991). Recently, Jaffe et al. (2002) have reviewed the status of the contemporary field of underwater optical imaging. Briefly, the modern revolution in new and affordable optical components has availed to underwater optical engineers the opportunities to create a whole new generation of such systems.

On a basic level, one usually discriminates between "passive" and "active" imaging systems. In the case of passive systems, the imager does not illuminate the subject matter. Thus, some other source of light, either ambient or other, is necessary. In the case of active imaging systems, the imager itself generates the light. Although passive systems can be substantially less invasive than active ones, unfortunately, except in some of the clearest waters, these systems are not capable of producing images of zooplankton. That this can be true in many cases is likely because these animals place a premium on being transparent, or difficult to see, in order to avoid predators. Consequently, in every case that this author can identify, imaging systems for optically observing underwater zooplankton all use an "active" source of illumination. This source can be either a CW (continuous wave) light source such as a underwater flashlight, a strobe light, or some type of laser, either pulsed or CW. Many of these systems will be reviewed in the section on applications, however first, some physics.

The propagation of light underwater can be described in a straightforward way via the concept of radiance. Simply put, radiance is the three dimensional directional intensity of light propagation at a give location and time. Given that radiance is the state variable for optical radiation, the radiant distribution (which also considers polarization) describes everything that is knowable about an optical experiment. Since this quantity is spatially, spectrally, and temporally variable, its measurement provides quite a challenge for the underwater instrument designer. As covered in companion chapters to this volume (Morel; Lewis), many sensors have been devised to permit the measurement of either this quantity or one its moments. Cameras measure radiant energy by integrating radiance over each pixel in the camera.

A considerable simplification in understanding the performance of underwater illumination systems can be accomplished by categorizing the different components of the image. Figure 6 shows that the light that has arisen from an artificial source can suffer several fates; (1) Absorption by the intervening water or the subject. (2) Scattering from the intervening water. (3) Reflection from the subject. (4) Scattering in the intervening water after reflection by the target. The goal of any underwater imaging system is to maximize the contribution of (3) while at the same time, minimizing the effects of (1), (2) and (4).

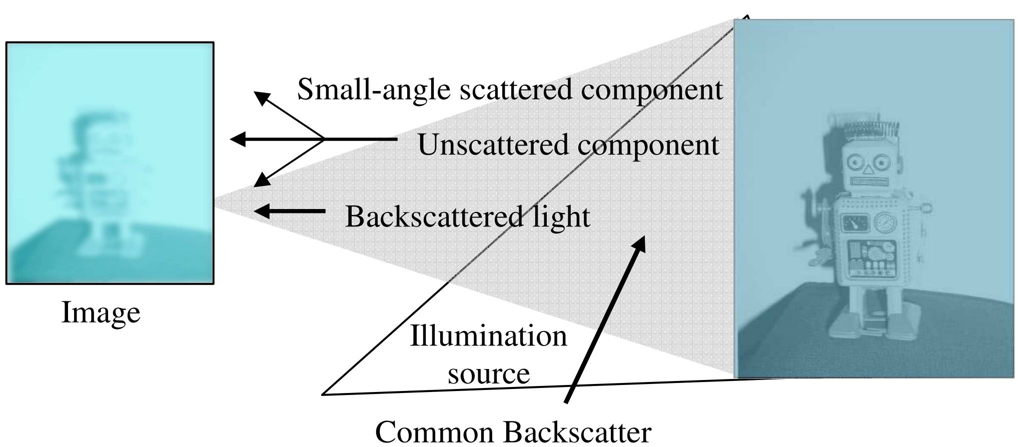

Figure 6: A schematic of an underwater imaging system and the components of an image that result from a categorization of the retrieved light

The environmental parameters that describe the propagation of light determine how well one can see. These parameters are the absorption and scattering of the water and the reflectivity of the subject. Although these parameters have been measured in some cases, the details of every environmental situation can play a predominant role in determining the outcome of an underwater optical imaging experiment. This is because, not surprisingly, the ocean can maintain large gradients in absorbers and scatterers (at for example, density discontinuities, or near the sea floor) that can also vary in time. Absorption, *a*, is a scalar. However, the more complicated situation of scattering must be described by a vector function that indicates the degree of scattered light as a function of incident angle and observation angle, $\beta(\theta_i, \varphi_i, \theta_r, \varphi_r)$. In most cases, in the water column, this function can be considered to be cylindrically symmetric (as above for acoustics), which reduces the dimensionality of the problem, however still leaves $\beta(\theta)$, the volume scattering function (VSF), to be measured.

An additional complication, which can be exploited in many situations, is the possibility of the absorbed light being re-radiated in some form. This can be via fluorescence or Raman scattering. Both elastic scattering (at the same wavelength of the incident light) and inelastic scattering (at some other, typically longer wavelength) can play a substantial role in the observations of an underwater optical imaging system.

The design of "efficient" underwater optical imaging systems can be aided by the development of computer models which can, in large part, mimic the propagation of light underwater and predict the outcome of a given situation. The computer model permits the user to place both cameras and lights at different locations with various orientations with respect to the subject matter. Predictions from the system can then be produced which will indicate the properties of the image field. This naturally assumes that the environment has been adequately characterized with respect to absorption and volume scattering. Although many absorption measurements have been performed and can be found in standard texts on underwater optics (Jerlov, 1976; Kirk 1994; Shifrin, 1988), information about the scattering function is rarely available. A web based resource for underwater optical parameters is now available (http://wood.jhuapl.edu/). The site contains many observations which can be used for obtaining absorption and scattering values which can be used to predict the quality of underwater images.

Although there have been relatively few measurements of the volume scattering function, a unique set of data (Petzold, 1972) have been used for many years and produce quite believable results when used in underwater models (McGlamery 1975; Jaffe, 1990; Jaffe et al., 1998b) and recent technological advances have yielded instrumentation so that volume scattering function observations will hopefully be routine in the near future (see Chapter by Roesler and Boss, this volume). The models can range from Monte Carlo simulations, which keep track of every photon, to those that use a semi-analytic formulation (Zege et al., 1991). The conventional formulation for understanding image propagation utilizes concepts from linear systems theory (Gonzalez and Wintz, 1987). This approach has been validated using Monte Carlo modeling (Jaffe, 1995b) and also semi-analytic formulations (Zege et al., 1991) for "reasonable" numbers of scattering lengths. The index which quantifies image blurring, the point spread function, describes the transformation in radiance distribution that occurs from object to image due to the small-angle scattering. Limited experimental determinations of these functions have been made and several parameterizations exist (Mertens and Replogle, 1977; Voss, 1991a, b; McLean and Voss, 1991). Unfortunately, there have been relatively few situations where the results of the models have been compared with the output of the camera systems when a complete environmental and instrumental suite of parameters was known. Nevertheless, in many cases, especially system design, the models can be used in a comparative sense, weighing the relative advantages and disadvantages of different geometries as a function of environment.

In considering the special case of the underwater imaging of zooplankton, a great unknown can be the animals' reflectivity. Many of the animals are transparent; this presumably conferring reduced visibility from predators. Therefore, for the most part, zooplankton imaging system designers typically use an empirical approach via the collection of animals and the experimentation with different imaging geometries. In some of these imaging systems a kind of "dark field" mode is used which images the scattered light (Davis et al., 1992; Strickler, 2000), however avoids the unscattered beam.

One desirable trait of an underwater imaging system is to possess a degree of "non-invasiveness", especially if behavioral observations are desired. This can be quite difficult for underwater optical imaging, since the options for propagating light over long distances are limited and for systems that are in close proximity, the animals are sensitive to hydrodynamic disturbances. Moreover, high light levels might affect the animals' behavior. The underwater lighting system designer whose goal is to image zooplankton needs to trade off all of these factors.

3. Applications: With special relevance to real time sensing

In the previous sections both the underlying physics for sound and light propagation in the sea have been discussed. In the next several sections, the practical implementation of such systems is described. In keeping with the general theme of this volume, we concentrate our efforts on describing systems that have some type of capability for reporting their data in real time.

3.1 Acoustical Systems for Sensing Zooplankton

Although there are a multitude of commercial acoustical systems that have been used in order to monitor fish, the commercial use of acoustics for monitoring zooplankton has been limited. This is likely due to the limited commercial marketplace for these animals. In turn, this has limited the types of acoustics systems that can be purchased off-the-shelf for measuring acoustic reflections from zooplankton.

In looking at the practical realizations of the acoustics we note that there are several different types of systems that have been used. One way of categorizing these systems is to consider both the number of beams and the number of frequencies that the system employs. Table 1 contains a list of systems known to this author. The notation commercial means that commercial versions of the system are available. N/K indicates that, to the knowledge of this author, there has not been either a research or commercial system used with these features.

	Single Beam	Split Beam	Dual Beam	Multi beam
Single Frequency	Commercial	Commercial	Commercial	Commercial FTV: Jaffe (1995)
Multiple Frequency	Maps/Taps: Holliday et al. Griffiths (1997)	Wiebe (1997, 2002) Demer (1999)	Wiebe (1994)	N/K

Wide Band System	Commercial (SciFish) Stanton (1998) Foote (1998)	N/K	N/K	N/K

Table 1: A categorization of acoustic systems. The table shows examples where commercial systems are available (commercial), and N/K = not known to have been built. Author's names are associated with mainly research systems.

3.1.A Single Frequency Systems

Certainly, the simplest type of system uses a single frequency and a single beam. In this category, one can find many of the economical consumer oriented acoustical systems for detecting fish that are available. Unfortunately, the use of these systems for scientific purposes has been hindered by a lack of calibration, which, in turn, prohibits their detection voltages from being turned into quantitative biomass.

One system which is widespread in the oceanographic fleet is the ADCP or Acoustic Doppler Current Profiler. The system is manufactured by RDI instruments of San Diego and it permits the user to estimate the underwater, range dependent, vector valued current velocity. The system works by sending four beams of sound into the water and subsequently measuring the range dependent Doppler shift and hence, from the four beams, some estimate of the vector-valued current.

Since these systems have become standard equipment for much of the oceanographic fleet, the possibility for using these systems to estimate biomass is appealing. In a classic study, Flagg and Smith (1989) used such a system to estimate zooplankton biomass. Although this early work attracted much attention, the difficulties of using such a system for quantitative estimation of animal abundance have precluded their routine use in the field. Thus, despite the fact that these systems operate at several frequencies, many of which are suitable for zooplankton, the systems have been used mostly for qualitative estimation of animal abundance. Nevertheless, the existence of concurrent information about both the physics (currents) and biology (animal abundance) presents interesting opportunities for analysis.

Both dual beam and split beam systems have been quite popular now for several decades. The systems have similar goals in that, since the sound fields that are projected by a sonar vary as a function of projection and receive angle, one must know the position of the animal in order to estimate the intensity of sound that in incident upon it so that the fraction of sound reflected can be inferred (and hence the target strength). Both of these types of systems are routinely calibrated and their field use has resulted in a many quantitative measurements. The split beam systems split a disk into four quadrants which each listen to the received signal. The time delay between quadrants is used to discern the direction that the sound is coming from. In dual beam systems the transmit and receive beam patterns (the intensity of sound with respect to direction) are cylindrically symmetric and the ratio between the intensity of the received sound for each target is used to locate the target and unambiguously allow the determination of target strength.

In comparing the performance advantages of the systems, Ehrenberg (1979) considered the relative advantages of split beam and dual beam systems from a signal processing point of view. The conclusions, at that time, supported the idea that the split beam systems were more robust in estimating animal position. This was especially true in the presence of other, possibly interfering targets. This may, in fact, explain the preponderance of split beam systems that are currently in use. There are several companies that manufacture bioacoustic systems (Simrad (Norway), Reson A/S (Denmark), Biosonics (Seattle, USA), Hydroacoustic Technology Inc. (Seattle, USA)). The systems can be purchased with a range of frequencies, some of which are suitable for observing zooplankton. Our purpose here is not to perform an exhaustive review of these systems and their use in zooplankton acoustics, but rather to alert the reader to the existence of such systems. Further information can be found in the article by Foote and Stanton (2000).

Multibeam systems offer an interesting set of advantages when considering field of view and the amount of power that can be projected. Since the acoustic reflectivity of zooplankton is typically low, these systems can offer substantial advantages for mapping these low reflectivity targets when a larger field of view is desired. Several commercial systems exist which were designed for multibeam bathymetry (Simrad, Norway and Reson, Denmark). The Simrad SM2000 system operates at a frequency of 200 kHz and projects sound in a plane that is 120 degrees wide by 1.5 or 3 degrees. The receiver beam forms the sound into 128 beams, each of a width of 1.5 degrees. The system has been used to map schools of herring off Nova Scotia with interesting capabilities to map three dimensional distributions of animals (Mayer, 2002). Several examples of the use of the Reson system, which operates at a frequency of 455 kHz with 60 beams each of width 1.5 degrees have illustrated its utility in characterizing the three dimensional distributions of animals (Gerlotto, 1998), (Gerlotto, 1999). To date, however, neither system has been used to characterize zooplankton distributions.

One multibeam system which has been used to map the behavior of euphausiids and copepods is the FishTV system (Jaffe, 1995b). Only two of these systems have been produced, one operating at a frequency of 435 kHz and the other at 1.57 MHz. Both systems incorporate similar designs and use multiple projectors that operate sequentially and are accompanied by parallel receipt. The 435 kHz system has a field of view of 16 degrees x 16 degrees and can map the reflections of up to 50 – 100 animals-m^{-3} (De Robertis, 2001) with a localization capability of one cm at frame rates of up to 4 Hz. At close range, the system records reflections from a 4 m^3 volume. A set of experiments in Saanich Inlet over two consecutive summer seasons resulted in the observation of several hundred thousand trajectories of the dominant taxa: euphausiids. The results have provided interesting information about the activity levels of the animals in the daytime (Jaffe et al., 1999) and the trajectories of the animals during their vertical migration (De Robertis et al., 2003). The 1.57 MHz system has been used to track copepods as small as 1-2 mm in the Gulf of Eilat (A. Genin et al. (2005).

3.2.B Multi-frequency Systems

The use of multiple narrow band frequencies in order to obtain more information than is available with a single frequency is a strategy for mapping animals that has been pursued by several groups. In one frequency regime (400 kHz – 3 MHz) a series of instruments by Holliday and associates over the last several decades have been used to estimate the size distribution of small animals (1 cm – 200 microns). In another frequency regime, there have been several systems whose design goals have been to map larger zooplankton (0.5 cm and larger) over larger volumes. As discussed above, the goal of these investigators has been to characterize animal size and, perhaps animal type, as a function of location.

Holliday and Pieper (1995) document many of the field situations that they have encountered with the MAPS system (at 21 frequencies) and the BITS system (at 165 kHz and 1.1MHz). In the use of two frequencies, the requirements for reasonable inversions can be quite

stringent. These include the existence of a single size organism that dominates the scatter at both frequencies, the existence of a validated model and other provisos that essentially imply that the animal densities are not so high as to introduce nonlinearities in the inversion process.
The latest version of the systems, TAPS ™ uses between four and eight frequencies. The system was designed to increase the discrimination in size over that of the BITS system without requiring the use of the large number of transducers that are used in the MAPS.

Holliday et al. (Holliday et al., 2003) describe the deployment of an 8-frequency version of the TAPS™ system that was extensively used in a series of studies in a shallow fjord in northern Puget Sound (between Seattle, WA, USA and Victoria, BC, Canada). During the summer of 1998 several multi-frequency acoustical zooplankton sensors (TAPS ™) were deployed. The sensor measured volume scattering strengths at 265, 4230, 700, 100, 1850, and 300 kHz with a 12.5 cm range resolution. The authors state that "useful" results were obtained at ranges up to 20 or 30 m (presumably with the lower frequencies). This new spatial resolution permitted the users to record a great deal of fine structure that existed in the water column. Inverse calculations for animal abundance as a function of equivalent size indicated that one of the "thin" layers seen was dominated by 1.2 mm copepods. Additional observations indicated the presence of mysid-shaped animals concentrated in an adjoining but distinct thin layer.

In looking at other multi-frequency systems, the Biomapper system of Wiebe et al. (1997) operating at two frequencies of 120 kHz and 420 kHz was used extensively in mapping plankton distributions in the vicinity of Georges Bank. The authors state "acoustics was used for *first order* estimates of the biomass of various taxa assuming that the proportions of taxa remained constant at constant depth". Building on experiences from the first BIOMAPPER system (and also a system called the Greene Bomber which employed a dual beam transducer operating at 420 kHz) the Woods Hole group has fabricated a new system, BIOMAPPER II (Wiebe et al., 2002). The acoustics for the system was manufactured by HTI (Seattle, USA) and it uses 5 upward looking and 5 down looking sonars at frequencies of 43, 120, 200, 420, and 1000 kHz. The system is well integrated with the resident vehicle and is completely digitally integrated into the complete package. Other examples of multifrequency systems are those of Foote (1998) and Griffiths et al (1997).

Note also that various users have combined multiple frequency split and dual beam systems themselves by simply deploying the various frequency systems together. The table here considers examples of systems where there has been a "tight integration" of the various frequencies so that identical volumes that have been insonified can be judged.

The conclusions of these workers seem to support the idea that there is, in fact, no ubiquitous solution to the acoustic detection of plankton. It seems, rather that based on the complexity of the scattering of the animals in a given ecosystem, the number of frequencies that are needed to describe the spatially dependent density of animals needs to be "tuned". That is not to say that valuable information cannot be obtained from acoustics, but rather, that the users of such systems must carefully choose their frequencies and inversion techniques based on the dominant taxa at the site of interest.

3.2.C Broad Band Systems

The use of broadband sound, is an area that holds much promise for zooplankton acoustics. One example where broadband sound was used in the lab by Chu and Stanton (1998) who showed that they could judge the size of the animals using a linear modulated FM chirp from 300 kHz to 700 kHz. Certainly, the coherent processing of broadband reflections represents a frontier that has great potential for providing valuable information about animal type. In addition, if the reflections were resolvable such that broadband sonar can be operated in an echo counting mode, it is possible that the features of the reflected waveforms will allow a higher degree of inference than the multiple (narrow bandwidth) sonars. A commercial system currently available from Scientific Fishery Systems Inc. (Scifish, 2003), Anchorage, AK, USA, uses a continuous sound source from 110 – 190 kHz. The manufacturer states that the system has been used successfully to classify among 20 different types of riverine and pelagic fish species.

3.2.D Prospects for real time acoustical monitoring of zooplankton

With special regard to the subject of this volume, we note that there are several examples of the use of acoustical systems for real time monitoring of zooplankton. In addition, the current trends in increased electronic power storage and processing efficiency lead to optimistic conclusions about the future of such devices.

In the realm of projects and equipment that have already been accomplished, the multifrequency systems of Holliday and co-workers have been used on moorings for many years.

The BITS system was placed on a mooring on the northern edge of the San Pedro Basin near the shelf slope break near Los Angeles, California. First installed in the fall of 1992 the system was composed of a two-frequency echo sounder. In one of the extended deployments, the system was deployed on a mooring for nearly 9 months. The system collected meteorological data on wind speed, light level and a two way packet radio communication system was used to automatically download the data to a shore station within line-of-sight of the mooring. The system of Holliday et al. that was deployed in Puget Sound is another example of the use of these TAPS^{TM}-like systems on moorings. Napp et al. (personal communication, 2003) have also deployed an eight frequency TAPS^{TM} system (TAPS^{TM}-8) on a biophysical mooring in the Gulf of Alaska. The system can discriminate animals into multiple size classes between 250 micrometers to 25 mm in total length by sampling a 2 liter volume at a range of 1.5 meters from the transducers. The lower frequencies were also used to estimate animal abundances at ranges as far as 15 meters. The system is designed to autonomously record data from the mooring over an almost 6 month period.

ADCPs have been extensively used on moorings, however, as explained above, the information currently available from these instruments has been primarily qualitative. In one example F. Nilsen and colleagues (University of Bergen) used moored ADCPs in order to analyze the daily vertical zooplankton migration in connection with mixed layer dynamics. Other opportunities currently being explored by several groups includes the deployment of either dual or split beam systems on moorings, and the adaptation of modern consumer level fish finders to remote moorings (D. V. Holliday, personal communication).

Future methods for acoustic surveys might incorporate different types of collection geometries or via the utilization of more information. An interesting demonstration of multi-frequency recordings from individuals (Demer, 1999) demonstrated that several animal types resident in the Antarctic ecosystem (euphausiids, siphonophores, myctophids) could be spatially discriminated (in some cases) using this approach. Alternative future methods could incorporate phase coherent processing of wide band echoes from individuals. Other alternatives would be to use Doppler processing techniques in order to glean information about the animals (Holliday, 1974, 1977) for those animals that actively propel themselves.

One interesting idea, which has seen little application, has been to use a “true” underwater acoustic imaging system to “see” the animals. An early use by Barans and Holliday

(1983) showed that, in fact, a high resolution side scan system could be used for imaging bottom fish. Currently, the DIDSON system of Belcher (Belcher, 2003) is being experimented with in order to monitor the length of fish that are progressing through a hydroelectric dam bypass. The extension of these methods to "imaging" of underwater zooplankton presents substantial challenges, especially when considering the high resolution that will be needed and the low reflectivity of many of the animals. As one final opportunity, it possible that other aspects of the scattered field like forward scattering or side scattering might provide additional information which could be used to improve species discrimination.

3.3 Optical Imaging of Zooplankton

The use of optical systems for observing zooplankton has been successful in many situations and there are a variety of systems that have been used for either routine survey or animal identification. In the case of survey, one must be prepared to argue that the assessment of animal populations is insensitive to the collection equipment. In some situations, both animal identification and abundance can often be judged. However, the small volumes that are necessary in order to obtain the resolution that is needed for animal identification can be partially compensated for via the use of fast frame rates and rapid surveys. Note that neither the optical plankton counter (OPC) nor the laser optical plankton counter (LOPC) are not considered here as they are not "imaging" systems. The interested reader is referred to the article by Wiebe and Benfield (2003) and the references contained therein for information about this system.

3.3.A The Video Plankton Recorder (VPR)

One system that has been used extensively in the field to image zooplankton is the Video Plankton Recorder (Davis et al., 1994). The system uses forward scattered light to image animals that are nearly transparent. Several cameras image several volume sizes simultaneously in order to provide information on different scales. The Video Plankton Recorder is an underwater microscope with a coupled video system that can be towed through the water column to make observations of small organisms in the approximate size range of 0.2 - 20 mm. Examples of animals which can be imaged are copepods, hydroids, and medusa. The quality of the images is generally sufficient to distinguish among species, and the same images can be classified automatically, as by a trained neural network, even at a video-image acquisition rate of 60 frames per second (60 Hz). Automated classification has the benefit of reducing the vast quantity of data to a description of the patterns of concentration (Tang et al., 1998).

The VPR was developed during the U.S. GLOBEC Georges Bank Regional Program (Davis et al., 1996; Benfield et al., 1996; Ashjian et al., 2001). Over the course of several years, the system provided extremely valuable data on the distribution of the mesozooplankton at the George's Bank region. Recently, (2001, 2002), it has been used in conjunction with the BIOMAPPER II system in the Antarctic.

3.3.B The ZooVis System

A new system is by Benfield (2001) uses a strobed sheet of white light. The system promises to yield images of zooplankton over an image field of view of 12 centimeters with a resolution of 50 microns. The zooplankton imaging and visualization system is a profiling instrument designed to collect quantitative images of mesozooplankton to depths of 250 m. The camera is aimed downward into a sheet that is 12 cm wide and 3 cm deep. By setting the depth of field to match or slightly exceed the depth of the light sheet, only targets that are in focus are illuminated.

3.3.C The Underwater Video Profiler System

The Underwater Video Profiler system (Gorsky et al., 1992) is an imaging system that consists of a Hi-8 video camera (with ultimate 512 x 512 resolution), controlling and data logging unit, batteries and several lighting systems. The lighting systems can either be a collimated light field in front of the camera which images 0.28 l via a 1.5 cm illumination sheet which is used for particle distributions, or a second system which uses four spotlights to image a volume of 70 liters of water. System calibration yielded a detection size of 100 microns. The system was designed for recording information about zooplankton and has been used successfully to image a variety of animals. In a typical deployment, the system was lowered at a rate of 1 m s^{-1} via a tether that resulted in a vertical sampling interval of 4 cm. Stemmann et al. (2000) present the results of a survey in the Northwest Mediterranean of particles greater than 150 microns. Several oceanographic studies illustrate the success of the system in the Western Mediterranean (Baussant et al., 1993; Gorsky et al., 2002) and the North Mediterranean (Gorsky et al., 2000). Figure 7 illustrates a collection of pictures of animals taken with the system.

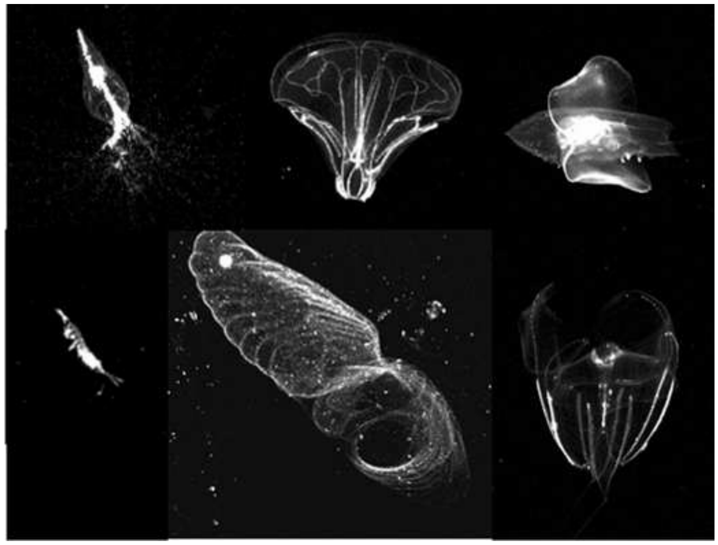

Figure 7: A collection of Macrozooplankton taken with the UVP from the northeast Atlantic, recorded during the French Pomme oceanographic cruise in 2001 (Gorsky, personal communication).

3.3.D The 3D Zooplankton Observatory

A system recently designed and built by Strickler and Hwang [2000] for obtaining information about 3-dimensional trajectories of zooplankton (3D Zooplankton Observatory) uses Schlieren imaging in conjunction with multiple cameras in order to obtain orthogonal projections of animals in a 1 liter volume. The system permits viewing aquatic organisms ranging from phytoplankton to fish and promises to provide interesting information about zooplankton behavior in the lab.

3.3.E Prospects for real-time imaging of zooplankton

Perhaps all of the systems considered in this section have the capability for real time imaging of zooplankton. In the case of the VPR system, the Autonomous Vertically Profiling Plankton Observatory (AVPPO) has been designed to collect data on the vertical position and taxonomic composition of the plankton together with ancillary environmental data. The system consists of the Video Plankton Recorder, which is buoyant, a transmissometer, fluorometer, downwelling light sensor, conductivity, temperature, absorption, and scattering meter and an

ADCP at 1.2 MHz that is used in an up-looking mode. Deployed at 100 meters, the device can operate in currents of up to 4 knots and medium seas (10 – 20' surface waves). The device is autonomous and can profile up to 500 times at a user-defined interval of days to weeks.

In a typical use, the VPR is released which is buoyant and it floats to the surface. It is then retrieved from the surface by a winch at a rate of 10 cm s^{-1}. Both video and environmental data are recorded. The system has the capability of transmitting a subset of the data to shore via a cell phone link. The system has been deployed on Georges Bank, and as part of the Front program (http://nopp.uconn.edu) between Montauk Point and Block Island and between Stellwegen Bank and Scituate (USA).

There is no doubt that the other systems considered in this section: ZooVis, UVP, and the 3D Zooplankton Observatory, could be configured to operate in a real-time mode on moorings. Since these systems have a very small volume of interrogation, the most advantageous use would be in an environment where the systems were profiling (as above) or in areas where there was adequate current to transport a volume of water through the field of view. As a final note, one should be aware of problems of avoidance by plankton. A general question arises about the non-invasiveness of any technology that uses light and creates a hydrodynamic disturbance. Thus, the user of any such system should consider the effects of their experimental configuration on the animals. From a hydrodynamic point of view, the deployment of such systems in a mode where they are either LaGrangian or 'downstream' that is, their imaging volume looks ahead of their hydrodynamic disturbance is desirable. In the case of light exposure, either the attraction or repulsion of zooplankton to light indicates that it is probably best to use a short exposure time on a "fresh" volume of interrogation if non-invasiveness is desired. This is not to say that valuable information cannot be collected from systems that use continuous illumination, but rather that such users should take into consideration the effects of such systems on the animals and on the types of conclusions that they are making.

3.4 Optical Imaging of Phytoplankton

In the previous section the underwater optical imaging of zooplankton was considered. Here, we survey a set of optical imaging systems that have the ability to observe phytoplankton. The demands which are put on the use of underwater optical imaging systems for observing phytoplankton are very similar to what one might consider in constructing a high magnification underwater microscope. If a true image of the organisms is desired then, considering that the cells sizes range from microns to 10's of microns, the resolution needed would be at a minimum several microns. As previously discussed, since light can be both attenuated and scattered, the observation of minute detail from cells that are suspended in water and therefore, *in-situ,* can be challenging.

3.4.A Holographic Imaging

A promising technique that has seen a great deal of progress in the last decade is underwater holography. Holographic imaging uses coherent light which is scattered from a collection of underwater particles and recorded on high resolution film to create an interferogram, or coding, of the three dimensional volume. The technique can be used in either an "in-line" or "off-axis-holography" which are different geometric configurations (Figure 8). In the "in-line" case, the scattered light is allowed to constructively and destructively interfere with the light that is unscattered. The "in-line" technique seems to work best when the water is only weakly scattering. As such, the scattered light can be combined with another, internally generated beam which coherently interferes with the scattered light

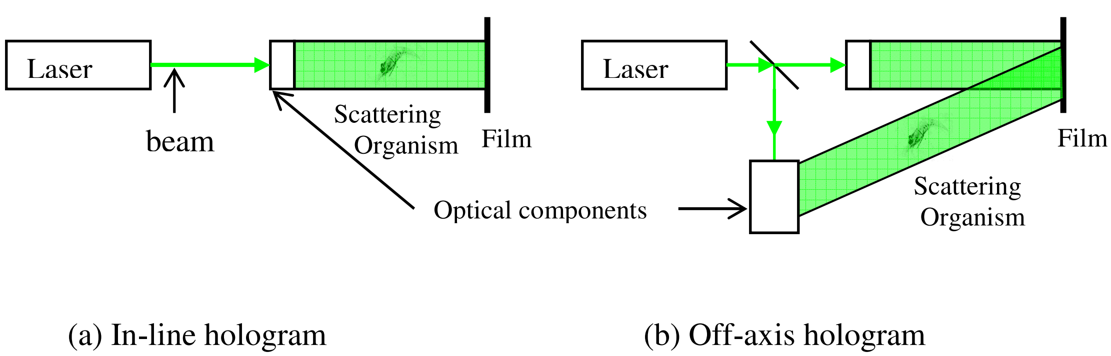

Figure 8. The basic geometry of an in line hologram (a) and off-axis(b) hologram.

Given the image of the interferogram, the three dimensional volume can be reconstructed via either analog or digital techniques. In the analog method an optical bench is used in order to physically reconstruct the three-dimensional volume that is then typically imaged with a digital camera. In the digital technique, the raw interferogram is digitized and the necessary image transforms are performed in order to synthesize the three dimensional image in the computer. One potential disadvantage of the method is that prodigious amounts of data are produced and necessary to process, so that the automated analysis of such data is imperative. A recent review of underwater holographic imaging principles and implementation by Hobson and Watson (2002) is recommended for further reading.

One of the first contributions to in-situ holography was due to Carder (1979). In the United States, a group that worked on underwater holograms for some time was under the supervision of A. J. Acosta at the California Institute of Technology. Several Ph. D. theses were authored in this area (O'Hern et al., 1988). In the last several years, a group at Johns Hopkins University have developed an in-situ holography system which uses an in line configuration in order to record coherent interference patterns that can be reassembled into 3-dimensional volumes (Katz et al., 1999; Malkiel et al., 1999). A lab system that uses both the in-line and off-line geometries has also been used extensively in the lab to measure the fluid fields around copepods. The system utilizes a ruby laser with an in-line recording configuration and has a sample volume of 732 ml. The reconstructed images have a resolution ranging from 10-20 μm for spherical particles and 3 μm for linear particles that lie within 100 mm from the film (in the plane of the camera imaging plane).

Most recently, a group in Aberdeen, Scotland has succeeded in deploying an underwater holographic imaging system that uses both in-line and off-axis geometry in order to form a set of images (Hobson and Watson, 1999; Watson et al., 2001). The system uses a pulsed Nd:YAG laser system which has the capability of very short exposure times which permits system operation in moderate flows. The interested reader should beware that the resolution that is usually quoted by the creators of such systems is the transverse or side to side (relative to the beam propagation direction. Hobson and Watson (2002) have noted that there is a fundamental limit to the axial resolution (along the beam axis) of such systems so that for a given system resolution of 5 microns, the depth of focus for a frequency doubled Nd-YAG laser-recorded holograph is 47 microns. One way around this complication is to fabricate a system that permits two orthogonal views of the subject (Sheng, 2003).

A system recently tested by Owen and Zozulya (2000) uses digital recording techniques and thus circumvents some of the complexities associated with film. The required digital image reconstruction techniques were developed and used to go directly from the camera image to the three dimensional volumes. The authors demonstrate resolution to 5 microns in small volumes and the technique seems to have promise because of the compactness of the instrument and its capability to circumvent some of the issues related to the recording media.

3.4.B The Flow Cam

A system that has been developed to image organisms from the sea is the Flow Cam system by Fluid Imaging Technologies, (Boothbay, ME, USA). The system, based upon flow cytometry technology, works by pumping samples through a relatively small volume that has both illumination and high resolution optics incident upon it. Having a small volume of interrogation allows the optics to be optimized for excellent image capability; however, this precludes using the system for imaging the in-situ distribution of animals and therefore restricts the system's use to analysis of samples that have been collected. The system has been used to image particles as large as 1 mm in size with a resolution of almost microns. The system comes in both a "lab bench" model for analyzing samples that have been retrieved and there is also a "Submersible FloCAM System" which can analyze particles from 5 to 1000 microns with a processing rate of up to 10 ml/minute. An interesting aspect of the system is that the manufacturers provide a suite of software programs that can be used to both examine and search the data base of inferred particle characteristics.

3.4.C FIDO-Φ

FIDO-Φ is a system that was designed to image the location and abundance of phytoplankton in volumes of approximately a half liter. The system does not "image" individual phytoplankton per se, however records either the scattered or fluoresced light from a volume that has been illuminated with a sheet of light that is 5-7 mm thick and subtends an angle of approximately 30 degrees. In a typical application an area of 25 cm x 25 cm at a range of 80 cm is viewed from a low light level CCD camera.

Palowitch and Jaffe (1993, 1995) demonstrated a lab based system for imaging the spatial distribution of phytoplankton which was subsequently used by Jaffe et al. (1998) and Franks et al. (2001) in the ocean in both a monochromatic and multi-spectral mode. Although this original system was tethered, in 2001, a new autonomous profiling system was used to collect information to 100 m depth on consecutive deployments. Preliminary processing of the data has revealed a large degree of spatial heterogeneity on scales of tens of centimeters. The primary advantages of the device are its ability to assay modestly large volumes (when compared with a conventional fluorometer) and to record the spatial organization of phytoplankton in their native, in-situ environment.

4. Combining Acoustics and Optics

As considered above, the lower frequency acoustical techniques for sensing zooplankton (50 – 200 kHz) have the advantage that they can be used at moderate distances (10's – 100's of meters) and survey large volumes. On the other hand, optical methods can be used for definitive animal identification (providing the magnification is large enough and the water clear). In consideration of this, several investigators have either deployed or synthesized *in-situ* devices that attempt to capitalize on the advantages of both optical and acoustical modalities. Since these sensing methodologies are complementary they can work together to permit a much more specific and valid set of inferences to be made. In the opinion of this author, the acoustics holds the promise of true non-invasiveness whereas the optics holds the promise of the detailed view of the organisms. The use of optics and acoustics in support of each other is documented in this section.

Over several field seasons, a very thorough set of surveys of the George's Bank area were performed using both acoustic and optical techniques. One of the acoustic systems used was the Biomapper system (Wiebe, 1997). This was complemented by the use of the Video Plankton Recorder. In addition, extensive net tows were performed in order to provide yet another degree of characterization. The advantage of the VPR, relative to the net tows, is that the device provides an instantaneous view of animals in its vicinity. As is widely appreciated, net tows integrate over the survey volumes. In the presence of high spatial variability the VPR can provide a degree of information that is not attainable with either acoustics or with the nets. In this set of surveys the optical information was used to complement the acoustic data, however, since the two platforms were being used from two different ships, the correspondence between the two was somewhat problematic. This motivated the development of the new BIOMAPPER II system (Wiebe, 2002), as described above. The system also has a VPR system mounted right on its bow so that some of the problems of judging animal correspondences that were encountered with the previous system could be avoided. Hopefully, the in-situ identification of animals by the VPR and its coupling with the acoustics will permit the inference of animal taxa to the volumes that are being surveyed by the acoustics but have not been imaged with the VPR system.

An example of an in-situ optical imaging system that was coupled to a system to measure target strengths at multiple frequencies (24 and 120 kHz) is documented in the article Warren and Stanton (2001). The authors deployed the systems on an ROV that was allowed to drift with the current for short periods of time and thus be relatively non-invasive. Gas-bearing siphonophores were optically imaged and corresponding target strengths were recorded. Although exact correspondences between organism and image were not achieved a great deal of in-situ target strength information was recorded which indicated that the siphonophores had large target strengths. A downward-looking sonar at a frequency of 120 kHz permitted the correlation between a more traditional echo-sounding system and the in-situ sensors.

The OASIS system combines the FishTV multibeam sonar system with a sensitive high resolution CCD camera. Using the system, exact correspondence between animal taxa, orientation, and backscatter intensity was obtained (Jaffe et al., 1998c). A special location, called the “magic voxel” was identified and when an animal was positioned in this exact 3-dimensional location (as sensed by the acoustics system) an optical image was taken of the animal using a red filtered strobe light for illumination. Deployment of the system over several weeks from a ship moored in Saanich Inlet, British Columbia resulted in somewhat greater than 100 optical images of animals with coincident target strength information. In order to promote non-invasiveness, a current vane was placed on the armature so that, in adequate current flow, the system could be pointed “upstream”, thus reducing the hydrodynamic signature upon the volume being imaged. Behavioral observations of the animal in 3-dimensions, in real time, were consistent with the conjecture that the animals were unaware of the device. Figure 9 shows a plot of animal target strength versus taxa and size for the animals which were simultaneously optically imaged and whose in-situ target strength was recorded.

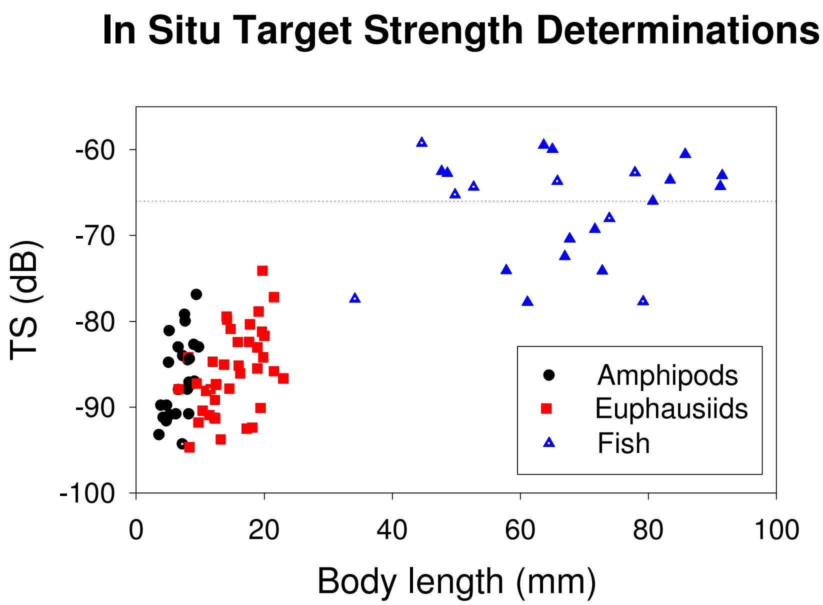

Figure 9: A graph of animal body length versus observed in situ target strength as a function of animal taxa (Jaffe et al. 1998c).

5. Summary and Conclusions:

In this chapter, the use of acoustics and optical imaging for monitoring *in-situ* zooplankton has been discussed. In addition, optical imaging of phytoplankton has been considered. A basic presentation of the underlying physical principals of systems for achieving this purpose has been made and a number of systems have been highlighted.

In the case of zooplankton, the primary contention of this article is that the "perfect" system for measuring zooplankton abundance and distribution does not exist. It is also somewhat unfortunate that taken together, the acoustical systems, the optical systems, and the net tow systems (which have not been discussed), do not completely fill in all the gaps. As all oceanographers know, the ocean is a space and time varying volume that can change rapidly. The characterization of large volumes at even moderate resolution remains a challenge.

It should be mentioned that the newest generation of deployment opportunities has great potential to enhance our view of oceanic processes. As demonstrated here, autonomous profiles, whether conducted from a vehicle, or a mooring, hold great promise. In addition, the newest generation of Autonomous Vehicles may permit a higher degree of space-time sampling than heretofore possible. The recent demonstration of the Autosub-II system (Brierly et al., 2002) which was deployed in the Antarctic and equipped with multiple frequency split beam sonars in order to detect the locations of euphausiids under ice at ranges of up to 27 km from the ice edge is an excellent example of new the kinds of new opportunities that these platforms present.

What can one say to the interested oceanic ecologist about which types of system are ideal for a given situation? In one set of circumstances, ecosystems with high species and morphological diversity, even the multi-frequency systems would not be a good choice. As inferred by Holliday and Pieper (1995) the use of very few frequencies can only be conclusive in the presence of very low diversity. For the two frequencies, only one dominant organism is necessary. The higher the diversity, the more frequencies will be needed. However, it cannot be said that for *any* given ecosystem, the use of a great many frequencies will provide an unambiguous outcome. Indeed it is likely that, given the current methods, obtaining animal abundance and species or even taxa identification simultaneously is not possible without using either optical or net tow techniques to supplement this information.

It seems therefore that a prerequisite to using acoustics is to have a certain amount of knowledge about what might be encountered in the field. This type of information can be obtained from both optical imaging and net tows. Afterwards, some guess can be made about an optimal acoustical package for survey and the range of acoustic frequencies can then be specified. Sample inversions should then be tried under assumptions about species diversity and location that allow the user to gain experience about what the results of the field expeditions may yield.

In the realm of new instruments, it is the firm belief of this author that broadband echo counting sonars which can obtain wide band spectra from individuals have the potential to greatly increase our ability to infer species and size. In addition, the prospects for using multistatic scatter in order to deduce animal size seem plausible. This will be especially valuable when coupled with the in-situ measurement of scattering functions with exact optical measurement of taxa and orientation. In the realm of optics, perhaps the newest generation of very low light level cameras coupled with infrared light will permit a new noninvasive and unambiguous view of these animals in their natural habitat.

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