A Component of the U.S. Global Change Research Program

Optics Technology Workshop Report

U.S. Global Ocean Ecosystems Dynamics

Report Number 8

March 1993

U.S. GLOBEC

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This is a report of the U.S. GLOBEC Workshop on Optics Technology held in Savannah, Georgia, USA at the Skidaway Institute of Oceanography on February 20-22, 1992 -- Gustav Paffenhöfer, convenor; Thomas R. Osborn, workshop chair.

The Editorial Committee for this workshop report included Hal Batchelder, Mark Berman, Percy Donaghay and Gus Paffenhöfer.

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EXECUTIVE SUMMARY

The goal of the U.S. GLOBEC Workshop on Optics Technology was to determine which existing, or soon to be available, optical technologies could be applied to U.S. GLOBEC's main objectives. Major advances in technology over the next decade are imperative if we are to successfully investigate biological and physical variables in the ocean at their respective spatial and temporal scales. This workshop was sponsored by the U.S. GLOBEC Steering Committee and had 14 participants whose background covered physics and biology (Appendix A).

The workshop focused on the potential of optical instrumentation to determine *in situ* (1) biomass and (2) rate processes of zooplankton. The workshop concluded that optical instrumentation will be required in conjunction with acoustics to quantify biomass, distribution and composition of zooplankton populations and communities. Optics and acoustics complement each other in studying spatial and temporal distributions of zooplankton; acoustics quantify the size distribution whereas optics are useful for taxonomic identification . This combination significantly enhances the probability of success of U.S. GLOBEC field studies of the interaction of zooplankton populations with their biotic and physical environments. The high spatial and temporal resolution of optical methods, plus continuous observation, make them an ideal tool for quantifying rate processes of juvenile and adult zooplankton, operating on scales from microns to meters. Two- and eventually three-dimensional, non-intrusive *in situ* observations should lead to a better understanding of rates of feeding, swimming and predation. Optics can also be applied to quantify other variables such as the distribution, abundance and types of potential zooplankton prey (both chlorophyll and non-chlorophyll containing) at the same time and in the same water parcel that the distribution and abundance of the zooplankton are measured.

Recommendations resulting from the workshop include the following: (1) Integrate acoustical, optical and physical sensors to simultaneously obtain from the same parcel of water information on zooplankton abundance and species composition, as well as the distribution of potentially controlling biological and physical variables. (2) Develop *in situ* configurations of sensors that can make non-invasive optical observations of zooplankton rate processes and biological and physical variables which control these rates. (3) Develop small, non-intrusive sensor platforms that can be used to follow small aggregations of zooplankton over time while rate processes are being measured. (4) Develop techniques for integrating multiple sensors into a sampling system that can be used to (a) identify and select locations for high resolution measurements and to (b) interrelate those measurements to rate processes and physical structures measured at other time and space scales. (5) Develop image analysis techniques that provide real-time, or near-realtime, identification of organisms from video images collected in measurements of both biomass and rate processes.

1 INTRODUCTION

1.1 Major U.S. GLOBEC Objectives

The U.S. Global Ocean Ecosystem Dynamics program (U.S. GLOBEC) is a component of the U.S. Global Change Research Program. Its major objectives have been stated in previously issued reports (e.g., GLOBEC Report No. 1, Initial Science Plan, 1991) and are as follows:

U.S. GLOBEC's overriding goal is to "address the question of how changes in global environment are expected to affect abundances, variations in abundance, and production of animals in the sea" (GLOBEC Rept. 1, p. 5). The approach chosen to accomplish this goal is to develop a fundamental understanding of the mechanisms that determine the mean level and variation in marine animal populations. Toward that end, U.S. GLOBEC is planning investigations of (1) how changes in ocean physics interact with biological processes to control the population dynamics of key species, and (2) how such population-level responses to physical processes affect the structure and stability of ocean ecosystems. Increasing our understanding of the mechanisms and linkages between physical processes, population dynamics and ecosystem structure will lead to improved understanding of how population dynamics and ecosystems will be impacted by climate change.

1.2 Goal of this Workshop

This workshop was intended to evaluate which optical technologies are presently available, which could be modified and which could be developed within the next several years to address some of the specific U.S. GLOBEC objectives mentioned subsequently. The workshop participants were asked to recommend technologies or instrumentation that would be near optimal to accomplish those objectives.

1.3 The Significance of Technology

The importance of advanced technology, specifically for biological oceanography, was emphasized in GLOBEC Report No. 1 (1991) and in the Marine Zooplankton Colloquium 1(1989). Many of the difficulties associated with studying plankton in the water column are obvious. Proto- and metazooplankton live in three dimensions and can respond instantaneously to external stimuli, feeding, swimming and darting about in ways which cannot be observed easily by humans without aid of instrumentation. To quantify and understand those organisms' behaviors, we need to sample or observe continuously, or at frequent intervals, and utilize approaches which do not alter natural behavior (non-invasive approaches). These observations need to be made at space and time scales relevant to the organisms' behaviors and appropriate for the rates being studied.

To make the requisite observations—high frequency, high spatial resolution, large dynamic range and non-intrusive—will require new technology (Marine Zooplankton Colloquium 1, 1989, p.198). Currently available instruments for quantifying the abundance, distribution, and most physiological rates of planktonic animals are inadequate to address the major goals of U.S. GLOBEC. At a U.S. GLOBEC Workshop on Acoustical Technology in April 1991 it was emphasized that acoustical and optical technologies were the "leading candidates from which the necessary tools could be drawn to support the pursuit of GLOBEC's science goals" (GLOBEC Report No. 4, 1991, p. 28).

1.4 Specific U.S. GLOBEC Objectives which could benefit from Optics Technology

To determine population dynamics of planktonic animals it is critical to quantify not only the abundance and distribution of the respective taxa over time, but also to quantify rates of birth, growth and mortality. Feeding rate measurements are valuable because they strongly affect the three above-mentioned variables. Neither acoustical nor optical instruments alone can be used to quantify all of these variables. Rather, these complementary technologies should be used together to provide the needed data (GLOBEC Report No. 4, 1991). Bioacoustic measurements of animals can perceive organisms larger than about 1 mm rather well, and can make accurate, frequent and rapid measurements of animal abundance and distribution. However, without ancillary information on target identification—either by the use of plankton nets, pumps, or optical sampling—current bioacoustical measurements are unlikely to provide the species specific data desired by U.S. GLOBEC. Nets and pumps are not adequate for target identification because in general they do not sample the same parcels of water sampled bioacoustically. Thus, optical instruments, which can provide target identification from the same parcel, will significantly improve the potential of acoustical methods to achieve the major goals of U.S. GLOBEC. Thus, the promise of optics is four-fold: (1) rapid identification of living organisms *in situ*, (2) quantification of organisms smaller than 1 mm, (3) observation of behavior and rate measurements directly and *in situ*, and (4) concurrent sampling of organisms, their prey and potential predators at spatial and temporal scales at which the physical environment can be sampled as well.

1.5 Organization of the U.S. GLOBEC Optics Technology Workshop

Workshop participants were provided a background document two weeks prior to the meeting which outlined the goals of the workshop and provided guidance for contributing. The workshop began with several presentations on existing optical instruments which are used to measure the abundance and distribution of zooplankton or to obtain rate measurements. The intent was to inform workshop participants of the status and capability of existing optical instruments to measure zooplankton structure and dynamics in the ocean.

Presentations were made by the following participants who described specific instruments and/or approaches.

P. Donaghay/J. Katz	Holographic Camera
R. Strickler	Holography and Schlieren Observations
D. Davis	Telemetry Developments
U. Kils	*In situ* technology, nested camera observations of predator and prey, video image processing
V. Holliday	Acoustical Estimation incl. MAPS and BITS; nesting of bioacoustical and optical technology; data reduction and analysis
R. Zaneveld	Coupling of physics and zooplankton food distributions

Discussion following the overview presentations resulted in the delineation of two major zooplankton issues to which the application of optical technology could contribute significantly. These issues are:

(1) the determination of zooplankton composition, biomass, and abundance, not only of target species, but also of their principal prey and potential predators, and

(2) the quantification of *in situ* zooplankton dynamics (i.e., rates), specifically studies of behavior, feeding, predation and conspecific interaction (e.g., mating).

Workshop participants formed two working groups to examine in detail the roles that current, planned and future optical based instruments could play in addressing these two issues. The results are provided in the working group reports which follow.

2 BIOMASS AND ABUNDANCE ESTIMATION WORKING GROUP

Chairman: Mark Berman
Rapporteur: Jeff Napp

Participants: Daniel Davis, Tommy Dickey, Van Holliday, Hein Skjoldal, Ron Zaneveld.

2.1 Task Statement

This group undertook to identify optical techniques which would facilitate quantification of the structure of plankton communities and their relationships to other biotic and abiotic variables over a wide range of temporal and spatial scales.

2.2 Scientific Problem

A basic objective of U.S. GLOBEC is to understand the ways by which physical and biotic factors govern the abundance and distribution of planktonic organisms over a range of temporal and spatial scales. Numerous studies have shown that zooplankton respond to certain physical (e.g., temperature, turbulence), biological (e.g., prey distribution, predators), and chemical (e.g., amino acids) variables. Our understanding concerning the range and extent of effects of these variables is severely limited because of the usual continuous motion of animals and water in three dimensions, and our inability to follow and sample or observe animal populations (e.g., Marine Zooplankton Colloquium 1, 1989) and water masses. To a certain degree this latter shortcoming can be solved by the application of improved technology. For phytoplankton to macrozooplankton sized marine (or aquatic) organisms optical methods appear to offer promising solutions to this problem.

2.3 Goal

The primary goal is to quantify the biomass of planktonic populations (including phytoplankton, meroplankton, holoplankton, and ichthyoplankton) and communities, and to describe the distribution and species composition of the plankton in relation to biotic and abiotic variables over a range of temporal and spatial scales.

2.4 Specific Objectives

(a) To measure total plankton biomass and its distribution on scales ranging from a few centimeters to tens of kilometers.

Biomass assessment should be sufficiently rapid and routine that surveys can be accomplished quasi-synoptically with results available in real, or near-real, time. This objective specifically includes the description of spatial, both horizontal and vertical, heterogeneity of plankton, its temporal persistence, and biomass distribution within a patch (i.e., distance to nearest neighbor).

(b) To relate the distribution of plankton to the structure of its biological, physical and chemical environment.

This would include description and evaluation of the physical and biological mechanisms responsible for the formation and maintenance of plankton patches. Animal behavior as it is affected by physical environment and biological neighbors is a variable whose importance must be assessed (see Rate Processes Working Group Report).

(c) To determine the composition of plankton communities in relation to their environment.

The structure of plankton communities can be crudely described by size-frequency distributions (e.g., Platt and Denman, 1978; Napp et al., 1993). At a more detailed level, plankton structure is described using relatively coarse taxonomic categories (e.g., separation of copepods, fish larvae, amphipods, and phytoplankton) (Berman et al., 1990). Ideally, the structure of planktonic assemblages is best described by species-specific, or even developmental stage specific identification of each planktonic individual.

2.5 Sampling Strategy

Eulerian and Lagrangian approaches have both been used in the past to describe the biomass and composition of plankton assemblages; rarely have both approaches been used simultaneously. The success of U.S. GLOBEC will eventually depend on the temporal and spatial resolution of biomass, abundance and species composition observations (e.g. Mackas *et al.*, 1985), and the rapidity of sample analysis. Traditional zooplankton sampling using nets and pumps provides relatively few and rarely replicated observations. The samples require a long time to analyze and provide poor temporal and spatial resolution. With the improvement of acoustical and optical technology for biomass quantification and the use of long-term moorings and/or drifters, the spatial resolution, frequency of observation, and rapidity of sample processing will improve. This improvement will provide more timely and detailed data on zooplankton abundance and distribution, and a better understanding of the structure and dynamics of zooplankton and ichthyoplankton populations (see reviews by Dickey, 1988, 1991).

2.6 Required Sensor Technologies

Achieving the objectives listed above will require the application of different combinations of sensor technologies and data analysis techniques. The objectives are listed in order of least (biomass and size only) to most (biomass, size and taxonomic identification, coupled with concurrent physical structure observations) sophisticated data return. These objectives will, therefore, be discussed separately.

(a) Technology to quantify total plankton biomass.

The most advanced optical sensor in routine use for zooplankton biomass and size structure assessment is the Optical Plankton Counter (OPC, Herman, 1988, 1992; Herman and Dauphinee, 1980) which is designed to count and size zooplankton in the size range from 0.25 to 30 mm. The OPC employs a parallel light beam of 640 nm wavelength and uses the maximum cross-sectional area of a zooplankter as it passes through the beam to estimate size. It is useful for identifying the dominant copepods in boreal environments where relatively few species dominate much of the planktonic biomass. Recent modifications to the OPC have extended its suitability for sizing small (down to 0.12 mm) zooplankton (Herman, 1992). It can be deployed in conjuction with net sampling systems, on remotely operated vehicles, or by itself. When the OPC is coupled with other sensors as a single deployable instrument package, it can provide reasonably detailed spatial resolution of the interaction between plankton and the physical structure of their habitat. In this regard, it is useful in addressing objective (b) below. It is commercially available and represents one of the more mature optical instruments available for the assessment of zooplankton biomass and distribution. It is not however, in its commercially available form, capable of imaging individual zooplankton, and thus is somewhat limited in its ability to define the taxonomic structure of zooplankton assemblages. It is adaptable for towing as part of an undulating vehicle, and a moored version is under development.

(b) Technology to determine distribution of plankton and their relation to the structure (biological, physical, chemical) of their fluid environment.

This objective focuses on the quantification of plankton distribution, supported by the quantification of major physical (e.g., temperature, salinity) and biological (e.g., distribution of food organisms, predators) variables. Distribution implies abundance measurements in up to three dimensions and through time. To accomplish this, various instruments employing acoustics and optics have been developed. Shipboard or moored Acoustic Doppler Current Profilers (ADCP) can measure acoustic backscatter, which if calibrated properly, can provide data on the vertical distribution of zooplankton biomass over extended periods of time (Flagg and Smith, 1989) or quasisynoptically for a spatial grid or transect (e.g., Heywood, et. al., 1991). The vertical distribution of 21 independent size classes of organisms can be measured using the Multifrequency Acoustic Profiling System (MAPS, Holliday *et al.*, 1989). The report of the U.S. GLOBEC workshop on Acoustical Technology (GLOBEC Report Number 4) provides an overview of sonar and acoustical sampling techniques and makes recommendations for further research in the area of acoustical instrumentation. However, as discussed below, a shortcoming of current acoustical instruments is their general inability to positively identify acoustic targets.

Several optical instruments currently exist that can provide data on plankton abundance (and to some extent size structure) at spatial and temporal scales similar to those at which physical structure is measured. The above-mentioned OPC in its towed version is commercially available and can be used to determine plankton abundance and distribution vertically and horizontally (Herman, 1988, 1992). The data can be immediately processed and displayed. When coupled with other sensors (e.g., for temperature, salinity, oxygen concentration, fluorescence, etc.) the OPC is useful for describing plankton distributions in relation to their physical, chemical, and biotic environment.

The Video Plankton Recorder (VPR, Davis *et al.*, 1992a,b) has been developed to quantify abundance of zooplankton on scales from microns to kilometers, and is now in the prototype stage. It consists of a video camera/strobe unit and an image processing system. Four video cameras are synchronized at 60 frames sec^{-1} to a red strobe light positioned 1 meter away. The field of view of each camera is adjustable from 0.5 to 10 cm, at 10 to 300 microns resolution, respectively, with

corresponding depths of field of 4 to 20 cm. Imaged volumes are concentric with their center located 0.5 m between camera and strobe. Each one microsecond strobe pulse permits highly resolved images of plankton and seston. Plankton abundance is determined by counting the number of animals per field of videotape and dividing by the field volume.

The Optical Plankton Recorder (OPR, Kils, 1981; 1989) is a compact, high-speed, underwater video microscope with optional preconcentration nets. It is designed primarily for small-scale, high-resolution observations of plankton distributions. Prototype instruments have been deployed free-falling in Antarctic krill studies (Kils, 1981); towed from small vessels in mesoscale monitoring of fish schools; anchored (moored) for plankton orientation and ecotoxicology studies; and used in aquaculture for particle flow quantification (Kils et al., 1991). When towed, free-falling, or hovering, each image is exposed to two short (10 μs) strobes separated by 20 ms. Three different cameras with nested magnifications allow for observation of both predators and prey simultaneously, and for taxonomic identification (Kils, 1989).

To understand the distribution of zooplankton in their physical and chemical environment requires simultaneous collection of physical, chemical and biological data at common spatial and temporal scales. The distribution of phytoplankton has been found to be closely correlated with physical phenomena such as density gradients and shear layers. Moreover, at times phytoplankton distribution influences the distribution of zooplankton (e.g. Paffenhöfer, 1983; Roman et al., 1986; Napp et al., 1988). Several bulk optical parameters are useful in describing the *in situ* distribution of phytoplankton or particulate biomass at scales comparable to physical structure measurements:

Beam attenuation: Beam attenuation at 660 nm has been used to estimate the volume of total particulate matter. For more than 20 years it has been applied to estimate total mass of autotrophic, heterotrophic and detrital particles. The standard instrument is the transmissometer.

Particle scattering: Back scattering of light from particles can be used to estimate total particle volume. Recent tests of prototype instruments have shown that particle scattering in a volume of 1 ml can be achieved for particle concentrations ranging over five orders of magnitude. Because the sensor is small it can be deployed in a variety of modes.

Red fluorescence: Stimulation of red fluorescence by ultraviolet and blue light has been widely used to estimate phytoplankton biomass (Yentsch and Menzel 1963). Low sampling rates (about 1 Hz) and internal averaging limit the spatial resolution of current *in situ* fluorometers.

Spectral fluorescence: The completion of development of *in situ* spectral fluorometers is about two years away. Tests of prototype laser-induced spectral fluorometers have shown their usefulness in measuring chlorophyll and other phytoplankton pigment signatures at vertical spatial resolutions on the order of 1-2 cm (Cowles et. al., 1989; Carr et. al., 1992). These instruments have the potential to estimate the biomass of major taxonomic groups of phytoplankton, such as diatoms, cyanobacteria and green algae.

Spectral absorption: Prototype measurements indicate that *in situ* absorption can be measured at a single wavelength. A prototype spectral absorption meter has been tested *in situ* (Moore et al., 1992; Zaneveld *et al.,* 1992). The device has been used to determine the concentration of chlorophyll, detritus and cyanobacteria at sufficiently high frequencies to delineate centimeter-scale structures.

(c) Technology to determine the species composition of plankton communities.

A major conclusion of the discussions at the U.S. GLOBEC Acoustical Technology Workshop (GLOBEC Report No. 4, 1991) was that while acoustical methods are very good for quantifying the abundance and distribution of plankton, they cannot currently identify organisms to species level. Identification to species is desirable within the context of plankton studies generally, and U.S. GLOBEC specifically, because animals of similar size but different taxonomy behave and perform differently (e.g., Paffenhöfer and Stearns, 1988). It became clear during the above-mentioned workshop that optics would be an excellent tool for identification purposes. This led to the statement that "the integration of acoustical and optical technology could yield synergistic benefits and that the technologies are complementary " (GLOBEC Report No. 4, 1991, p.28).

Zooplankton can be organized in various taxonomic groups ranging from phylum to species. We consider here a range from family to genus, species and developmental stage. Morphometric features, mainly body shape and dimensions have been used for computer-automated identification of net collected, preserved zooplankton (Berman, 1991). To identify living organisms *in situ* several optical approaches have been used with varying degrees of success and identification: cameras (Ortner et al., 1981), video adapted nets (Welsch et al., 1991), video/OPC (Ortner et al., pers. comm.), the VPR (Davis et al., 1992a,b), and the CritterCam (Bergeron et al., 1988).

The Plankton Camera (Ortner et al., 1981) is towed at approximately 2 kt and obtains a silhouette photograph every two seconds. The film records are quantified manually after the cruise. In addition, measurements of temperature, conductivity, depth and fluorescence are obtained every second.

The Video Adapted Gulf III Net (Welsch et al., 1991) was designed for surveys of herring recruitment in the North Sea. A videocamera images organisms as they pass into the cod end of a plankton net; the video is transmitted in real-time via conducting cable to the research vessel. The images enable the identification of organisms from 0.5-20 mm in length to major taxa (Schulze et al., 1992). This device is expected to be commercially available within one year.

The Video/OPC (Ortner et al., pers. comm.) is a device combining the Optical Plankton Counter (OPC) with a frame-synchronized strobe light silhouette camera. It employs "smart sampling". When the OPC detects the presence of an organism or particle, it triggers the video camera to image the particle during passage through the instrument. The video image is used for specific target (taxonomic) identification. Not every possible target needs to be visualized, but merely a sufficiently large random subsample.

The towed VPR (Davis et al., 1992a; 1992b) has been tested as a prototype. Images from the video cameras on the VPR are digitized and processed in real-time by an image processor and transmitted to a host computer where morphometric indices (e.g., lengths of major/minor axis, area, etc.) are computed, and organisms sorted to major taxa (e.g., copepod, euphausiid, chaetognath, etc.) using discriminant analysis (Berman et. al., 1990).

The CritterCam (Bergeron et al., 1988; Schulze et al., 1992) is a video system developed by Rudi Strickler for imaging plankton distributions and behavior underwater. It is based on modified Schlieren optics and achieves very high resolution at sufficiently long working distances (0.15 to 0.4 m) so that organisms' behaviors are minimally affected by the instrument. It views a field of ca. 6 x 4.5 cm with a resolution of 5 μm and uses a pulsed (<1 μs) near-infrared diode illumination to freeze the motion of organisms. In moored configuration it can produce images of zooplankton

sufficient in quality for the organisms to be taxonomically identified. The CritterCam is presently being readied for commercial production.

The 3-D Bioluminescence Mapping System (Greene et al., 1992; Widder, 1992; Widder et al., 1992) is used to identify and map bioluminescent organisms based on the spatial and temporal patterns of their stimulated bioluminescent displays. Using species-specific bioluminescent displays enables bioluminescent organisms ranging in size from 50 μm to 1 m to be mapped simultaneously with a single video camera.

2.7 Recommendations

A variety of optical sensors designed to study the distribution and behavior of planktonic plants and animals are currently available or in development (Schulze et al., 1992). Support for new optical samplers should be based on their ability to meet a need not filled by those systems already under development. Existing optical sensors should be improved and adapted to sampling platforms that will allow them to sample on the time and space scales needed to meet U.S. GLOBEC's basic scientific goals. This improvement should include interfacing optical sensors for both zooplankton and phytoplankton with acoustical and physical oceanographic sensors to allow simultaneous collection of various types of data from the same parcel of water (e.g., using moorings and drifters).

From the discussions of this working group, it is apparent that the increasing sophistication of video sampling technology is outpacing improvements in post-collection data analysis (e.g. automated image analysis and pattern recognition). Video sensors typically collect 30-60 samples (images) per second. Even though most of these images (depending on the magnification of the image and the depth of field) may be devoid of animals, visual examination of each image soon becomes impractical. Higher priority should be given to the development of systems to analyze the output of the video sensors. Ultimately, we need integrated optical collection, analysis and recognition systems that can measure and identify plankton animals to the species level in real time. However, a more realistic near-term goal is the near-real time identification of video images of plankton to major taxonomic groups.

3 *IN SITU* RATE PROCESSES WORKING GROUP

Chairman: Percy Donaghay

Participants: Joseph Katz, Uwe Kils, Gustav Paffenhöfer, Rudi Strickler

3.1 Task Statement

The *in situ* rate processes working group was given the task of identifying optical techniques that would allow direct or indirect estimation of rate processes controlling the dynamics of key zooplankton species. Specific tasks for optics utilization outlined in the Background Document were: (a) obtain estimates of feeding, birth, growth and mortality rates of planktonic animals *in situ* through continuous observations of individuals or small swarms over seconds to minutes; (b) obtain information on swimming behavior; (c) obtain predation data on zooplankton by using 2 or 3 different scales of observation simultaneously; (d) observe small-scale distribution of zooplankton, especially aggregations of animals smaller than 1 mm; and (e) obtain data on small scale physics and biophysical interactions. Each of these tasks was evaluated both from the perspective of using optics to directly address key scientific issues of U.S. GLOBEC (see Scientific Problem) and as tools to routinely collect data on rates. There was a general consensus that efforts should initially

focus on developing optical techniques to directly address key scientific issues. The rationale for this choice is summarized below.

3.2 Scientific Problem

A fundamental objective of U.S. GLOBEC is to understand how biophysical interactions at the individual level control zooplankton population dynamics and fish larval recruitment. Small-scale biophysical interactions can potentially affect zooplankton population dynamics through their effects on feeding, swimming and predation vulnerability. The nature of these effects is currently a matter of considerable controversy. In particular, recent experimental and theoretical studies have suggested that increased small-scale mixing may (1) alternatively decrease feeding through reduction of micropatches (e.g., Lasker, 1975; Davis et al., 1991), or increase feeding through the effects of increased small-scale shear on encounter rates (e.g., Rothschild and Osborn, 1988); (2) alter swimming patterns through effects on fine-scale food structure or induction of escape responses (e.g., Donaghay, 1990); and/or (3) modify predation vulnerability through effects on predator encounter rates (e.g., Rothschild and Osborn, 1988), predator detection mechanisms, and zooplankton aggregation (e.g., Kils, 1992). Although there is sufficient theoretical and experimental evidence to indicate that these biophysical interactions may sometimes control individual success and population dynamics, we do not have the technical capability to test these biophysical interaction models *in situ*, to evaluate the validity of current rate measurements, or to test new techniques that could dramatically improve *in situ* rate estimates (Price et al., 1988; Marine Zooplankton Colloquium 1, 1989; Schulze et al., 1992). Just as important, these same gaps in technical capability have limited our ability to relate *in situ* responses to potentially controlling factors and thereby identify "signature" properties that can be used in surveys to link fine to coarse scales and to identify areas where specific processes should dominate.

3.3 Goal

The primary goal is to develop a combination of techniques that will allow the *in situ* measurement of zooplankton rate processes (feeding, swimming and predator interactions) along with the potentially controlling fine-scale biological structure, physical structure and biological-physical interactions. Optical techniques will be important in achieving this goal because they provide direct evidence of the interaction of individual organisms with their conspecific neighbors, their prey and potential predators.

3.4 Objectives

(a) Measure the feeding, swimming and/or predation interactions of a group of zooplankton while simultaneously measuring in 3 dimensions the fine-scale (μm to cm) particle and shear fields immediately surrounding these individuals. These measurements must be repeated periodically as the zooplankton move through the diel cycle.

(b) Interrelate the fine-scale structure and responses to small-scale (mm to m) vertical and horizontal gradients in those biological (food, zooplankton, predators), physical (shear fields, temperature, salinity, density), optical (light) and chemical (oxygen) properties that could influence the rates or induce directional responses in the fine-scale measurements. These gradient measurements must be made in conjunction with the fine-scale measurements (above) so that the two can be interrelated.

(c) Link fine to coarse scales by measuring biological and physical "signature" properties over a broad range of scales covered by the daily ambit of the zooplankton of interest (i.e., from scales of

centimeters to 100's of meters covered by migrating zooplankton). These measurements should include the vertical structure of temperature, salinity, density and oxygen as well as the distribution and migration of foods, zooplankton and predators.

3.5 *In Situ* Rate Process Sampling Strategy

Two very different strategies could be used to estimate critical rate processes in zooplankton First, a single individual zooplankter could be followed (tracked) over time (15 minutes or so) while measurements of individual feeding, swimming and predator interactions are recorded. Although this technique provides ideal data for analysis of the mechanism controlling feeding and swimming behavior, the costs of developing equipment for individual tracking *in situ* appear to be prohibitive at this time. Second, a group of zooplankton can be followed over time while measurements of feeding, swimming and predator interactions are repeatedly recorded for the group. This approach provides statistical estimates of rate processes such as percent of time feeding, percent swimming upwards, average swimming velocity, etc. These statistical estimates are critically needed for parameterizing models of population dynamics and for identifying factors that control these behaviors. In contrast to tracking individuals, the acoustic technology required for tracking groups of individuals is readily available. The following sections therefore presume this latter strategy.

3.6 Required Sensor Technologies

Four major types of optical sensors will be required. Each of these types of sensors are summarized briefly below. For a more detailed discussion of these sensors see the short sensor descriptions in the Biomass Working group report and the instrument descriptions in Schulze et al. (1992).

(a) Imaging optical sensors that can provide real time and permanent 2D or 3D records of zooplankton feeding, swimming and predation interactions.

A variety of excellent two- (2D) and three-dimensional (3D) video systems have been developed for making these measurements under controlled conditions (see reviews by Price et al., 1988; Dickey, 1988; Schulze et al., 1992). These systems range from low-resolution systems designed to measure swimming behavior (Bugwatcher, e.g., Buskey and Swift, 1983; 1985), to high-resolution systems designed to measure feeding appendage motions and particle capture (Alcaraz et al., 1980). These techniques have recently been extended to allow tracking of single individuals over time (CritterCam-2D and CritterSpy-3D), as well as examination of the behavior of groups of individuals (Bugwatcher). Extensive motion analysis software has also been developed. High-resolution systems for *in situ* two-dimensional video measurements have recently been developed for measuring feeding (CritterCam, Bergeron et al., 1988) and predator-prey interactions (ecoSCOPE, Kils, 1992) in the ocean. The critical factor limiting application to *in situ* rate measurements is the lack of development of stable, nonintrusive deployment platforms. High resolution three-dimensional systems have not been developed for use in the ocean primarily because they are far more intrusive than 2D systems, although 3D videography using multiple orthogonally-oriented cameras has been recently used to study the behavioral interactions of relatively large organisms (fish and shrimp) (W. Hamner, pers. comm.).

(b) Imaging optical sensors that can measure particle characteristics, distributions (inter-particle distance), and motions (from swimming, sinking and small-scale mixing) in three-dimensions in the immediate vicinity of the zooplankton (20 μm to cm).

The technology for making these measurements does not currently exist, but motion-sensing holographic systems are under development to make these measurements in the ocean and in the

lab. Prototype hardware testing should begin in two years. The critical factor limiting application to *in situ* rate measurements is the development of the sensor hardware and data processing techniques. The lack of development of stable, non-intrusive deployment platforms will also limit some applications.

(c) Imaging sensors that can measure gradients in zooplankton and their resources over small- to course-scales.

Various arrays of the required video sensors are undergoing prototype testing in the field (e.g., Video Plankton Recorder (Davis *et al.*, 1992a; 1992b); ecoSCOPE (Kils, 1992)). These systems provide sufficient quality images that visual methods can be used to identify two-dimensional spatial patterns at the species level. Routine use of these systems for spatial mapping is severely limited by image analysis software development (see Biomass Estimation Working Group Report). The holographic systems currently under development could also be used to measure gradients.

(d) Bulk optical sensors that can measure food distribution on both fine- and coarse-scales simultaneously (thus providing links between scales).

Bulk optical sensors measuring transmission, scattering, fluorescence and absorption at single or multiple wavelengths that could provide the required information exist or are under development . The utility of these measurements for estimating "signature" properties should greatly increase as spectral optical devices move from prototype to commercial instruments. Prototype tests have demonstrated the feasibility of developing biological/physical microstructure profiling systems that can be deployed free-fall in survey mode (laser/fiber optic profiler (Cowles et al., 1991)) or from stable platforms (bio-optical surface profiler (Donaghay et al., 1992)). The primary factor limiting routine measurement of fine structure during both surveys and rate experiments is the development of combinations of non-intrusive sensor configurations and non-intrusive sampling platforms.

3.7 Recommendations

The progammatic goal of *in situ* rate measurements can only be met by the nonintrusive application of a combination of optical and non-optical sensor technologies. Although most of the required sensor technologies have been developed for use in the laboratory or for coarse-scale sampling in the ocean, their successful application to *in situ* rate process measurement will require major efforts in the five areas discussed below.

(a) Develop *in situ* configurations of the required sensors.

Accurate measurement of an *in situ* rate process and fine-scale structure is dependent on developing non-intrusive sensor packages that do not disturb the structure and processes they are measuring. These problems are particularly critical for systems designed to collect time series data on behavior. Recent prototype work, however, has demonstrated that major advances can be made in this area by reducing instrument size through use of micro-optics and micro-electronics. Major progress can also be made by using optical relay techniques (fiber optics, relay lenses, range gating) to increase the distance between the sensor and the sensed volume. The working group strongly recommends that these technologies be exploited not only in developing new sensors, but also in reconfiguring existing and prototype sensors into operational instruments.

(b) Develop non-intrusive techniques for testing and routine deployment of the multiple sensor systems needed to make the required measurements.

This is a first-order problem that severely restricts the use of both existing and future optical systems for measuring *in situ* rates. Small sub-surface platforms (including manned submersibles and remotely operated vehicles) hold the greatest potential for rapidly developing the capability to follow a single group of zooplankton over time while making behavioral observations. This capability is essential to making the *in situ* rate process measurements discussed above. The working group therefore strongly recommends that non-intrusive techniques be developed and tested for deploying these sensor systems from both existing small manned submersibles and from small unmanned platforms associated with those submersibles (Donaghay, 1989).

(c) Develop techniques for integrating the multiple sensors into a system that can be used effectively under *in situ* conditions.

Two closely related sensor integration problems must be resolved. First, techniques must be developed that ensure that all recorded data can be interrelated in time and space. Although this problem sounds trivial for the systems with a fixed (i.e., Eulerian) frame of reference, the problem is more difficult when the coordinate system is constantly moving with the group of plankton being observed. Second, "smart sampling" techniques must be developed that allow real time analysis of data from one or more sensors to be used to control the timing and location of sampling by higher resolution systems. Prototype tests (bio-optical and density surface sampling, OPC triggered video camera) have demonstrated that the development of "smart sampling" techniques is an excellent way to reduce post-experiment data analysis to manageable levels, yet still insure that critical data are collected.

(d) Conduct cooperative engineering of sensors and sensor configurations.

Many of the imaging optical sensors under development have the potential to provide information critical to the interpretation of bulk optic and acoustic sensors. At the same time, the bulk optic and acoustic sensors have considerable potential for guiding high-resolution sampling. System integration is needed, i.e., electronic coordination of data acquisition from different sensors which cover the same water volume at the same time.

(e) Improve data and image analysis capabilities.

Current capabilities for analyzing images to identify species are clearly inadequate to allow automated processing of the large numbers of images that will be produced by these systems. Although it is doubtful that deficiencies in data handling and pattern recognition will limit progress in rate measurements in the near term (where problems (a)-(d) are of greatest concern), the development of these capabilities should begin immediately since they will require years to perfect.

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