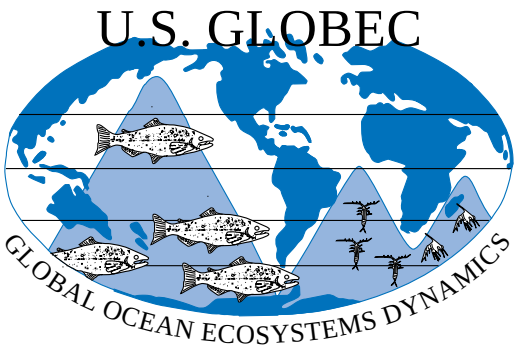
U.S. GLOBEC: A Component of the U.S. Global Change Research Program



U.S. GLOBEC NEWS

Number 4 August 1993

Models, Physics and Predictive Biological Oceanography: KNOW Your Organism

by Marc Mangel

From its inception the U.S. GLOBEC program has rightly recognized that modeling should be an inherent conceptual component, particularly if we want to connect biological and physical oceanography. In fact, there is considerable merit to the argument that the harder it is to get the data, the more important it is to do the modeling. Michael Sissenwine (1983), considering the Convention for the Conservation of Antarctic Marine Living Resources, wrote,

> Modeling should be an integral part of research on Antarctic marine living resources. It is the process of formalizing thought. Mathematical models express ideas in concise and universal language...Like thinking, modeling is an ongoing process which is stimulated by observations (i.e., data). Models in turn stimulate additional data collection, usually followed by modeling. The process of modeling forces consistent thinking. This process is particularly important for multi-disciplinary, multi-national situations where observations are made and ideas evolve independently. A model is a synthesis of these observations and ideas.

Simply put: before spending lots of time at sea, we should think carefully about the kinds of data that should be collected and what they can tell us; here models can play an enormously important role.

But, what kinds of models should be used? I differentiate between models based in "physical mathematics" and those based in "biological mathematics." The former are essentially rooted in classical mechanics, and assume large numbers of identical individuals. The latter differ most importantly in recognizing the role of natural selection in shaping organisms and, particularly, that organisms can facultatively respond to their environments, often in unanticipated ways.

In its early stages, our field benefited greatly from the use of "physical" mathematics (ordinary and partial differential equations) to describe biological settings. For example, the Lotka-Volterra predator prey equations illustrate how purely biological interactions between predators and prey can lead to oscillations in population numbers. Similarly, the Lotka-Volterra two-species competition equations show how competitors might either co-exist or not, depending upon the strength of inter- and intra-specific interactions. Nonlinear reaction-diffusion equations show how species-specific interactions such as predation or competition, melded with diffusion, can lead to spatial pattern. However, such models

[biological mathematics]...differ most importantly in recognizing the role of natural selection in shaping organisms...organisms can facultatively respond to their environments, often in unanticipated ways

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Modelling—(Cont. from page 1)

are difficult to use as predictive tools if they involve parameters that are fit from the data series which are being modeled (as is often the case). They do not provide a means for predicting how the parameters, and thus the dynamics, will change in changed circumstances. An exception could be the situation in which organisms are transported by passive diffusion or currents (so that we understand the transport parameters very well), but even simple organisms can behave in complex ways and modify their transport.

Physical mathematics is especially limiting, for two reasons, if we want to predict the response of organisms to changed environments. First, the historical emphasis of physical mathematics has lead to what might be called "backward hypotheses": workers often formulate a "null" hypothesis that is exactly contrary to what would be concluded by careful thinking about the biological situation. For example, the inappropriate assumption that the experimental subject and observer share the same perceptions of the environment causes one to assume global information as a null hypothesis when, in fact, exactly the opposite should often be the underlying assumption. To illustrate this, a common assumption is that predators in a spatially distributed system will aggregate in regions having highest prey densities. This idea is based on the assumption that the predators "know" the global distribution of prey and are wide ranging. When organisms demonstrate such global environmental knowledge, the phenomenon needs to be explained, but it is not the starting point. This is particularly important if we want to use models of organisms to help identify the correct scales of measurement for physical studies. Second, because of the enormous numbers of particles usually studied in physics, diversity is not important. But one of the great attractions of biology is the enormous variation of living organisms. In fact, R. J. Berry (1989) has said, "Variation is the core of biology." Removing diversity from biological descriptions renders those descriptions nearly devoid of meaning.

To include such diversity and develop means for predicting the parameters in the equations of population dynamics, we must adopt the principle "KNOW Your Organism" as a guidepost for biological modeling, and for coupling of biological and physical models in the U.S. GLOBEC program. The principle asserts that organisms can display a wide range of behaviors in response to their environments and that we must thoroughly and deeply understand the particular organism before constructing a quantitatively predictive model. Approaches that focus on the state of the organism (Mangel and Clark, 1988; Mangel and Ludwig, 1992) allow this to be done. Furthermore, by considering the state, we link physics and biology in a natural way since physical factors such as temperature, photoperiod, and flows generally affect and constrain (because of physical and chemical laws) the physiological condition of organisms. Physical mathematics underlies these couplings.

The principle "Know Your Organism" is almost antithetical to what guides us in physics. After all, the laws governing ocean physics are the same in the California or Benguela Currents, or in the Antarctic or at the Equator. And it is true enough that biological organisms must obey the laws of physics. But these laws merely act as a constraint on the main law of natural selection that governs organisms. "Know Your Organism" means that, in general, we will not be able to develop highly predictive and general "zooplankton" or "fish" models, although we should be able to develop qualitative models which provide insight by using physical mathematics. Highly predictive models must treat the organisms as ones which can behave and respond to changed environments in novel ways, and it is here that biological mathematics is needed. In general, the challenge still awaits us.

(Marc Mangel is a professor in the Department of Zoology at the University of California, Davis).

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U.S. GLOBEC

GLOBAL OCEAN ECOSYSTEMS DYNAMICS

U.S. GLOBEC NEWS

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First U.S. GLOBEC Field Program Set to Begin on Georges Bank

by William Peterson

The U.S. GLOBEC Georges Bank program was initiated in 1991 with funding from the NSF (Biological Oceanography) for two modelling projects (described in U.S. GLOBEC News No. 1, Spring 1991) and with funding from NOAA Climate and Global Change, Marine Ecosystems Response program for a pilot field project and some retrospective data analysis (summarized in U.S.GLOBEC News No. 2, Fall 1991). An additional modelling project was added in late 1991 as were two biotechnology projects.

In January 1992, a team of academic and NOAA scientists began meeting regularly at Woods Hole to write a plan which would lead to the implementation of a full-scale study of the physical and biological dynamics of Georges Bank. As a result, the Northwest Atlantic Implementation Plan was published in June 1992 (U.S. GLOBEC Report 6, 1992). Subsequently, a request for proposals (RFP) was issued by the newly-established NOAA/NSF Interagency Program Coordination Office, with a September 1992 deadline. Two review panels were convened in December 1992—one to provide advice on the long-term research goals outlined in the Northwest Atlantic implementation plan, and the other to provide an evaluation of the Northwest Atlantic proposals and their peer reviews. A total of 37 proposals, with over 100 co-principal investigators requesting $13,000,000, were received and evaluated. Eighteen projects have been recommended for funding (approx. $5,000,000) to 72 scientists at 24 institutions in the U.S. and Canada. Accompanying tables list the projects by title and PI.

The goal of scientists working in the U.S. GLOBEC program is to predict the effects of changes in the global environment on the abundance, variation in abundance and production of marine animals (particularly zooplankton and fish populations), through a fundamental understanding of the mechanisms that control variations in abundance in time and space. Our approach is to determine which (and how) physical and biological oceanographic processes control populations, and how variations in abundance might be partitioned between natural and anthropogenic causes. This will require:

- an understanding of the response of ocean physics to climate change
- an understanding of how physical processes control the biology and ecology of zooplankton and fish populations.

Thus the overall program goals for all U.S. GLOBEC studies are:

- to determine linkages and degree of coupling between atmospheric forcing, and physical and biological

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RETROSPECTIVE ANALYSIS	
Jim Bisagni, John O'Reilly (NMFS-Narr.) Jim Yoder (URI)	Satellite-derived sea surface temperature and ocean color variability on Georges Bank
Steve Bollens, Cabell Davis, Andy Solow (WHOI)	Georges Bank zooplankton and storm effects: historical data analysis and comparison with model results
Carol Meise, Wallace Morse, John Green (NMFS/Narr) Ted Durbin (URI)	Life history patterns of *Calanus finmarchicus* and *Pseudocalanus* in the Gulf of Maine: effects of temperature and water column structure

WATER COLUMN STRATIFICATION: PHYSICAL AND BIOLOGICAL DYNAMICS	
Bob Beardsley, Julio Candela, Jim Churchill, Cabell Davis, Scott Gallager, Steve Lentz, Bob Weller, Albert Williams (WHOI) Dave Mountain, Greg Lough, Jim Manning, George Bolz (NMFS/Woods Hole) Mark Berman, John Green (NMFS/Narr) Lewis Incze (BLOS) Neil Oakey (BIO/Nova Scotia) Dave Hebert (URI) Charlie Flagg (BNL) Brad Butman (USGS)	Seasonal development of stratified water on Georges Bank: dynamics of zooplankton and larval fish
Ted Durbin, Ann Durbin (URI) Jeff Runge (IML/Quebec)	Recruitment and production rates of *Calanus finmarchicus* and *Pseudocalanus* on Georges Bank
Dian Gifford (URI) Mike Sieracki (BLOS) Scott Gallager (WHOI)	Phytoplankton and protozoa in the diets of copepods and larval cod on Georges Bank
John Stegeman, Michael Moore (WHOI)	Analysis of short-term growth in copepods and larval fish using molecular markers of cell proliferation

BANK-WIDE SURVEYS OF PHYSICAL AND BIOLOGICAL PARAMETERS	
Peter Wiebe (WHOI) Dave Mountain, John Green (NMFS/Woods Hole) Wallace Smith, Peter Berrien, Michael Fahay, Wallace Morse (NMFS/Sandy Hook)	Ichthyoplankton and zooplankton in the Georges Bank region: distribution, abundance, life history characteristics of zooplankton and larval fish in relation to hydrography, and ecological and climatic factors
Charles Miller (OSU)	Copepod population biology on Georges Bank
Pamela Blades-Eckelbarger (U.Maine) Nancy Marcus (FSU)	Investigations of environmental cues and physiological processes that regulate diapause in *Calanus finmarchicus*
Peter Wiebe, Tim Stanton (WHOI) Charles Greene (Cornell)	Broad-scale and time-series acoustic measurements of zooplankton and nekton in the Georges Bank region
Larry Madin, Steve Bollens (WHOI) Barbara Sullivan, Grace Klein-MacPhee (URI) Marv Grosslein,Mike Fogarty(NMFS/Woods Hole)	Effects of predation by fish and invertebrates on target species of fish and copepods on Georges Bank
Ann Bucklin, Wendell Brown (UNH)	Biological and physical evidence of source regions and transport patterns of zooplankton on Georges Bank

Georges Bank—(Cont. from page 3)

processes that control the population dynamics of marine zooplankton and fish

- to translate this understanding into assessments and predictions of the impact of climate change on marine ecosystems.

These goals will be accomplished through an interdisciplinary effort involving physical and biological oceanographers and fisheries biologists, carrying out modelling studies, field studies, retrospective data analysis and long-term observations.

Why Begin With A Study Of Georges Bank?

Georges Bank was selected for several reasons. First, it is situated just north of a faunal boundary which separates subtropical species from temperate species. Global warming could result in a northward shift of this boundary, which would completely change the species composition, and, consequently, the ecosystem dynamics of the Bank. Such a shift would be immediately detectable and would serve as an early indicator of climate change. The shift could occur because of general warming, changes in coastal circulation driven by increased buoyancy of coastal waters (due to warming and ice melting), or shifts in the location of the core of the Gulf Stream. Other processes that could impact the Bank are changes in the frequency of severe storms that cross the Bank and frequency of collisions of Gulf Stream rings on the Bank. Another result of a shift of the faunal boundary could be that large schools of filter-feeding mackerel (a southern species) would arrive earlier than usual on Georges Bank and severely impact larval fish and copepod populations through predation. All of these topics are being studied by U.S. GLOBEC investigators.

Secondly, the Bank supports an economically valuable fishery for cod. Recently, all cod stocks along the western Atlantic have fallen into a state of decline, which many scientists believe can be attributed partially to subtle changes in climate. Fishing pressure may be a factor contributing to the decline as well, so we need to sort out the effects of fishing vs. environmental influences on the observed declines in cod stocks. Third, the Bank is of sufficient size and has a physical circulation (an anticyclonic retention gyre) which enables distinct populations to develop and persist for long periods (time scale of months), making them amenable for time-series study. Thus, we know that we can make repeated cruises to the Bank and study the dynamics of the same populations. Fourth, a 10+ year data base exists for interannual variations in cod and copepod abundance, hydrography, circulation, and SST and ocean color from satellites. These records need to be reanalysed in a climate change context. Finally, the target species selected for study (the codfish, *Gadus morhua*; haddock, *Melanogrammus aeglefinus*; and the copepods *Calanus finmarchicus* and *Pseudocalanus*), are subjects of intense ecological study by many nations around the north Atlantic, including Canada, Iceland, Norway, the United Kingdom and Denmark. This gives all scientists the opportunity to mount collaborative comparative studies of these species in a basin-wide oceanographic context. Such activities are moving forward through the ICES Cod and Climate Change program and through a "*Calanus* and Climate Change" program recently proposed to ICES.

The Georges Bank Research Program (In A Large Nutshell)

The Georges Bank Study contains these components: retrospective data

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LONG-TERM MONITORING/IN SITU INSTRUMENTATION	
Ken Brink, Jim Irish, Bob Beardsley, Richard Limeburner (WHOI) Charles Flagg, Creighton Wirick (BNL)	Long-term moored and Lagrangian measurements as part of the Georges Bank study
Rick Pieper (USC) Van Holliday (Tracor)	Applications and new technologies for bio-physical interdisciplinary trophic studies [Moorings for monitoring biomass of zooplankton using acoustics]

NUMERICAL MODELLING	
Dan Lynch (Dartmouth) Francisco Werner (UNC) Mike Sinclair, Peter Smith, David Greenberg, John Loder (BIO) Ian Perry (DFO/Nanaimo) Fred Page (DFO/St. Andrews) Greg Lough (NMFS/Woods Hole) Wallace Smith (NMFS/Sandy Hook)	Importance of physical and biological processes to population regulation of cod and haddock on Georges Bank: a model-based study
Glen Gawarkiewicz, David Chapman, Cabell Davis, Changsheng Chen (WHOI) Don Olson (U.Miami) Peter Franks (Scripps) Glen Flierl (MIT)	Modelling studies of coupled physical/biological processes affecting recruitment on Georges Bank
Benoit Cushman-Roisin (Dartmouth)	Numerical investigations of currents on Georges Bank

Georges Bank—(Cont. from page 4)

analysis, fine-scale process studies, broad-scale surveys, long-term monitoring/*in situ* instrumentation, and numerical modelling.

Projects concerned with retrospective data analysis are looking at a 1939-1941 zooplankton data set from Georges Bank, the 1977- 1988 MARMAP data set on winds, hydrography and plankton abundance, and at the entire SST and ocean color data sets from satellites for the Bank. These studies will provide both the climatology, and, importantly, the degree of interannual variations to be expected for winds, SST, salinity, phytoplankton and abundances of copepods and larval fish.

Broad-scale survey cruises and fine-scale process cruises will be conducted each month, from January through July 1995. Broad-scale cruises of 16-day duration are followed by 12-day process cruises. Thus, at least one ship will be on the Bank nearly continuously for seven months. The broad-scale surveys provide information on bank-wide variations in temperature, salinity, currents, phytoplankton, zooplankton and fish using CTD, ADCP, satellite imagery, fluorometry, plankton nets and towed acoustic sensors. The zooplankton sampling contributes to the description and modelling of population dynamics of copepods, larval cod and larval haddock, a key component of the overall program. A companion laboratory study of factors controlling diapause in *Calanus finmarchicus* will contribute to an understanding of the dynamics of overwintering populations. Sampling during the broad scale surveys will be sufficiently intense to allow comparison of dynamics of populations in the Gulf of Maine (GOM) with those on Georges Bank (GB). Genetic structure of GOM, GB and other *Calanus* populations living around the North Atlantic will be compared to determine degree of isolation of populations. In addition to the hydrography and zooplankton work described above, monthly surveys of the distribution of schools of predatory fish (mackerel and herring) and invertebrate predators will provide data on the zooplankton predator fields. This last project is a collaborative effort co-funded by the NOAA Coastal Ocean/Coastal Fisheries Ecosystem program.

An added value of the monthly surveys is that they provide "snapshots" of the hydrography and the distribution and abundance of plankton which will aid greatly in the planning of each of the fine-scale processes cruises. The fine-scale studies planned for 1995 will focus on the processes controlling stratification of the water column, seasonal evolution of stratification, and effects of stratification on food chain dynamics. Physical oceanographic work includes study of currents using current meters, shipboard ADCPs, and drifters, and measurement of turbulence and mixing both within the water column and in the benthic boundary layer. Much of the

(Cont. on page 20)

BIO	Bedford Institute of Oceanography
BLOS	Bigelow Laboratory of Ocean Science
BNL	Brookhaven National Laboratory
Cornell	Cornell University
Dartmouth	Dartmouth University
DFO/Nanaimo	Dept. of Fisheries and Oceans/Nanaimo
DFO/St. Andrews	Dept. of Fisheries and Oceans/St. Andrews
FSU	Florida State University
IML/Quebec	Institut Maurice-Lamontagne
MIT	Massachusetts Institute of Technology
NMFS/Narr	National Marine Fisheries Service/Narragansett Lab
NMFS/Sandy Hook	National Marine Fisheries Service/Sandy Hook Lab
NMFS/Woods Hole	National Marine Fisheries Service/Woods Hole Lab
OSU	Oregon State University
Scripps	Scripps Institute of Oceanography
Tracor	Tracor, Inc.
U.Maine	University of Maine
U.Miami	University of Miami
UNC	University of North Carolina
UNH	University of New Hampshire
URI	University of Rhode Island
USC	University of Southern California
USGS	United States Geological Survey
WHOI	Woods Hole Oceanographic Institution

(Editors Note: Technology Forum is intended to stimulate thought and discussion on diverse oceanographic technology issues. We welcome contributions on technological issues relative to ocean science, but particularly to U.S. GLOBEC.)

3-D Bioluminescence Mapping

by Edith A. Widder

An intensified video transect technique has been used to identify and map bioluminescent organisms based on the spatial and temporal patterns of their stimulated bioluminescent displays (Greene et al., 1992; Widder et al., 1989, 1992).

This technique evolved as a by-product of an investigation of unstimululated background bioluminescence levels in the Monterey Canyon. The intensified video transect technique employed during that investigation, using the single person submersible DEEP ROVER, was originally designed to measure the abundance of potential luminescent sources in the water column. During horizontal transects, a video recording was made of bioluminescent displays from organisms which were mechanically stimulated to luminesce as they contacted a 1-meter diameter screen mounted in front of the submersible. An automated computer image-analysis program was then used to count the number of sources stimulated during the transect. During the course of this investigation, it became apparent that it was frequently possible to identify the sources of these displays, often to the species level, based on the temporal and spatial patterns of their bioluminescent displays (Widder et al., 1989). In this initial investigation, identified displays were limited to gelatinous sources. More recently the technique has been adapted for use on the JOHNSON-SEA-LINK submersible (Figure 1) and was employed to map the micro-scale distribution patterns of the euphausiid *Meganyctiphanes norvegica*. Simultaneous estimates of krill abundance and patchiness were made with a dual-beam acoustic method. Comparison of abundance and patchiness estimates made with these two very different mapping techniques demonstrated no significant differences between these estimates (Greene et al., 1992). In addition, the bioluminescence technique simultaneously mapped a population of co-occurring ctenophores (Widder et al., 1992).

Data Analysis

Through a collaboration with MRJ, INC., image recognition algorithms are now being developed that will automate the identification of organisms based on their bioluminescent displays, and then map their locations in three-dimensional space. Using parallel processing, the video frames from a bioluminescence transect are stacked, one on top of each other, to create a solid volume. With this data format the high-contrast video images can then be thresholded and the background made transparent so that only the bioluminescent events are visible in three-dimensional space. This volume data structure is used to identify luminous events and extract features such as intensity; duration; size; kinetics (rise time, decay rate, pulsing rate, if any); release of extracellular material if any (glowing particles, scintillating particles, diffuse clouds); and the coordinates of the point of impact. This information is then sent to the luminous object data structure, where it is stored and organized into flash categories. Densities of different flash categories are then calculated from the volume scanned. Distances to nearest neighbors, etc. can then be calculated.

Advantages

1) Bioluminescence mapping is both a high-frequency and high-resolution technique which samples a statistically significant volume. Using the species-specific label of bioluminescence, dinoflagellates as small as 50 µm can be identified in real-time in a field of view of one meter. Therefore, multiple bioluminescent species of sizes extending from 50 µm to 1 m can be mapped simultaneously with a single video camera.

2) Bioluminescence recordings are very high contrast, thus edge detection is much less of a problem compared to illuminated video recording. As a result, the algorithms for categorization and identification of bioluminescent signatures are extremely simple compared to image recognition algorithms, which require much higher resolution images and must deal with issues like multiple

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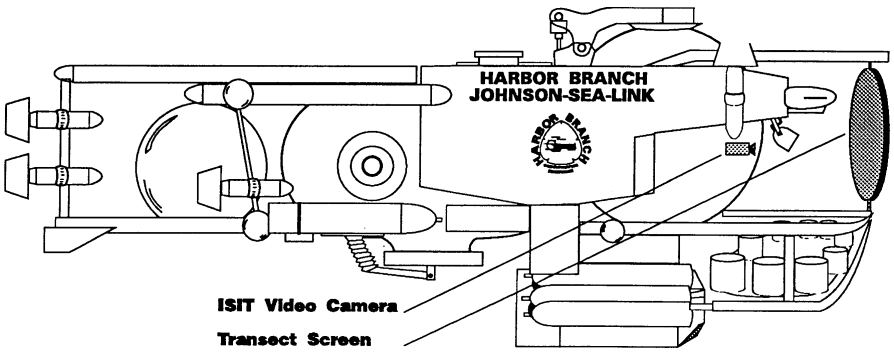

Figure 1. Configuration of the JOHNSON-SEA-LINK submersible for making intensified video recordings of stimulated bioluminescence during horizontal transects.

In situ Behavior of Deep-Sea Animals: 3-D Videography from Submersibles and Motion Analysis

by Peggy P. Hamner and William M. Hamner

Movements of animals in three-dimensional (3-D) space cannot be measured accurately with standard two-dimensional (2-D) photographic systems. To understand the behavior of individual animals and their interactions in a three-dimensional medium, it is absolutely essential to use three-dimensional videography. We describe a 3-D video system for use on either manned submersibles or ROVs, and provide an example from the deep sea that demonstrates the necessity for three-dimensional measurements. Animals recorded simultaneously on two videotapes as they swim within a calibrated volume of water in front of the submersible are automatically tracked though time and in three-dimensional space with a commercially available 3-D tracking system.

Rationale for 3-D Measurement

One obviously important facet of every organism's existence is its patterns of movement within its habitat, both in space and through time. Ethologists routinely record animal behavior optically in the field for later analysis. However, the usual single camera view provides only a two-dimensional image of behavioral phenomena that often occur in three-dimensional space, particularly if the animal moves through the air or through water. Tracking undisturbed animals in 3-D provides far more accurate information about movements than do estimates derived from 2-D measurements. We have developed and tested a two-camera video system which can be mounted on a submersible to record objects in the deep sea for subsequent automated 3-D analysis of behavior.

Video System for a Submersible

We use two monochrome video cameras corrected for underwater parallax (Walton, 1988). The cameras are fitted with fixed-focus lenses and wide angle adapters and are permanently mounted in custom housings. Wide angle adapters are necessary because the cameras on the submersible toe in toward each other so that their fields of view intersect approximately 2 m in front of the submersible. Each camera's field of view must be as wide as possible because images outside the volume of water viewed by both cameras are recorded on only one videotape, and are useless for 3-D analysis.

The camera housings are rigidly mounted on either side of the submersible because the positions of the cameras relative to each other must remain constant to track an object accurately. A control box remotely

Humans see the world with stereo optics, and the ability to view three-dimensional events in 3-D would assist us in understanding complex behaviors.

powers both cameras and electrically controls the irises. When operated inside a manned submersible, the control box is connected to the cameras via a cable which penetrates the submersible's hull to transmit video and control signals (Fig. 1). Video recording is controlled from two VCRs inside the submersible. Sequences on the two videotapes must be synchronized for subsequent identification of identical frames. We use a time-code generator which lays down a time-code signal simultaneously on one audio track of each VCR. When the original tapes are dubbed onto work tapes, the auditory time code is transformed into a visual code in minutes, seconds, and frame numbers at 30 fps, that appears on every frame. The greatest advantage of the time code over periodic synchronizing signals, such as a strobe flash or tone, is that the continuous visible code allows the user to be sure that identical sequences of frames on multiple videotapes are selected for digitizing.

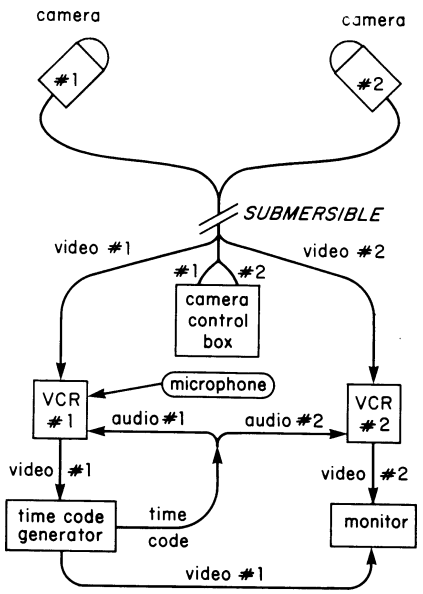

Figure 1. Drawing of the 3-D video system on the JOHNSON-SEA-LINK submersible. The two cameras are mounted outside the submersible, on either side of the sphere, and are linked by cable to the video equipment inside the sphere.

We use Super-VHS or Hi8 videocassette recorders because of their high video resolution. For recording from the J*ohnson Sea-Link* , where space was limited, we used 2 portable VCRs. The model we chose had 2 audio inputs, allowing the observer to record commentary on the second audio track while the time code was recorded on the first. A portable

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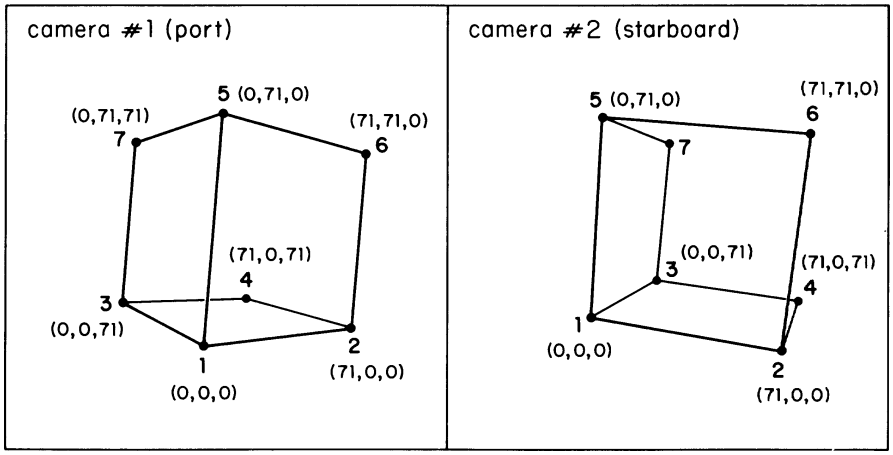

Figure 2. The digitized calibration grid in port and starboard camera views. White cubes at the 8 corners were used as targets. One cube was not well illuminated and its video image could not be digitized. Known spatial coordinates for each target establish the x, y, and z axis for subsequent 3-dimensional measurements. Any single target may be selected as the origin and the remaining targets are then identified in relation to that target.

Behavior—(Cont. from page 7)

monitor attached to the outputs of the two VCRs displayed what was actually recorded and permitted the operator to hear the time-code signal being recorded on the audio tracks.

Photogrammetric Data Collection

In order to track identified targets, the spatial dimensions of the volume under observation must be known. For our automated tracking system, discussed below, each camera view is calibrated with a minimum of 6 non-coplanar targets within the fields of view of both cameras (Fig. 2). Calibration provides information about spatial relationships between the cameras and the recorded targets which is used to compute the 3-D trajectories of objects moving through the calibrated volume.

For dives with the J*ohnson Sea-Link* we calibrated the cameras with a "stick" box made of 12 black rods, each 71.0 cm long, inserted into 8 white plastic cubes which served as calibration targets on each corner of the box (Fig. 2). The cameras on the *Johnson Sea-Link* were positioned so that the grid filled approximately 3/4 of the monitor screen in each camera's view when it was held in front of the submersible. The two cameras were focused approximately 2 m in front of the submersible's sphere. Once the cameras were positioned, the motionless calibration grid was recorded with both cameras. The cameras were calibrated at depth from the submersible by holding the stick box in front of the cameras with the claw. Calibration at depth is easier than calibration on deck because of the uniform black background beyond the targets. We recorded animal behavior with the 3-D video system from the *Johnson Sea-Link* both in midwater and while the submersible rested on the ocean floor. One example is presented below.

Data Analysis

Once multiple, synchronized images are recorded, they must be analyzed photogrammetrically. Manual computations for such temporal sequences, particularly of individuals living in groups, is tedious. We found a company, Motion Analysis Corporation, Santa Rosa, California, that sells a three-dimensional tracking system (ExpertVision) that digitizes multiple target images in real time, automatically computes x,y,z coordinates from their positions on synchronized frames of multiple tapes (Table 1), and plots their 3-D trajectories over time, using the digitized views of the calibration cubes (Fig. 2) for spatial reference.

Data Example

One example of target tracking, from the LEO750 project in September 1990, is presented to illustrate the

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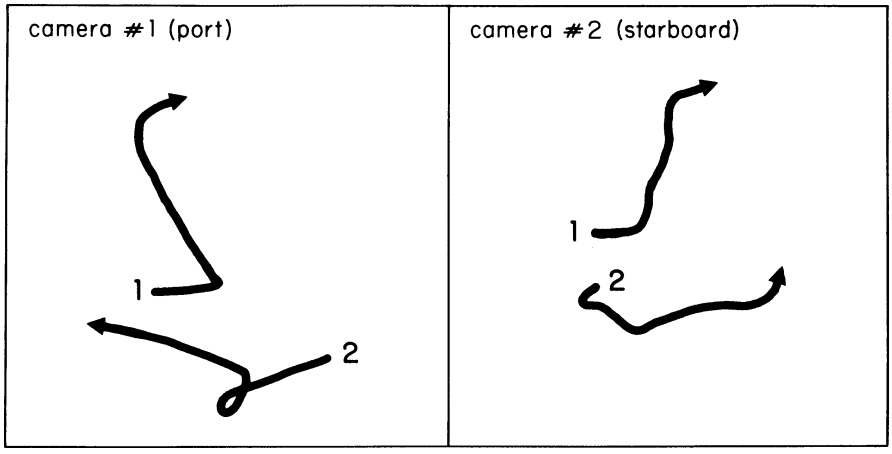

Figure 3. 2-dimensional paths of a sergestid shrimp (1) and a fish (2) as viewed from port and starboard cameras. Because of the camera angles the sergestid appears to lobstertail at two different angles and the apparent paths of the fish are diametrically opposed, but when the x,y coordinates of the two animals' positions in both views were combined to calculate 3-dimensional positions, the correct paths could be determined.

Table 1. Spatial coordinates for the location of a swimming sergestid over 1.5s (45 frames at 30 frames s^{-1}). The 3-dimensional measurements were calculated by the ExpertVision 3D tracking program from x,y coordinates provided by two camera views of the shrimp. The cruising sergestid lobstertailed upward (between frames 18 and 25) when it encountered a fish, then resumed a slower swimming pattern.

Frame No.	X (cm)	Y (cm)	Z (cm)
1	-23.674	33.728	-3.529
2	-23.332	33.775	-3.527
3	-22.915	33.855	-3.465
4	-22.522	33.922	-3.648
5	-22.013	34.024	-3.373
6	-21.799	34.094	-3.576
7	-21.346	34.153	-3.573
8	-20.932	34.219	-3.397
9	-20.597	34.257	-3.472
10	-20.208	34.317	-3.684
11	-19.734	34.434	-3.604
12	-19.342	34.575	-3.608
13	-18.831	34.685	-3.504
14	-18.472	34.799	-3.664
15	-18.031	34.830	-3.531
16	-17.784	34.825	-3.414
17	-17.427	34.880	-3.650
18	-17.045	35.067	-3.487
19	-17.264	37.135	-1.799
20	-18.547	40.211	2.131
21	-19.583	42.967	5.112
22	-20.325	45.316	7.038
23	-20.914	47.357	8.052
24	-21.318	48.640	8.432
25	-21.702	49.521	9.053
26	-21.583	49.934	8.914
27	-21.431	50.214	8.737
28	-20.764	50.424	8.179
29	-21.180	50.602	8.699
30	-21.293	50.761	9.373
31	-21.047	50.894	9.144
32	-20.783	51.057	9.456
33	-20.646	51.177	9.380
34	-20.027	51.427	9.266
35	-19.975	51.640	9.800
36	-19.649	51.807	9.617
37	-19.215	52.124	9.793
38	-18.815	52.313	9.660
39	-18.564	52.515	9.972
40	-17.979	52.729	9.900
41	-17.797	52.871	10.346
42	-17.455	53.105	10.328
43	-16.899	53.328	10.368
44	-16.704	53.541	10.789
45	-16.221	53.787	10.379

Behavior—(Cont. from page 8)

importance of using a 3-D rather than two-dimensional system for measurements of motion. On dive no. 2257 we set the *Johnson Sea-Link* on the bottom at about 710 m and recorded the interaction between a sergestid shrimp and a fish when their swimming paths converged. The sergestid swam into view from the port side and the fish from the starboard side. The sergestid was slightly above the fish when they met, and from the original video it appears that at least one of the sergestid's long, trailing antennae touched the fish. The encounter startled both animals and they both abruptly changed course at increased speeds.

The data presented encompass 45 frames of video, or 1.5 seconds. The paths of both the sergestid and the fish initially continued in the same directions they had been swimming since they first appeared. Fig. 3 shows the 2-D paths of the sergestid and the fish as they were computed from each individual camera angle. Note that the computed path directions of the animals are strikingly different in the two views. Nonetheless, after the x and y coordinates for these two views were combined to track the animals in three-dimensional space, the true paths and other motion-related parameters could be calculated.

Fig. 4 is a plot of the sergestid's speed over 1.5 seconds, calculated by the ExpertVision system from these coordinates. For the first 17 frames, the sergestid's speed averaged 12.2 cm s-1. Its lobstertail response to the proximity of the fish reached a maximum speed of 140.0 cm s^{-1} in 3 frames (0.1 sec). In the same sequence, the fish averaged 15.6 cm s^{-1} for the first 17 frames. In response to the sergestid, the fish abruptly changed course and accelerated to a maximum speed of 90.8 cm s^{-1} in 5 frames (0.7 sec). By frame 33, the last frame in which its image could be digitized, the fish had decelerated to 53.0 cm s^{-1}.

(Cont. on page 10)

Behavior—(Cont. from page 9)

In this example of three-dimensional behavioral quantification, the inaccuracy of relying on 2-D measurements is clear. When the swimming sergestid and fish converged, the fish and shrimp reacted at almost the same time, changing speeds and directions. While each camera view individually suggests that interaction affected the two animals, taken separately they provide conflicting information. Fig. 3 shows the paths of the sergestid and fish calculated by the EV3D program for each view. Because of the camera angles, the sergestid appears to dart upward vertically along 2 different diagonals, and the fish actually appears to swim in opposite directions. Any behavioral measurements based on either one of these 2-dimensional views alone would be wrong.

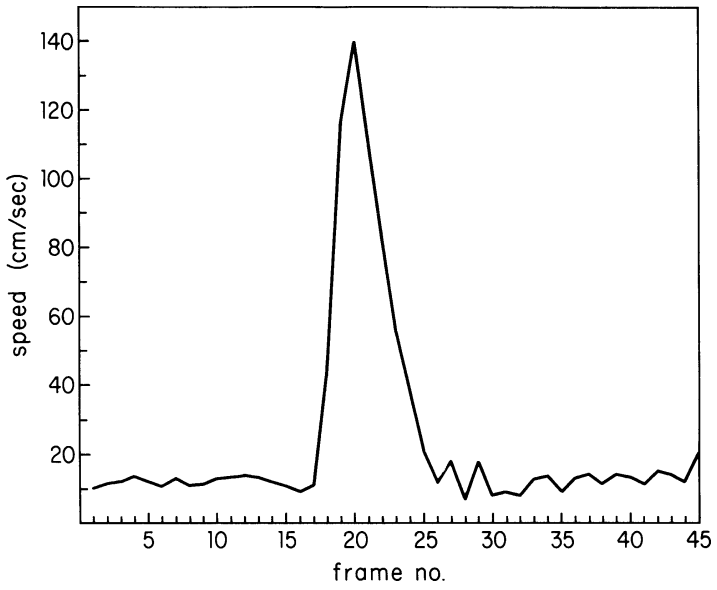

Figure 4. Speed of the sergestid in cm s^{-1}, calculated from its 3-dimensional spatial coordinates in the calibrated volume of water in front of the submersible.

Recommendations for Future Developments

To obtain quantitative *in situ* behavioral information about animals that live in a three-dimensional medium, 3-D sampling tools are absolutely essential. In the deep sea optical sampling is limited to short distances because of lighting difficulties; but where it is applicable, it is the most accurate method available for recording behavioral phenomena.

Correct lighting is important for subsequent digitization of recorded images. The digitizing computer outlines target images by "thresholding", that is, setting the grey level transition that defines the edge of the targets against the background. If lighting is uneven, the threshold level will change from frame to frame because contrast between the target and the background changes and the computer is unable to digitize the target throughout the sequence. Broad-beam lights are essential because the light field must be as even as possible.

Cameras on the submersible should be spaced as far apart as possible. Increased separation improves the accuracy of measuring coordinates along the axis perpendicular to the plane of the cameras. When possible, additional cameras should be used to increase the probability of the target remaining in more than one view. With additional cameras, two could be aligned side by side to provide a stereo view which could be projected in 3-D. Humans see the world with stereo optics, and the ability to review three-dimensional events in 3-D would assist us in understanding complex behaviors.

In an earlier report (Hamner et al. 1988) we discussed three-dimensional videography and described several systems of 3-D video projection. Once the elements of three-dimensional image collection, viewing, and computer analysis are combined, we will have an extremely powerful new tool for analyzing behavior in the deep sea. *(Peggy and Bill Hamner are scientists in the Department of Biology at UCLA)*

Acknowledgments

We thank the crews of the *Johnson Sea-Link* submersibles for their able and indispensable assistance in operating the 3-D video system. We thank Ms. Sadie Harrison for her help in analyzing behavioral sequences. The work described here was funded by NSF grant OCE 86-16487, NOAA/NURP contract NA88AA-H-UR020, and NOAA/NURP subcontract SC 02791.

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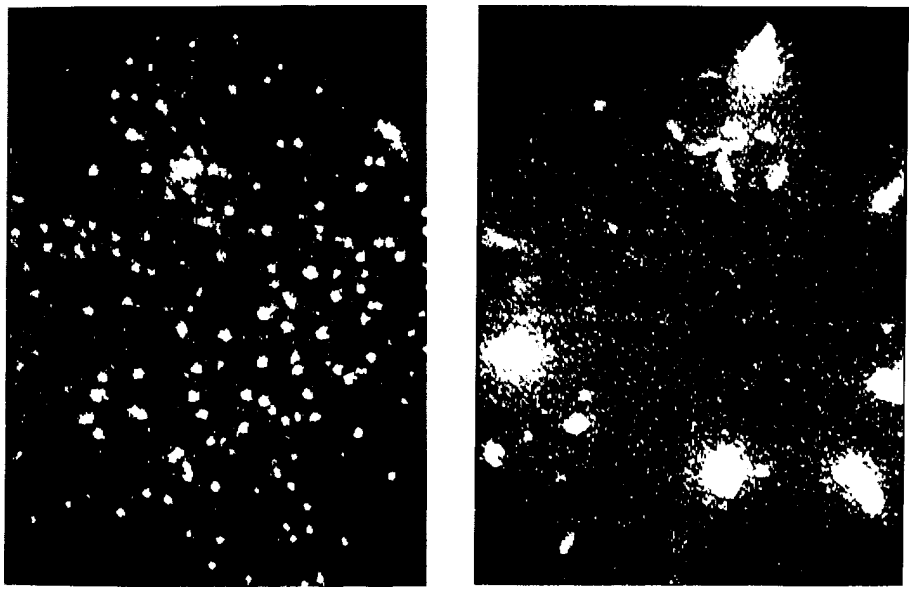

Figure 2. Examples of bioluminescence mapping.

Left Image. Bioluminescent flashes recorded in a dinoflagellate layer.

Right Image. Bioluminescent flashes recorded in a ctenophore layer. The figure-8 at the top of the image is characteristic of the lobate ctenophore Bolinopsis infundibulum. The other bright displays are extracellular emissions characteristic of the cydippid ctenophore Euplokamis sp. The field of view in these images is 0.74 m by 1.0 m.

Bioluminescence—(Cont. from page 6)

possible orientations of specimens and the identification of types and numbers of appendages.

Disadvantages

1) Not all marine organisms are bioluminescent. However, many of the dominant species in the ocean are light producers and virtually every cubic meter of the ocean contains some bioluminescent organisms. These include all pelagic krill as well as many species of copepods, dinoflagellates, ostracods, larvaceans, amphipods, mysids, decapods, polychaetes, fish, squid and most of the gelatinous zooplankton. Due to the enormous complexity of the marine environment, it has been suggested that investigators should concentrate on a few key species. Therefore, the fact that not all marine organisms are bioluminescent can be used to advantage.

2) Bioluminescence in some taxa, esp. dinoflagellates, is inhibited by high-light intensities and the majority of bioluminescent zooplankton undergo vertical migration, occupying surface waters only at night. Furthermore, bioluminescent displays provide the greatest contrast and are thus most easily detected when it is dark. For these reasons, three-dimensional mapping of near-surface waters of the oceans is done only at night or at dusk and dawn while following migrating layers.

3) Some mechanical disturbance is required to stimulate bioluminescence. However, because of the slow transect speed (0.6 kt) and the large mesh size of the screen (1800 μm), the pressure wave in front of the screen is small, as evidenced by the lack of screen avoidance by krill (Widder et al., 1992).

4) Definitive identification of a particular organism with a particular bioluminescent display must be based on *in situ* measurements, since displays from captured specimens are often radically different from those recorded *in situ*. Therefore a dual camera system, which records high-resolution images of organisms superimposed on their bioluminescent displays (Widder, 1992) is being adapted for *in situ* work. *(Edith Widder is a marine scientist at the Harbor Branch Oceanographic Institution).*

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Estimation of Zooplankton and Ichthyoplankton Growth and Condition Using Nucleic Acid Probe Techniques

by Lawrence Buckley and Peter McNamara

One of the key biological processes controlling population dynamics of marine organisms is zooplankton growth and production rate. Estimation of growth and production *in situ* is a major goal of biological oceanography, and a better understanding of these parameters as they may be affected by global climate change is a primary objective of the U.S. GLOBEC Program. Presently, a variety of approaches are being used to evaluate copepod growth and condition including cohort analysis, methods based on estimation of egg production, molting frequency, feeding incidence, and metabolic rates. None of these approaches are entirely satisfactory due to drawbacks associated with the required handling and incubation of organisms following shipboard collection. The development of improved biochemical or molecular techniques for estimation of growth and production rates of zooplankton would represent a significant improvement over existing methods.

At least three main biochemical approaches have been applied to the study of zooplankton growth and production: (1) incorporation or metabolic uptake of a labelled precursor, (2) measurement of the rate of a metabolic pathway or enzyme, and (3) estimation of RNA content. The first approach has been used to evaluate both primary production and secondary production. The incorporation of ^{14}C-labeled carbonate by phytoplankton has served as a functional definition of primary production and uptake of ^{14}C-labeled algae by zooplankton has also been investigated. Currently efforts are underway to determine the relation between incorporation of bromodeoxyuridine (BrdU) into DNA (an index of cell proliferation) and growth of zooplankton, including larval fish (see Moore and Stegman, 1992).

The activity of the respiratory electron transport system in marine zooplankton has been related to feeding condition and other environmental variables. Currently, studies relating zooplankton growth with the activities of specific enzymes comprise an active area of research. Enzyme activities are known to be sensitive to nutritional status in adult fish, and to scale with body length in both adult and larval fish. Activities of the key metabolic enzymes lactate dehydrogenase and citrate synthase (representative of anaerobic and aerobic metabolism, respectively) have recently been measured in larval fish and appear to be useful in assessing condition (Clarke et. al., 1992). Citrate synthase activity has also been estimated in individual zooplankton and was found to correlate with feeding (Clarke and Walsh, 1993). Additionally, DNA polymerase activity is known to be responsive to changes in growth rate of *Artemia salina* nauplii, and may provide another useful marker of cell proliferation and growth in copepods and larval fish.

The third biochemical approach to assessing growth and production rates—estimation of RNA content—has been under active investigation in a number of laboratories and is the primary topic of this article. The RNA content of any tissue or whole organism consists primarily of ribosomal RNA (rRNA), the nucleic acid component of ribosomes. Ribosomes are the protein synthesizing complexes of the cell. Ribosome number, and, therefore, the concentration of rRNA, at any given time is directly related to the protein synthesizing activity of a cell. Since the DNA content of a somatic cell is constant, RNA levels may be normalized to a per cell value after dividing by DNA concentration. The resultant RNA-to-DNA ratio value has proven to be a useful indicator of both nutritional status and growth in larval fish. The relationship between RNA content and growth in copepods, however, appears to be more complex, due to the nature of the molt cycle. (Ota and Landry, 1984).

The successful application of the RNA-to-DNA ratio method to evaluate condition and growth in larval fish can

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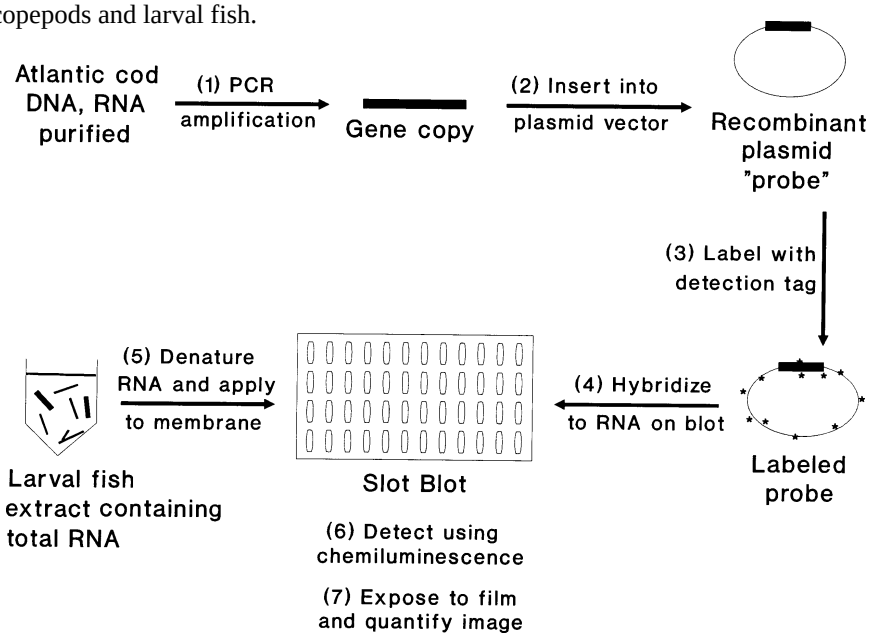

Figure 1. Flow diagram showing the production of DNA probes for use in the quantitation of specific RNA levels in larval Atlantic cod.

Growth—(Cont. from page 12)

be attributed to several factors. First, there is a direct link between RNA concentration and growth which, in larval fish, involves primarily the generation of muscle tissue (Houlihan et al., 1988). A relationship between the ratio of total RNA to DNA and the growth of a wide variety of temperate marine fish larvae reared in the laboratory has been established (e.g. Buckley 1979). A general model relating RNA-to-DNA ratio and water temperature to larval growth rate was developed from data on 8 laboratory-reared fish species (Buckley, 1984). Second, the larval period in fish is a single, well-defined life stage characterized by rapid growth. Analysis of growth and nutritional condition in fish larvae, therefore, has been relatively free of complicating factors such as age, gender and reproductive status. Finally, extensive laboratory experiments have been conducted to calibrate the method. Consequently, the RNA-to-DNA ratio method (with refinements and variations) has become widely accepted and utilized for the estimation of short-term (days) growth in larval fish. In a recent study, for example, a variation of the approach employed flow cytometry to estimate the RNA and DNA content of individual brain cells from fish larvae (Theilacker and Shen, In press). Two distinct fractions of cells were identified in which RNA levels were sensitive to either feeding or growth.

Growth, as gauged by the weight of fish larvae, is exponential through the larval period, with the majority of protein synthesis directed toward the accretion of muscle tissue. Unlike other life history stages in fish, little metabolic energy is spent in building energy reserves or in reproduction during the larval stage. A consideration of zooplankton growth and physiology as a whole, however, presents a much more complicated picture. Even when considering a single species such as the copepod *Calanus finmarchicus*, one net haul taken in the field might contain multiple life stages of the organism. Complicating the problem are discontinuous growth and the molt cycle that accompany each developmental stage. These factors can have a profound effect on the biochemical composition of the organism.

The Potential for Nucleic Acid Probe Techniques

Production and growth are a function of gene expression. Techniques are currently available to estimate the concentration of individual RNA molecules, including rRNA and specific mRNAs. The use of nucleic acid probes can provide information on the identity, growth rate, physiological status and reproductive condition of individual organisms. In addition, information on the genetic relationships among individuals, cohorts and populations is available using this approach (Powers, 1993). Doug Crawford and Lew Incze, for example, are currently using the polymerase chain reaction (PCR) to quantitatively amplify mRNAs from the copepod *Calanus finmarchicus* which encode enzymes that are indices of

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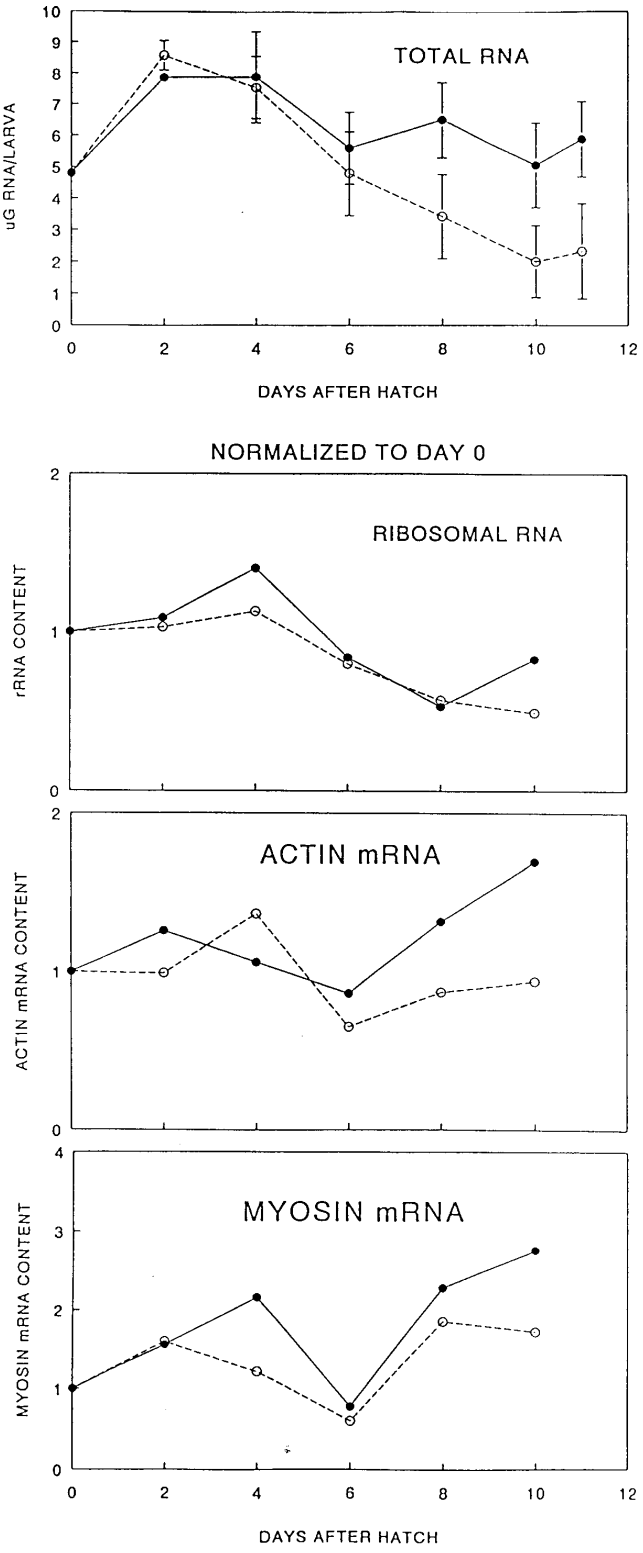

Figure 2. RNA levels in first-feeding Atlantic cod larvae. Starved fish (dashed line) had food withheld since hatching. Solid lines were fed fish. Total RNA measurements were made using standard fluorometric methods. Ribosomal RNA, along with actin and myosin mRNA levels, were determined using specific DNA probes as described in the text. Because these data sets represent single measurements, no error bars are shown.

Molecular Approaches to Identify a Species and Assess its Physiological Status In OceanicPlankton

by Douglas L. Crawford

Molecular techniques are useful for identifying species and defining distinct populations and for quantifying cellular or physiological status. A variety of molecular methods, sequence analysis, and Restriction Fragment Length Polymorphism's (RFLPs) have been successfully applied to marine problems. Carlton and Geller (1993) demonstrated that many bays and estuaries are constantly invaded by plankton carried in ship ballast water. Finnerty and Block (1992) demonstrated that although marlin are found world wide, they are not panmictic—i.e., different ocean basins consist of genetically distinct populations.

These studies are only a few examples of how molecular techniques can be used to address important biological and conservational questions in the ocean. Quantitative molecular analysis is similarly useful. A few researchers have used gross measures of RNA and DNA concentrations to ascertain an index of growth or physiological status (see article by Buckley and McNamara). More specific measurements, e.g., determination of specific gene products, are almost exclusively performed on culture organisms where there is little or no problem with identification. These endeavors have been applied most successfully to the identification of developmentally specific gene expression, growth-specific gene products or genes characteristic of disease states. For cultured oceanic species, Clarke et al (1992) were able to demonstrate that the amount of certain enzymes is indicative of the recent feeding regimes and, therefore, likely linked to growth or other important physiological indices.

What is needed is a way to combine these two seemingly disparate technical goals, that is, to be able to both (1) identify specific species or populations and (2) quantify specific genes or their products as an index of physiological status. My laboratory is currently working toward this goal by utilizing quantitative PCR to both identify the copepod *Calanus finmarchicus*, and to quantify its expression of specific genes. The level of enzyme expression from a single species is determined by isolating nucleic acids from these copepods, converting its mRNA into cDNA, and quantitatively amplifying the cDNAs that code for key metabolic enzymes. The PCR products for each enzyme's mRNA are of different size and are electrophoretically separable. As a first step, we have cloned and characterized genes that code for several key metabolic enzymes and used them to design species specific primers. This research makes the assumption that mRNA concentration is highly correlated to the concentration of the enzyme they encode. Although this has to be verified experimentally, it has been demonstrated in a number of laboratory studies. For example, diet-related changes in enzyme concentrations in rabbits can be accurately determined by measuring mRNAs (Granner and Pilkis, 1990). Additionally, enzyme concentration in the small minnow *Fundulus heteroclitus* is a function of its mRNA concentration, and both of these measures of enzyme expression are sensitive to environmental temperature experienced by these fish (Crawford and Powers, 1989). Thus, by designing the proper molecular probes, it will be possible to identify a specific species and determine its physiological status by quantifying mRNA expression.

This approach has several advantages over other methods. First it does not depend upon identifying and sorting adults; the level of enzyme expression can be measured in any stage of the life cycle, even the most difficult to identify. Second, many gene products can be measured simultaneously, each separated by size electrophoretically. And, finally, many individuals may be assessed simultaneously without any sorting at all. In a mixed species sample, the number of *Calanus finmarchicus* could be determined by measuring the concentration of a nuclear gene (this is really a measure of the number of cells), and the average physiological condition could be assessed by measuring the concentration of an enzyme's mRNA. Both nuclear DNA and mRNA can be assayed in the same reaction because their PCR products are of different size. The ratio of these two measures may vary due to changes in recent feeding conditions, temperature, developmental state or other physiological conditions. By grouping of many individuals, one loses information concerning inter-individual variability in physiological condition, but gains the ability for rapid survey of many populations. Where populations (samples) differ, individual organisms would need to be analyzed using the same methods outlined for mixed populations to determine the source of the differences.

This goal of simultaneously identifying a species and quantifying gene expression faces many hurdles. There are obvious molecular and biochemical assumptions to be tested (e.g., the relationship between enzyme concentration and its mRNA, the correlation between dry weight and nuclear DNA concentration, etc.). Just as important are problems arising when taking a basic laboratory technique into a field setting. To design probes that are species-specific yet inclusive of all individuals within a species, one has to know the sequence variation both within and between species. Thus, it is not adequate to sequence a single clone from an individual. One must measure the sequence variation within the species and compare this variation to the phylogenetically most similar species. If a good phylogeny is not available, one must be determined. Fortunately, for *C. finmarchicus* the most closely related species have been

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Molecular—(Cont. from page 14)

identified, and the relationship between these sister groups is reasonably well established. Theoretical considerations suggest that much of the genetic variability will be observed if approximately ten individuals are sequenced for each gene. Aligning these sequences will identify species-specific oligo-nucleotide that will be used to design primers. Another challenge is to make sure the molecular probes do not exclude genetic variants that may exist in different populations. Primers could be too specific and may not work on all the allelic variants at a locus. If the molecular probes did not assess all alleles, one might find a variation in gene expression due to a change in allelic frequency. This can be addressed by examining the sequence variation between widely dispersed populations and by judiciously choosing molecular probes that are unlikely to vary between populations (sites).

By melding two fields of molecular biology—molecular population genetics and molecular physiology—we hope to streamline the assessment of zooplankton physiological status. There is a vast potential in such an approach, but caution must be taken to verify all the assumptions. Applying known molecular techniques to field problems shows promise, but one must understand the underlying premises inherent in these techniques before applying them to questions in the field. *(Douglas Crawford is an Assistant Professor in the Department of Organismal Biology and Anatomy at the University of Chicago)*

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Second Annual PICES Meeting

PICES (The North Pacific Marine Science Organization) is holding its second annual meeting October 25-30, 1993, at the NOAA Western Regional Center in Seattle, Washington. The program is being organized by the Science Board and contains sessions on the following topics.

Ocean Circulation and Climate Variability in the Subarctic Pacific. Convenors for this session are Prof. Paul LeBlond, Dr. James E. Overland, and Prof. Stephen C. Riser. This session will focus on formation of subarctic intermediate water, absorption of CO2 and its circulation in the subarctic, status of numerical modeling, long-term variation in the water properties and circulation, and characteristics of the subarctic gyre and their impact on climate.

High-Resolution Paleoecological Studies in the Subarctic Pacific. Prof. Michael M. Mullin will convene this session, which will consider studies of foraminifera and fish scales in sediments, isotope analysis, tree rings and other proxy techniques to reconstruct ocean conditions, species dominance and biological productivity in the North Pacific over the last millennium.

Priority Chemical and Biological Contaminants in the North Pacific Ecosystem. This session, convened by Dr. Ushi Varanasi, will include national overviews and new approaches to assess impacts of sewage discharge, waste dumping, anthropogenic chemicals, fisheries and agricultural processing wastes, and ballast water contaminants in the North Pacific. Impact of transport of these contaminants from nearshore to offshore waters, and on natural biogeochemical processes and cycling, will also be considered.

Shifts in Fish Abundance and Species Dominance in Coastal Seas. Convenors for this session are Prof. Qi-Sheng Tang and Dr. Alec D. MacCall. The session will emphasize low frequency, long-term fluctuations in plankton and fish stocks, rates of resource production, and coincident changes in their ecosystems. These fluctuations may last for decades, and may be punctuated by sudden changes, creating unmanageable "boom-and-bust" cycles with severe social and economic consequences. Relationships to ocean and global climate will be discussed. Problems of prediction or detection, and strategies for adaptive resource management may also be explored.

Long-Term Monitoring from Platforms of Opportunity. Dr. Charles B. Miller will convene this session, which will address possibilities for long-term monitoring of physical and biological conditions in the subarctic gyre and North Pacific coastal ecosystems. This monitoring would utilize satellite remote sensing, X-CTD mapping, drifting buoys, pollution monitoring, collection of trans-Pacific continuous plankton recorder data from ships of opportunity, and other potential monitoring sources, along with associated statistical and data management issues.

Scientific sessions will include invited and contributed papers on these topics, as well as contributed papers on other subjects of interest to the committees. Contributed papers will be selected for oral or poster presentation. Registration materials may be requested from the PICES Secretariat c/o Institute of Ocean Sciences, P.O. Box 6000, Sidney, B.C., Canada V8L 4B2 (Phone: 604-363-6366; FAX: 604-363-6827; Omnet: PICES.SEC; Internet: pices@ios.bc.ca). Deadline for registering is September 24, 1993. ΔΔΔ

U.S. GLOBEC Calendar

1993

13-15 September: U.S. GLOBEC Workshop on Dynamics of Open Ocean Populations, Woods Hole, MA. Contact: L. Madin, WHOI, Woods Hole, MA (Omnet: L.Madin; Phone: 508-548-1400; FAX: 509-457-2169)

22-24 September: Marine Technology Society meeting on Technology Requirements in the Nineties., Long Beach, CA. Contact: MTS '93 (Phone: 703-631-6200; FAX 703-818-9177)

7-8 October: U.S. GLOBEC Scientific Steering Committee meeting, Woods Hole, MA. Contact: H. Batchelder, Division of Environmental Studies, University of California, Davis, CA. (Omnet: H.BATCHELDER or T.POWELL; Phone: 916-752-4163; FAX 916-752-3350).

21-23 October: Eastern Pacific Oceanic Conference Annual Meeting (EPOC), Fallen Leaf Lake, CA. Contact: G. Lagerloef (Omnet: G.LAGERLOEF; Internet: lagerloef@frazil.nw.saic.com).

25-30 October: Second Annual Meeting of the North Pacific Marine Science Organization (PICES), Seattle, WA. Contact: PICES Secretariat, Institute of Ocean Sciences, P.O. Box 6000, Sidney, BC, Canada V8L 4B2 (Omnet: PICES.SEC; Phone: 604-363-6366; FAX 604-363-6827).

2-4 November: California Cooperative Fisheries Investigation Annual Meeting, Lake Arrowhead, CA. The symposium will deal with the genetics of California Current Organisms. Contact: George Hemingway, Marine Life Research Group, Scripps Institution of Oceanography, UCSD, 9500 Gilman Drive, La Jolla, CA 92093-0227 (Internet: ghemingway@ucsd.edu; Phone: 619-534-4236; FAX 619-534-6500)

1994

21-25 February: AGU/ASLO Ocean Sciences 1994 Meeting, San Diego, CA. Contact: E. Hofmann, Old Dominion University, Norfolk, VA. (Omnet: E.HOFMANN

15-18 August: ICES Symposium on Zooplankton Production: Measurement and Role in Global Ecosystems and Biogeochemical Cycles, Plymouth, U.K. Contacts: R. P. Harris, Plymouth Marine Laboratory, or J. C. Gamble, Sir Alister Hardy Foundation for Ocean Science, Prospect Place, Plymouth PL1 3DH, UK (Omnet: PML.UK or J.GAMBLE.CPR; Phone: + 44 752 222772; FAX +44 752 670637).

Growth—(Cont. from page 13)

metabolic flux (see Crawford article, this newsletter). It is anticipated that PCR primers specific for this copepod will be identified, allowing the indirect quantitation of enzyme concentration (through mRNA concentration) from only *C. finmarchicus*, even in a mixed sample.

We are using quantitative RNA slot blots to estimate the concentration of rRNA and selected mRNAs in larval fish and copepods. Nucleic acid probes have been developed which are complementary to selected RNA target sequences. These have either been synthesized directly (oligonucleotide probe) or produced using the PCR reaction and labeled with a nonradioactive tag. Extracts containing the RNA from individual copepods or larval fish are blotted and fixed onto a nylon membrane and the labeled probe is allowed to hybridize to the immobilized target RNA molecules. The amount of bound probe is quantified using a chemiluminescent detection procedure followed by film exposure and densitometry.

In a preliminary analysis we have examined changes in the levels of a small subunit rRNA and two abundant mRNAs, coding for the muscle proteins actin and myosin in Atlantic cod larvae. Since growth in larval fish is primarily accomplished by protein synthesis and accumulation as muscle tissue, we reasoned that the levels of these RNA molecules should change in response to changes in feeding conditions and growth rate. Results with first-feeding cod larvae reared in the laboratory indicate that levels of 18S rRNA follow a pattern similar to that of total RNA—peaking around day 4 and then remaining relatively constant from day 6 to day 10 (Figure 2). After day 8, the total RNA content of fed larvae is significantly greater than that of larvae which have been starved since hatching. No appreciable difference, however, is evident in the levels of 18S rRNA from fed and starved fish until day 10. Messenger RNAs from the actin and myosin genes each reach a minimum at day 6, after which levels increase relatively rapidly compared to the levels of 18S rRNA and total RNA over the same time period. Actin and myosin mRNA levels also increase more rapidly in fed than in starved larvae from day 6 to day 10. It should be noted that these experiments are preliminary and it is therefore premature to assess the significance of these apparent trends. In general, however, it does not appear that the patterns of 18S rRNA and actin and myosin mRNA abundance differ greatly from that of total RNA over the time course studied.

An interesting observation made during the course of this work is that the probe for myosin mRNA produced from cod muscle RNA does not hybridize with haddock RNA. This specificity for cod may be useful in distinguishing between cod and haddock eggs that co-occur on Georges Bank (and other spawning grounds) and cannot be distinguished by microscopic examination until just before hatching. This finding highlights one of the major advantages of the nucleic acid hybridization approach to estimation of biological rates (growth, egg production, etc.) and physiological status (nutritional, reproductive, developmental, etc.) of marine organisms. With the appropriate probes available, and the optimal hybridization conditions known,

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U.S. GLOBEC PUBLICATIONS

Theory and Modeling in GLOBEC: A First Step. February 1991.

Initial Science Plan. February 1991. Report Number 1.

GLOBEC: Northwest Atlantic Program. GLOBEC Canada/U.S. Meeting on N.W. Atlantic Fisheries and Climate. February 1991. Report Number 2.

GLOBEC Workshop on Biotechnology Applications to Field Studies of Zooplankton. February 1991. Report Number 3.

GLOBEC Workshop on Acoustical Technology and the Integration of Acoustical and Optical Sampling Methods. September 1991. Report Number 4.

GLOBEC: Southern Ocean Program. GLOBEC Workshop on Southern Ocean Marine Animal Populations and Climate Change. November 1991. Report Number 5

Northwest Atlantic Implementation Plan. June 1992. Report Number 6.

Eastern Boundary Current Program: Report on Climate Change and the California Current Ecosystem. September 1992. Report Number 7.

Optics Technology Workshop Report. March 1993. Report Number 8.

Implementation Plan and Workshop Report for U.S. GLOBEC Studies in the Arabian Sea. May 1993. Report Number 9.

Copies of these reports are available from:

U.S. GLOBEC
Scientific Steering Committee Coordinating Office
Division of Environmental Studies
University of California
Davis, CA 95616-8576

Phone: 916-752-4163
FAX: 916-752-3350
Email: T.POWELL (OMNET)
Internet: SELYNCH@UCDAVIS.EDU

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varying degrees of specificity can be obtained from nucleic acid probes.

One of the challenges of using nucleic acid probes lies in identifying a target mRNA that is responsive to feeding conditions and growth rate. We are presently in the process of preparing a subtracted DNA library (a collection of DNA fragments copied from mRNAs) that should be enriched for genes from which transcription is induced or enhanced during starvation in cod. We will use this and other strategies to identify additional mRNAs that are responsive to changes in food availability and growth rate.

Questions concerning zooplankton growth and condition are currently being addressed through the application of both biological and biochemical methods. The use of biochemical and molecular biological techniques offer the advantages of unparalleled sensitivity and specificity at the level of a single gene or gene product. *(Larry Buckley is a marine researcher at the National Marine Fisheries Service Laboratory and University of Rhode Island and Peter McNamara is a post-doctoral fellow at the University of Rhode Island)*

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Summary of Cod and Climate Meeting in Lowestoft

by Keith Brander

The Cod and Climate Change Working Group of ICES met for the first time June 7-11, 1993, in Lowestoft, to address the terms of reference described earlier (U.S. GLOBEC NEWS No. 3). Thirty-five scientists took part, and the fact that this was a joint ICES/GLOBEC group broadened the geographic and scientific boundaries of the meeting.

The question being investigated by the working group—the effect of climate variability on cod stock fluctuations—is simply stated, but involves many different scientific subdisciplines and scales of investigation, from the effects of small-scale turbulence on encounter rates between individual fish larvae and their prey, to the effects of interdecadal changes in windfields on large-scale circulation and transport of heat and young fish. In spite of the complexity of the processes by which variable physical forcing may affect cod stocks, the effects of climatic variability on fish abundance has been demonstrated for several cod stocks. For example, periods of low temperature cause stock decline at the northern limits of the range (Barents Sea, Greenland); particular hydrographic and wind conditions result in unusual transport of eggs and larvae (Iceland-Greenland) or flush and re-aerate deoxygenated basins where cod spawn (Baltic). These examples combine empiricism, an increasing understanding of ocean/climate variability, and detailed knowledge of processes during the life history (mainly early life history) of cod. They provide reason for believing that the question is not wholly intractable, and that we may be able to predict at least the broad direction of changes in stocks under different physical regimes.

The GLOBEC approach provides a conceptual framework within which studies at different scales can be nested. It is obvious from the reports of work in progress or in planning (for U.S. GLOBEC, see Report by W. Peterson, p. 3 of this issue) that a great deal of research is already underway that is very relevant to Cod and Climate Change, because of the focus on cod recruitment and studies of copepod dynamics. The challenge for this working group was to identify themes and approaches which strengthen and facilitate the scientific programs being followed at individual, laboratory or national levels, and to propose and initiate relevant regional or international studies which can be better implemented within ICES and GLOBEC.

A number of unifying themes were discussed relative to the scale—large, intermediate, small—of the physical processes affecting them. Large-scale processes range from global to regional (e.g., Georges Bank, the North Sea) and include long-term changes in atmospheric and oceanic dynamics which affect cod stocks through changes in heat and transport. Intermediate-scale processes include eddies, rings and fronts, which have effects due to localized aggregation, retention and enhancement of plankton production. Small-scale processes include water column stability and turbulence, which affect plankton production and encounter rates of predator and prey. The working group attempted to identify key interactions between physical and biological processes at all of these scales. The intent was to strengthen the rationale for physical and biological oceanographers and fisheries biologists to work together from an early stage in developing models and observation programs. The categorization by scale is not rigid, because, for example, large-scale events, such as anticyclones, are often a major source of variability at smaller scales, and models of intermediate-scale features, such as fronts, may be sensitive to boundary conditions generated at larger scales.

The need to consider cod in the context of the full multispecies ecosystem and possible models and methods for accomplishing this task were discussed. Although variability in cod stocks is the principal issue, there are compelling reasons to include other species in the investigations when their dynamics interact with cod, or if they are more sensitive indicators of ecosystem change than cod.

The working group recommended that two special workshops be held in 1994 to plan and carry out retrospective analyses of physical and biological data, including considering the case for setting up a Cod and Climate database. Other recommendations propose topics for research which should be considered as themes for future workshops, or joint sessions of committees at ICES meetings. There are also a number of proposals for specific action in relation to existing monitoring programs (e.g., the CPR program), or the initiation of new regional programs. Finally, the consensus of the meeting was that the activities of the working group could be carried forward effectively by the special workshops in 1994, and that the group should meet next in 1995 to review progress and carry out further coordination, if required. Copies of the discussions held at this meeting can be obtained from Ms. Amy Freise, GLOBEC.International Secretariat, Chesapeake Biological Laboratory, P.O. Box 38, Solomons, MD 20688, USA (Internet: freise@cbl.umd.edu). ΔΔΔ

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GLOBEC Related Ocean Sciences 1994 Sessions

One contributed and two special sessions at the February 1994 Ocean Sciences Meeting in San Diego (see Calendar) will be of particular interest to marine scientists investigating potential effects of Global Climate Change on marine populations.

U.S. GLOBEC Pilot Study of the Effect of Stratification on Larval Fish and Zooplankton Populations on Georges Bank

Results of the 1992-1993 pilot study of the effect of seasonal stratification on the growth and survival of larval fish on Georges Bank will be presented. Variations in water column structure have been shown to affect larval growth and condition through changes in availability of zooplankton prey. This project also involved the application and intercomparison of a variety of new instrument systems and biochemical techniques. *Convenors for this contributed session are David G. Mountain, Northeast Fisheries Science Center, 166 Water St., Woods Hole, MA 02543; Phone: 508-548-5123; FAX: 508-548-5124; Omnet: D.MOUNTAIN; Internet: dmountai@whsun1.whoi.edu and Peter H. Wiebe, Department of Biology, Woods Hole Oceanographic Institution, Woods Hole, MA 02543; Phone: 508-548-1400 x2313; Omnet: P.WIEBE; Internet: pwiebe@cliff.whoi.edu.*

Modelling Physical-Biological Couplings In The Ocean

Many biological processes in the ocean, operating at a wide range of spatial and temporal scales, are affected by fluid motion. These processes include nutrient uptake, feeding and metabolism by planktonic, nektonic and benthic organisms, and dispersal, settlement and recruitment. This session will feature predictive, quantitative models of links between these biological processes and the physical processes that may govern them. This session will include, but not be limited to, reports from the U.S. GLOBEC (GLOBal ocean ECosystem dynamics) program. *Convenor for this session is James E. Eckman, Skidaway Institute of Oceanography, P.O. Box 13687, Savannah, GA 31416; Phone: 912-598-2467; FAX: 912-598-2310; Omnet: J.ECKMAN; Internet: eckman@skio.peachnet.edu*

Decadal-Scale Variability in the Ocean, Lakes and the Atmosphere

This session solicits papers that discuss low-frequency fluctuations in oceanographic, limnologic and atmospheric variables, in the Pacific Ocean and lakes influenced by Pacific weather patterns. Papers may be based on retrospective analysis of long time series of physical and biological (including paleoceanographic and paleolimnologic) data sets, on modelling and/or theoretical work. Results should be discussed in the context of climate change. Our overall goal is to identify linkages between atmospheric forcing and ocean/lake response, and to determine if (and how) aquatic ecosystems respond to low-frequency changes in the physical environment. *Convenors for this session are Tim R. Baumgartner, CICESE, P.O.Box 4844, San Ysidro, CA 92073; Phone: 011 52 667 45053 (Ensenada) or 619-534-2171 (Scripps); e-mail: trbaumgartner@ucsd.edu and William T. Peterson,U.S. GLOBEC Interagency Program Coordination Office, NOAA/NMFS-F/RE3, Room 6276, 1335 East West Highway, Silver Spring, MD 20910; Phone: 301-713-2367; Omnet: W.PETERSON.* ΔΔΔ

Workshop in Ukraine to Discuss Arabian Sea

The Woods Hole Oceanographic Institution (Joel Goldman and Hugh Livingston) will co-host a workshop in Sevastopol, Ukraine, together with the Marine Hydrophysical Institute and the Institute of Biology of the Southern Seas, September 20-24, 1993. The purposes of the workshop are, first, to disseminate data and literature from previous research activities of Former Soviet Union (FSU) oceanographers in the Arabian Sea and Indian Ocean to U.S. scientists, and, second, to provide the opportunity for U.S. scientists to describe to FSU colleagues the current U.S. and other international interests in the forthcoming Arabian Sea initiatives of JGOFS, ONR, and GLOBEC. These interactions will greatly aid planning efforts underway in the U.S. for research in the Arabian Sea in 1995 and 1996.

The workshop will afford the opportunity for FSU scientists to establish scientific contacts with their U.S. counterparts, and to form connections which will hopefully lead to scientific cooperation both in the Arabian Sea and in other ocean regions of mutual interest. The workshop is being funded jointly by NSF, ONR and NOAA as part of the JGOFS, ONR/Arabian Sea, and U.S. GLOBEC programs. Those attending from the U.S. GLOBEC program include Drs. Sharon Smith, Charles Miller, David Stein and Bill Peterson, representing zooplankton and myctophid fish interests. For details on the results of the workshop and information on future research opportunities, contact Bill Peterson at the U.S.GLOBEC Interagency Program Coordination Office at (301) 713-2367, Omnet: W.PETERSON.

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biological work will involve following drogued patches of water for a number of days, during which larval fish and zooplankton are sampled for study of birth, feeding, growth and mortality rates in relation to hydrographic features. Growth rates of copepods and larval fish will be estimated using molecular techniques, as well as by conventional techniques (moulting rates for copepods; otolith analysis for fish larvae). Detailed studies of vertical distribution of larval fish in relation to their prey field—copepod eggs and nauplii and other microzooplankton—are planned using plankton pumps, nets, acoustics and video plankton cameras.

Long-term monitoring of circulation, phytoplankton and zooplankton biomass will be accomplished with continuous *in situ* measurements at three mooring sites: in the center of the Bank, at the northeast corner and along the southern flank. Currents will be monitored with bottom-mounted, upward-looking ADCPs (operating at 300 kHz) with several VMCM current meters for calibration. There will be two bio-optical packages on each mooring as well. Zooplankton biomass will be obtained from the ADCP data, but will also be provided by several dual beam/dual frequency and eight-frequency acoustic sensors developed by Tracor, Inc.

Modelling studies focus primarily on numerical simulation of circulation patterns on the Bank, particularly on the dynamics controlling the observed anticyclonic gyre which persists on the Bank. Modelling studies include analysis of the effects of varying wind fields on circulation and the effects of storms on the distribution and abundance of zooplankton. Modelling projects include both finite-element and spectral-primitive equation approaches. These modelling studies are a prelude for the fine-scale process studies planned for 1997 which will focus on sources, sinks and retention mechanisms of water and planktonic animals on the Bank. Most of the circulation studies carried out at the long-term mooring sites, and during shipboard surveys, have the goal of producing dynamic descriptions of the mean and variance of the around-bank flow patterns. An understanding of the processes controlling circulation patterns must be gained before we can reliably predict the effects of climate change on dynamics of animals populations on the Bank, and provide accurate assessments of the impact of climate change on the Georges Bank and other Northwest Atlantic bank ecosystems. ΔΔΔ

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U.S. GLOBEC Scientific Steering Coordinating Office
Division of Environmental Studies
University of California
Davis, CA 95616-8576

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