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GLOBEC Workshop on Biotechnology Applications to Field Studies of Zooplankton

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This is a report to the GLOBEC Steering Committee from the GLOBEC Workshop on Biotechnology Applications to field studies of Zooplankton held at the Rosenstiel School of Marine and Atmospheric Science, University of Miami in Miami, Florida on November 14-16, 1990-- Lewis S. Incze and Patrick J. Walsh, conveners.

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I. INTRODUCTION

At a GLOBEC (Global Ocean Ecosystems Dynamics) Workshop held in Halifax, Nova Scotia (U.S.-Canada Workshop on Climate and Fisheries, June 1990), several discussion groups met to consider desirable directions to take in the study of zooplankton (including holoplankton, meroplankton and ichthyoplankton) dynamics as part of NSF's GLOBEC program. GLOBEC's goal is to understand the factors that affect population abundance in the sea and to predict, based on this understanding, population and community changes likely to arise from climatic change. Several working groups concluded that advances in biological sampling and methods of measurement are urgently needed if we are to make substantial progress in our understanding of population dynamics in the oceans, It was felt that biotechnological approaches might hold particular promise in two key areas: 1) molecular identification of zooplankton taxa as it relates to improved methods of sample sorting *(i.e.*, greater speed and accuracy) and population genetic structure; and 2) molecular proxies for rapid assessment of physiological rates and condition. On behalf of many participants in the Halifax meeting, we forwarded the recommendation that a follow-up biotechnology workshop be convened specifically to address these topics related to zooplankton field studies. The GLOBEC Steering committee accepted that recommendation and provided guidance and support that ultimately resulted in this workshop on biotechnology applications.

This workshop consisted of 24 scientists (Appendix I) in the disciplines of marine biology/oceanography and molecular biology/biochemistry/genetics. The goal of this workshop was to draft two RFPs on biotechnology development and application in the areas of population genetics and physiological rate measurements as applied to GLOBEC research. These two drafts are included as appendices II and Ill. Two other considerations were unavoidable. First, since feeding rate and dietary composition are central factors in animal physiology, means of enhancing sampling methodologies would be relevant to our discussions of assessing physiological condition and establishing cause. Second, new approaches (biotechnology-enhanced sampling) would have to be implemented and integrated within a sampling scheme that is both realistic and useful in the oceanographic context. Goals and obstacles will have to be discussed between the various disciplines. Thus, four working groups were formed: Physiological Rates and Condition; Genetics; Feeding Rates and Dietary Composition; and Sampling. Groups were interdisciplinary and were jointly led by one "marine biologist/oceanographer" and one "molecular biologist/biochemist". The agenda and working group assignments are given in Appendix IV.

Chapters II through V summarize discussions held by the various working groups as reported by the group leaders. They comprise a record of major topics and conclusions of the groups and are not intended as research documents. Recent publications by Joint Oceanographic Institutions (1990) and Powers *et al.* (1990) provide useful overviews of how developments in molecular biology may be applied to problems in the ocean sciences. Both papers can be consulted for introductions to relevant literature.

II. SUMMARY OF DISCUSSION, WORKING GROUP I: BIOCHEMICAL AND MOLECULAR METHODS FOR MEASURING PHYSIOLOGICAL RATES AND CONDITION

Discussion Leaders: George Somero and Joseph Torres

Overview

Numerous biochemical and molecular "indicator" techniques are available for determining physiological capacities (rates) of organisms and their physiological status (condition). Indicator methodologies may be used to generate a "calibration curve" which relates the physiological trait of interest, *e.g.*, metabolic rate, to a readily measured biochemical or molecular characteristic, *e.g.*, activity of a respiratory enzyme. The physiological rates *(e.g.*, metabolism or growth) or status *(e.g.*, nutritional or reproductive status) of large numbers of individuals from field populations can then be determined simply by making a biochemical (or molecular) measurement, without need for difficult and time-consuming experimentation with living specimens.

Several biochemical or molecular indicator methodologies are well established for use in species of terrestrial and freshwater organisms, but they have not been applied widely to marine species. Below we briefly discuss several methodologies which are appropriate for addressing questions of central importance to GLOBEC, specifically, questions concerning rate processes and physiological states of zooplanktonic organisms. This information is intended to serve as background material for the draft "Request for Proposals" (RFP) ("GLOBEC Call for Proposals for Research Concerning the Exploitation of Biochemical and Molecular Techniques for Gauging Physiological State of Marine Zooplankton", Appendix II of this report).

General Performance Capacity

Metabolism and Locomotory Ability. Two critical rate processes of interest to physiological ecologists are rates of metabolism and locomotory capacities of organisms. Metabolic rates may be used to estimate energy turnover and flux in an ecosystem. Locomotory capacity is critical in determining predator-prey relationships in motile species.

Enzymatic analyses can provide accurate estimates of the metabolic potential--both "basal" and "active" rates--of organisms. The enzymes of energy metabolism, *e.g.*, those of ATP generation, are common to most marine species. Assay of these enzymes under physiologically realistic conditions, *e.g.*, of temperature and pH, can provide an estimate of the maximal ATP-generating potential of an organism or a specific tissue. Likewise, in the locomotory muscles, activities of ATP-generating enzymes in both aerobically- and anaerobically-poised pathways can provide an estimate of capacities for both low-speed (aerobically-powered) and high-speed (anaerobically-powered) swimming.

To exploit enzymatic methods successfully, one must have an understanding of the pathways of ATP generation in different species. For fishes this information is available; for many invertebrates data are lacking. It is imperative that initial efforts to adapt enzymatic analyses to marine systems more completely characterize the metabolic capabilities of ecologically important invertebrate species such as copepods. A general theme of this document is that much additional background information on the physiology, biochemistry, and molecular biology of marine species must be determined before appropriate biochemical indices for evaluating ecologically important characteristics can be fully developed.

Several factors must be considered in designing effective methods for using enzyme activities as estimators of metabolic rate and locomotory capacity. Among these are the following.

First, enzymatic activities tend to be size-dependent. Thus, size must be incorporated into experimental design and data analysis. Second, enzymatic activities are highly dependent on diet (a fact which makes these methods very useful for estimating physiological state). In laboratory calibration studies, *e.g.*, studies in which oxygen consumption rates and metabolic enzymes are measured, dietary effects should be considered as an experimental treatment. Third, the stability of the enzymes of interest during storage should be determined. Although many enzymes are generally stable during long-term storage at -80°C (or even in conventional freezers), it is appropriate to test for stability whenever a new enzyme, or new type of organism, is to be analyzed.

Specific choices of indicator enzymes for gauging metabolic and locomotory capacities must be based on knowledge of the primary pathways used by the species or tissue of interest and of the stabilities of these enzymes during storage. The aerobic metabolic potential of an organism or tissue can be estimated by determining citrate synthase (CS) activity. CS is a good indicator of the Krebs citric acid cycle activity of an organism or tissue, and CS is generally stable during long-term storage of frozen specimens. Cytochrome c oxidase is another appropriate enzyme, but its stability is typically not good when subjected to freeze-thaw cycles. Lactate dehydrogenase (LDH) is a very good indicator of anaerobic locomotory power in fishes, and is stable during long-term frozen storage. However, in copepods and other planktonic invertebrates, other enzymes might be better indices of locomotory capacity, especially if the planktonic invertebrates rely more on aerobic than on anaerobic schemes for supplying the ATP required for locomotory processes.

Nutritional Status. Enzymes of digestive processes may provide an important source of information about physiological state. Individuals living under conditions of food shortage may contain reduced levels of substrate-inducible digestive enzymes. To use digestive enzymes in analyses of nutritional status, it is necessary to determine the appropriate enzymes for analyses. For example, protease activity may be appropriate for carnivorous species, whereas enzymes like laminarinase are more suitable for herbivores. Possible diel variations in digestive enzyme activities should be considered in designing experiments. Nutritional status also could be indexed by measuring the amounts of stored nutrients, *e.g.*, glycogen or triacylglycerides, deposited in storage sites such as muscle and liver.

Molecular Biological Approaches. Although enzymatic analysis has the benefits of being well-developed, rapid, usually inexpensive, and relatively simple, there may be circumstances under which molecular methods offer increased sensitivity and resolving power. Using specific primers to quantify the amounts of specific nucleic acids in natural samples is one example of a situation where molecular approaches may be especially powerful. Methods of this type will depend on the generation of specific primers of use to workers studying zooplankton.

Other Considerations. Depending on the question being asked, sorting of organisms may or may not be necessary. if the total metabolic potential of all organisms in a given volume of water is of interest, then measurement of, for example, CS activity in an entire community (made via separating the organisms by centrifugation, settling, or screening, and, then, homogenizing the entire community to obtain a source of soluble enzyme) is possible. This type of measurement obviously is crude, but it seems likely to give information about the integrated capacity for ATP generation in the entire community of organisms in the water sample. Refining the sampling and sorting regimes could involve separation and analysis of individual sizes or species, and the analysis of single tissues, *e.g.*, muscle tissues or digestive organs, to obtain more specific information about swimming ability or digestive capacity, respectively. Analysis of diagnostic tissues can provide especially clear biochemical signals about the organism's physiological state.

Morbidity

In many contexts it is helpful to have an index of the degree to which an organism has been stressed, and the extent to which an organism is living near the limits of its tolerance, *e.g.*, to anoxia or temperature. At present there are no clear cut methods for quantifying stress level or "morbidity." However, several biochemical attributes would seem to offer promise in this context

Stress Proteins. A diverse family of proteins known as stress proteins ("heat shock proteins" was the expression formerly used to describe these molecules) can be induced in response to a variety of environmental stresses, including high temperature, anoxia, heavy metal pollution, and exposure to ethanol. The appearance of stress proteins typically occurs within an hour of exposure to the environmental stress, and a high level of stress proteins may be maintained in the cell for extended periods. The appearance of stress proteins is an indication of two things: the occurrence of an environmental stress, and the ability of the organism to respond adaptively to this stress. Thus, the synthesis of stress proteins does not indicate morbidity *per se;* it may reflect just the opposite condition, *i.e.*, an organism which is coping well with an altered environment. However, stress protein appearance could serve, at least in theory, as a useful biochemical indicator of exposure to stress, even though other physiological and biochemical indicators would be needed to gauge accurately whether that stress leads to some level of morbidity.

Choice of Tissue. Tissues and organs differ in their environmental sensitivities, and the choice of appropriate tissue for examining morbidity is important. In fishes, brain tissue seems to be the most highly "defended" tissue, showing physiological decline only after more "expendable" tissues, *e.g.*, locomotory muscle, have begun to show physiological deterioration *(e.g.,* in protein synthetic capacity and ATP generating potential). Thus, physiological studies under controlled laboratory conditions should be conducted to determine which tissue(s) are most appropriate for use as indicator tissues of morbidity.

Diapause

Diapause, whether it occurs during early development of copepods (diapause eggs) or during later stages (CII-adult) is a very important stage in the life history of many important calanids. From an ecological standpoint, the absence of important metazoan grazers in the trophic pyramid changes the character of the ecosystem. From a physiological standpoint, it is an important (and perhaps flexible) response to times of environmental stress, a metabolic refugium that may increase the odds of species survival during global warming periods.

Our current lack of knowledge about the physiology and biochemistry of marine invertebrates is perhaps best illustrated by the minimal understanding we possess about factors regulating diapause in copepods. Regulatory mechanisms that affect the reversible shut-down of active metabolism are totally unknown. Metabolic costs/benefits of the diapause state likewise need much additional study. Biochemical methods should be used in background studies to elucidate these regulatory mechanisms, and to provide insights into the metabolic benefits (and costs) of these states of reduced activity. Perhaps the large data base on brine shrimp (*Artemia*) diapause and quiescence could provide insights into the most appropriate models for copepod diapause. Changes in tolerance of anoxia might also be examined for species that settle into low-oxygen water *(e.g.,* oxygen minimum layers) during periods of diapause.

Egg Production Rates

Biochemical and molecular approaches for quantifying egg production rates are currently not in use. Sorting and counting methods are time-consuming, but are accurate and effective. Several interesting biochemical alternatives were suggested as worthy of further exploration.

First, immunological tests for the occurrence and/or abundance of eggs of a particular species in theory could provide a rapid and accurate means for surveying egg production. For example, if highly specific antibodies could be generated to a species-specific protein such as a sperm-binding protein, then sensitive antibody tests could be developed for characterizing unsorted populations of eggs. Such antibody tests might be restricted to species that spawn unfertilized eggs. Species with internal fertilization might not be amenable to this type of analysis.

Second, chemicals *(e.g.,* pheromones) that induce spawning by stimulating reproductively competent individuals to release eggs over a short period of time might provide an index of the reproductive activity that is occurring. Sensitive analytical methods, *e.g.,* HPLC techniques, will be needed to detect, identify, and quantify these highly dilute chemicals.

Growth Rates

Biochemical and molecular indicators of growth rate potentials have been used for over a decade with considerable success. Further refinement of these methods will enhance their accuracy, speed, and utility for use in generating "real time" data on board ships.

RNA:DNA Ratios. The ethidium bromide method for quantifying RNA and DNA in small (<0.5 mg) samples is now commonly used for determining RNA:DNA ratios. Dozens of samples can be analyzed per day by an individual worker, so this method is well suited for sampling large numbers of individuals. Microtitre plates and readers could speed-up the analysis of numerous samples. The method needs more testing and development with invertebrates, but its general applicability to animals seems likely.

There are several precautions that must be taken in using this method. Among these are: (i) DNA concentration may be size-dependent, *e.g.,* in fish locomotory muscle DNA concentration may decrease with size, and (ii) DNA content per nucleus varies widely among species. Thus, there is no single term for the denominator of the RNA:DNA expression.

DNA Polymerase. One enzyme involved in DNA replication, DNA polymerase, could in theory serve as an excellent indicator of growth rate, *i.e.,* of DNA synthetic rate. With the advent of non-radioactive fluorescent substrates for quantifying DNA synthesis, the measurement of DNA polymerase activity at sea seems a very reasonable and attractive strategy for measuring growth rates. This method requires further development for marine species for which growth involves increases in cell number (DNA synthesis). For species in which growth involves increases in cell size, but no increase in the amount of DNA per cell, *e.g.,* later stages of copepods, the DNA polymerase method is clearly not applicable.

Chitin Polymerization Enzymes and Molting Hormones. For species in which chitin synthesis correlates strongly with growth, measurement of the activities of one or more enzymes of chitin synthesis could be a useful biochemical method for estimating growth potential. Assay of molting hormone concentrations in seawater could also be useful for estimating growth. These potentially useful methods require development, including detailed laboratory calibration.

Developmental Stage

For fishes, identifying developmental stage appears to provide few major problems, and biochemical or molecular approaches appear unnecessary. For invertebrates, screening large populations for developmental stage is more problematical. Here, 2-dimensional (2-D) gel electrophoresis methods could be a real boon, assuming that each life stage has a unique "protein signature" that could be detected by highly sensitive 2-D gel methods.

Age

There is no known biochemical or molecular method for estimating age. No chemical species appears to build up merely as a consequence of age. Lipofuscin methods appear unreliable. Size-related changes in biochemical properties should not be confused with age-related changes.

Applicability of Biochemical and Molecular Methods to Shipboard Study

Several factors must be taken into account in evaluating the applicability of biochemical and molecular methods for use on board ships, *e.g.*, for gathering "real time" data. Some of these considerations are briefly discussed below.

Stability of Samples. Many--probably most--enzymes and RNA and DNA are generally stable during prolonged storage at freezing temperatures. As a general rule, the colder the temperature of initial freezing and storage, the more likely is the biochemical or molecular system to be stable. Freezing in liquid nitrogen is optimal, but often not necessary to ensure adequate stability. The presence of a -80°C freezer on board ship is almost certain to benefit biochemical and molecular analyses.

The decision as to whether samples should be worked-up on board ship must be based in part on considerations of stability. Labile samples should, if possible, be worked-up immediately, without freezing. More stable samples can be collected in large numbers *(i.e.*, in larger numbers than can be conveniently assayed on board ship during an expedition) and returned to the home laboratory for analysis.

Weighing and Storage of Reagents. Because most biochemical and molecular methods employ several labile reagents, often in minute quantities, it is advisable to pre-weigh as many reagents as possible before going to sea. if a motion-compensated shipboard balance is not available, then pre-weighing of reagents is a must. On board ship the reagents must be stored under appropriate conditions to ensure stability.

Centrifugation. Table-top centrifuges ("microfuges") work well at sea, and even larger centrifuges work well if placed on gimbals. Thus, preparation of high-speed supernatants for enzymatic analysis should pose few problems.

Other Considerations

1. Normalization of Enzymatic Activities. No one normalization format may be suitable for all purposes. Very small organisms, *e.g.*, individuals with fresh (=wet) weights of less than *0.5* mg, may be difficult to weigh accurately when wet. Thus, normalization of enzymatic activity to dry weight or to total body protein may be more suitable than normalization to fresh weight. For larger

individuals, tissue samples can be run and normalization to wet weight may be the best means of expressing enzymatic activity per mass of organisms. Whatever the normalization procedure adopted, procedures must be followed that will allow comparisons to be made between different organisms and, when possible, between data from different laboratories. Often the lack of consistent normalization procedures between laboratories makes data sets difficult, if not impossible, to cross-calibrate.

2. Standardization of Analytical Procedures. The caveats raised above apply equally strongly in the context of designing precise analytical protocols for biochemical and molecular work. Assay temperature, pH, ionic strength, substrate concentration, *etc.* will affect the results of *in vitro* experiments. Ideally, different workers should agree on common in vitro conditions, thereby making their data sets as amenable to cross-calibration as possible. A particularly strong caveat applies to assay temperatures for enzymatic analyses: a 1°C change in assay temperature typically leads to a 12% change in enzymatic activity. Thus, it is imperative to use thermostatted cuvette holders for running spectrophotometric enzyme assays. Running assays at "room temperature," *i.e.*, without precise temperature control, will lead to enormous variation in the data, and a reduced ability to compare samples from different days or laboratories.

3. Minimal Data Base on Marine Invertebrate Species, Especially Zooplanktonic Species and Life Stages. A major theme running through our discussions of biochemical and molecular indicators of physiological state is the dearth of information on the biochemistry and molecular biology of marine invertebrates. Emphasis should be given to enlarging this data base, to enable informed decisions to be reached concerning the best biochemical indicators to study, and the best technical approaches to be developed for specific applications to invertebrates.

4. Field *versus* Laboratory Populations. In several contexts it was pointed out that laboratory-reared individuals may differ in physiological or biochemical status from field-caught individuals of similar size, age, or life stage. This difference, which may reflect differences in diet, exercise level, predation, or other factors, should be taken into account when laboratory studies are conducted to generate "calibration curves," *e.g.*, of metabolic rate *versus* enzymatic activity. Fortunately, however, it seems likely from what is currently known that extrapolation of such calibration curves to higher or lower values of the variable(s) of interest is apt to be valid.

Ill. SUMMARY OF DISCUSSION, WORKING GROUP II: ZOOPLANKTON GENETICS

Discussion Leaders: Dennis Hedgecock and Michael Lynch

The GLOBEC initiative seeks fundamental information about basic mechanisms that determine the abundance and distribution of zooplankton populations, including holoplankton, meroplankton and ichthyoplankton. Understanding the processes that cause present-day variability of these populations about their average values should assist in the prediction of population responses to global environmental change. Obtaining fundamental information about mechanisms depends critically on the ability to characterize zooplankton field samples according to taxonomic and population genetic criteria. The following information was discussed as background in formulating the draft "Request for Proposals" ("GLOBEC Call for Research Proposals Concerning Genetic Identification and Characterization of Zooplankton Populations", Appendix Ill of this report).

Prospects for Real-Time, Automated Taxonomic Identification of Plankton Samples. An "idealized" schema for sampling zooplankton that had been developed earlier by some members of the GLOBEC Steering Committee was presented as a starting point for discussion in this working group. This schema depicted in-line coupling of a plankton pump to reaction vessels for molecular probing and then to flow cytometers for sample sorting. The output would be taxonomically sorted in real time, providing intact and possibly even living specimens for physiological analysis.

It was recognized that this was a hypothetical set-up employed to stimulate discussion, and the working group quickly agreed that such a schema could not be achieved in the foreseeable future. Although molecular methods are indeed powerful tools for systematic biology and taxonomy, they do not yield results in "real-time" and they are destructive. Two hours is a more realistic minimum time required for characterization of a specimen by current molecular methods. DNA probing, for example, requires at least three basic steps: (1) extraction of DNA, or making permeable the intact specimen; (2) hybridization of the probe; and (3) washing under stringent conditions to reduce background or non-specific probe binding. The biochemistry involved in these steps constrains shortening of the time required. Moreover, for organisms as small as zooplankton, the whole organism would generally be consumed in this process, making it unavailable for physiological studies. This is not to say that molecular methods cannot play an important role in GLOBEC studies. Characterizing species-specific markers through studies of molecular systematics will provide a foundation for unambiguous identification and enumeration of zooplankton species, and assessing intraspecific variation in molecular markers will allow characterization of conspecific zooplankton populations on spatial/temporal scales appropriate to physical oceanographic features or processes that may influence population mixing or recruitment success. However, it must be recognized that current molecular methods are likely to provide only retrospective information on plankton composition, either in parallel subsamples taken at a station or on specimens already used for physiological analysis.

A "Spread and Probe" Strategy for Zooplankton Identification. The working group considered two alternative strategies for determining the species and genotypic compositions of zooplankton samples: quantitative analysis of bulk samples *vs.* qualitative analysis of individuals. Quantification of molecular species in bulk samples is difficult even in simple systems such as mammalian cell cultures; quantification of species in a batch sample of mixed zooplankton would be fraught with error. The alternative strategy of spreading individuals out in two dimensions for molecular probing would probably prove more reliable for species or genotype characterization.

Size Limits. The small sizes of zooplankton should not prove to be a limitation for molecular technology. Conventional analysis of restriction fragment length polymorphisms in DNA (RFLPs, detected by restriction enzyme digestion of sample DNA, electrophoretic separation of resulting fragments, transfer of fragments to a membrane, and hybridization of membrane-bound fragments to a radiolabeled probe) requires nanogram quantities of high molecular weight DNA but have been applied successfully to species identification of large fish eggs. The polymerase chain reaction (PCR), on the other hand, can amplify a particular segment of DNA by a factor of several million or even a billion; enough DNA can be obtained from as little tissue as a single cell for analysis or characterization. Moreover, DNA can be extracted and amplified from alcohol-, and in some cases formalin-preserved, material. Thus, molecular studies could be done retrospectively in samples preserved during a cruise or on samples in historical zooplankton collections.

Adapt Existing Molecular Methods to Zooplankton; Obtain Basic Population Data. The working group felt that most methods of molecular biology probably could be adapted to zooplankton studies. To bring the power of molecular biology to bear on the problems of taxonomic identification and population genetic analysis of zooplankton, at least in the retrospective sense described above, we do not need to develop new technology. It will, however, be critical for GLOBEC to insure the support of the basic laboratory bench work required to *adapt* existing molecular methods to marine zooplankton.

Following this, the working group identified a great need for basic information on the amount of molecular genetic variation within and between populations and species of marine zooplankton. Present knowledge does not allow for the design of taxon-specific probes or markers for most of the organisms likely to be important in GLOBEC studies, nor does it allow an evaluation of which molecular techniques are likely to be the most useful in a particular context. A range of existing molecular techniques -- allozyme electrophoresis, RFLP analysis, sequence-specific oligonucleotide probing of PCR products, DNA fingerprinting, and immunofluorescent probes -- are available to discriminate morphologically similar species and to analyze population genetic structure. These techniques differ greatly in ease and efficiency of application and yield information of relevance in proportion to the amount of underlying variation in the particular species being investigated. **The working group recommended that GLOBEC support basic studies of molecular genetic variation, using the full range of existing molecular methods, so that decisions regarding the most appropriate method could be based on informational content and sampling efficiency.**

Scope of Sampling. An additional problem identified by the working group concerned the necessary scope of sampling for population genetic analysis. In order to understand the capacity of animal populations to adapt to environmental change in an area, one must understand the genetic diversity and structure of the total species population. Limiting genetic or physiological studies to only one local population *(e.g.,* Georges Bank) might not allow prediction of the capacity of that organism *(e.g.,* cod) to remain in that area under a regime of changing climate; the local stock might simply go extinct and be replaced by a stock with a different genetic composition from another area. A shift in gene frequencies in the local population under study might be difficult to interpret without background information on this species from a much broader geographical area.

Genetic Basis of Adaptation to Global Climate Change. An additional area of genetic research was felt to be important to the overall aims of GLOBEC -- studies of the genetic bases of variation in phenotypes likely to determine the responses of zooplankton populations to climate change. These studies primarily would utilize the methods of quantitative genetics rather than molecular biology.

Two categories of phenotypic traits that are likely to be important in adapting to environmental change and that might be influenced directly by factors such as warmer temperature are: (1) characters relevant to recruitment *(e.g.,* duration of pelagic larval phase, competency for metamorphosis, response to environmental cues for settlement); and (2) juvenile and adult physiological, behavioral or life-history traits *(e.g.,* age- or size-specific fecundity).

New Technology. While prospects for automated real-time plankton sorting by existing molecular methods appear dim, breakthroughs in biotechnology are difficult to anticipate. Research on the development of new technology for rapid and accurate identification of preserved, intact, or live zooplankton ought to be encouraged in a call for proposals. Such new technology also might utilize physical methods, such as optical image analysis, perhaps in combination with molecular labeling of intact zooplankton. Any research that might reduce existing or new biotechnologies to shipboard practice also should be solicited.

IV. SUMMARY OF DISCUSSION, WORKING GROUP III: FEEDING RATES AND DIETARY COMPONENTS

Discussion Leaders: Gary Kleppel and Steven Hand

The discussion in this group was intended to contribute to the sections on physiological condition and rates. In the context of Global Change, the intent of GLOBEC is to understand fundamental mechanistic processes that are responsible for: (1) abundance of marine animals (2) fluctuation in abundance, and (3) secondary production in ocean ecosystems. In order to approach these broad issues, much more basic information is needed on the following topics:

I Physiological condition and rates; population genetics and genetic markers

II Viable biotechnology that can be applied at sea *(i.e.,* on shipboard) to speed data acquisition in these research areas

One underpinning of physiological condition is the process of feeding and the nature of the associated dietary components. Definitive data and new experimental approaches to three simple questions are needed, particularly as related to zooplankton:

(1) What methods are available to determine whether or not a population of organisms is feeding?

(2) What are the most effective approaches for identifying dietary components?

(3) What is feeding rate of populations *in situ?*

The first question is intended to stimulate technological development in high frequency data acquisition, so that feeding data can be interfaced more rapidly with oceanic physical/chemical data that are acquired on real time and near time bases. Because variations in both concentration and composition of food in the environment may influence zooplankton production, data on the composition of the diet are needed (question #2). Diet tends to be among the most poorly quantified aspects of feeding, yet it may be one of the most important.

Identification of gut contents by using taxon specific DNA probes (particularly above species level) is one promising approach. Probes should at least differentiate between carnivory and herbivory; additional levels of taxonomic detail are desirable. The ability to analyze small samples within a time scale of hours on board ship is an important goal. Various potential problems need to be addressed and resolved. For example, we need to evaluate the effect of transit time of food through the gut, the acidic gut pH, and the actions of nucleases and proteases on the degradation of the DNA and protein used for food identification. It may not be feasible to prepare large numbers of probes for use on predator species with a broad dietary intake. Application of this approach to adult stages which feed on the development stages of the same species *(e.g.,* copepods eating their own nauplius larvae) may not be feasible. Are these approaches quantifiable? For example, can the number of gene copies be related to the number of cells ingested, providing the appropriate controls are performed *(i.e.,* laboratory verification and calibration)?

Immunological identification of food also may be applicable. For example, antibodies raised against the yolk protein of fish eggs or yolksac-stage larvae could be used to follow ingestion by euphausiids. Antibodies raised against parvalbumin (lower vertebrate specific

contractile protein in white muscle), against whole phytoplankton cells, or against ciliates may be useful. Will gut dissections be required in all cases to acquire a clean immunological signal? If so, the number of analyses performed per day would be compromised. The relative effectiveness of polyclonal *vs.* monoclonal antibodies should be considered.

Application of flow cytometry to zooplankton feeding studies may be helpful in speeding up the processing of gut content analysis, as well as for monitoring the rate of food removal by predators in "closed container" experiments. Other optical screening techniques may be useful.

Analytical techniques for identification of pigments, including measurement of carotenoids for determining diet of predators, already exist and are relatively rapid. Pigment content can be related to total carbon ingested. Further refinement of these techniques is necessary. The feasibility of developing immunofluorescent markers for carotenoprotein complexes would increase the sensitivity of pigment analyses enormously.

Finally, reliable indicators for feeding rates of populations in the field need to be developed. Attempts to use the level of digestive enzymes measured in predators as an indicator of recent feeding activity has not been overly successful. Often the induction of these enzymes in response to food intake is not detectable. The successful application of this approach will likely require laboratory verification for each predator species in question. New and more reliable enzyme/macromolecular markers that are induced rapidly and that can be used as indices of feeding and/or assimilation need to be identified. For example, can the level of enzymes associated with synthesis of the peritrophic membrane be used as an index of feeding activity? Unquestionably, more basic research on the physiology of zooplankton feeding and on the biochemistry of the zooplankton gut is absolutely essential to accomplish this objective.

V. SUMMARY OF DISCUSSION, WORKING GROUP IV: SAMPLING

Discussion Leaders: Bruce Sidell, Peter Ortner and Lewis Incze

General

Developing techniques in molecular biology now make it possible to assess certain physiological properties of individual marine planktonic organisms. New discoveries and methodological advancements almost certainly will expand the present list of capabilities to include smaller samples sizes, additional physiological parameters and more convenient procedures that can be taken to sea. There should be collateral development of techniques for sample collection and processing so that the methods are complementary and, to the extent possible, can be physically combined to enable frequent sampling in the field. There are three parts to the problem: sample acquisition, identification and sorting of taxa, and application of desired chemical or molecular assays. The latter two topics were discussed by the other three working groups at this workshop. It was the objective of this Sampling Working Group to consider how the molecular tagging and various assays might be combined and to consider how these jointly might be incorporated into a sampling scheme that could satisfy the needs of the oceanographic field program.

As background and stimulus for discussion, a schema of an "ideal" sampling system was presented. This "pipe-dream" schema had been developed earlier for general discussion by members of the GLOBEC Steering Committee. It was recognized that the final technology might not even closely resemble the depicted flow diagram, but the central goals were clear and should be maintained. To wit: small-volume samples should be obtainable from well-defined depths; biological sampling should be combined, as much as possible, with physical and optical instrumentation; samples delivered to the surface should be "tagged" in some manner to enable rapid and accurate sorting; and tagged (that is, identified and sorted) animals should be used for the numerous shipboard analyses that might be desired. The central questions which arise from this sampling and processing strategy are: 1) what sampling frequency is desirable and feasible for the physiological/ecological work; 2) can the experimental and physiological work be done in tandem with the taxonomic marking and sorting; and 3) are there genetic or physiological objectives that cannot be met by such a sampling scheme? These questions formed the basis for our discussions, which are summarized accordingly.

Specific Comments

Sampling Frequency, Scale and Methods. The ability to sample physical properties of the ocean with fine spatial (especially vertical) and temporal resolution is recognized. It also is recognized that biological sampling must routinely be conducted at finer spatial and temporal scales than presently possible in order to meet GLOBEC objectives. Developing applications such as multi-frequency acoustic devices, imaging systems and acoustic current profilers are approaching the scales of physical observations with respect to estimates of zooplankton biomass and approximations of community composition based on size and shape. A pumping system could deliver samples with fairly small spatial resolution and could be used to sample frequently. It was felt that animals probably could be delivered to the ship's laboratory relatively unharmed. The question is: is it reasonable or desirable to try to approach this frequency of observation with the genetics or physiological assessments?

Most discussants felt that initially they should examine physiological parameters at only a

few depths per station and concentrate on sampling a larger number of individuals from each depth. This is because differences between individuals even under the same conditions can be great and may play a significant role in survival and recruitment. More must be learned about these differences before it will be useful to sample for physiology at more depths. Group members were more positive about the immediate benefits of fine-scale genetic (taxonomic) information. This could provide valuable insights by itself and would be useful in considering how to plan physiological and experimental work in the future.

The advantages of a pumping system were recognized as saving ship time and providing small volumes from well-defined depths with accompanying physical and other *(e.g.,* bioacoustical, fluorescence) information. Such systems should prove useful in many respects for the types of field objectives being discussed here. However, such systems probably are inadequate for sampling larval fish and other comparatively sparsely distributed fauna, and they likely introduce unacceptable levels of trauma to most organisms for subsequent live experimentation on board. The latter should be investigated, but the consensus was that specialized sampling designed for specific organisms and questions will remain a necessary feature of field programs. It was felt that most enzyme assays are relatively robust with respect to pump sampling *per se*, but that macromolecules in zooplankton may be subject to rapid degradation due to release of lytic enzymes *(e.g.,* proteases, lipases, RNAses, *etc.)* and the total handling time may be a problem. Consequently, protocols for processing (and/or storage) of samples will be dictated by the particular organism under study and/or the nature of measurement being performed. Much has yet to be explored. Consolidation of sampling design, instrumentation and techniques should be sought wherever possible, but flexibility also is needed. A generalized and unified sampling scheme probably cannot be devised *a priori.*

Joining of Taxonomic Tagging with Physiological Assays. Molecular labeling of zooplankton is needed for more detailed and accurate taxonomic identification. This can be the basis for increased sample sorting speeds *(e.g.,* with optically labeled tags) and for asking more detailed questions about the physiology and behavior of zooplanktonic organisms and their response to environmental conditions and change. There would be obvious benefit to performing behavioral studies and molecular assessments of physiology on animals that have already been sorted and identified. Specifically, this would reduce the number of "superfluous" tests and would ensure that you obtained intended sample sizes for the targeted taxa. The question is to what extent and in what manner can taxonomic tagging and sorting be coupled to the other procedures. It was felt that there may be ways to execute molecular physiological assays on individuals that had been tagged previously for identification (a serial coupling of the two procedures). For example, it is possible that taxon-specific molecular surface tags *(e.g.,* optically active immunochemical tags) may be compatible with some physiological assays *(e.g.,* enzyme activity determinations) that could be executed after tagging and identification. Alternatively, because of the high sensitivity of many molecular techniques, separate sample aliquots from moderately small organisms may be able to support both types of measurement. For example, taxonomic identification by 2-dimensional electrophoresis could be done in parallel with some enzyme assays requiring little tissue. However, this cannot be predicted *a priori* even with extant assay techniques because it depends on the chemical nature of the tag(s) used, and these tags have not yet been determined for the taxa of interest. This suggests that development of genetic tags and physiological assays will have to proceed independently for awhile, recognizing that a marriage of the two ultimately is wanted.

Present tagging techniques require destruction of the organism. Consequently, it would not be available for studies which require live or at least intact animals. In the near future, such tagging would have to take place after the behavioral or other experimental work was conducted. This would impose the same difficulties cited above with respect to superfluous samples and/or

inadequate sample sizes. Some assays may themselves be destructive of the animal tissue. For these reasons, it is important to develop techniques for tagging of live and intact zooplankters to permit identification before, and without interference to, other analyses.

Genetic or Physiological Objectives Not Met by the Above Sampling Scheme. Discussions held by the three other working groups at this meeting focused on measuring the response of individual organisms to physically forced conditions of the environment. The sampling scheme discussed above attempts to make such measurements feasible within the framework of an oceanographic cruise and the desired coupling of biological and physical data. The focus is clearly on the small-scale for the individual, the medium-scale (that is, the study "site") for the population-level response *(e.g.*, recruitment) and the present. Not all of the study can be restricted to these scales, however. Significant variations in biomolecular characters probably will be encountered not only within the study area, but also beyond its boundaries. For the development of reliable genetic markers and for understanding possible shifts in genetic structure and physiology of local populations in response to climatically related changes in the environment, it will be necessary to conduct some sampling well beyond the boundaries of specific study sites. GLOBEC must recognize these needs in addition to the focused effort on processes within designated areas.

Conclusions

The following summarizes the main points of the above discussions.

1. We are not ready to try to couple completely physiological measurements to the meter-scale depth resolution achievable in the physics. The next generation of developing molecular techniques should concentrate on measuring the range of individual variations at a relatively few depths per station, but doing so rapidly and efficiently enough to enable much improved resolution in the horizontal and over short time intervals. The use of a pump attached to a CTD package is valuable (see discussion for details) but should not be construed to suggest that physical and biological data will always be obtained at the same spatial and temporal scales. Abundance and compositional data (see discussion) are better suited to such 1:1 comparisons in the near future.

2. Some specialized sampling probably will be necessary regardless of efforts to streamline operations. A view to such flexibility must be maintained. Even within the realm of molecular techniques, it may not be possible to identify a generalized and unified sampling scheme *a priori*. Considerable work must be done first on the tagging and assay methodologies.

3. Genetic labeling techniques capable of supporting small-scale vertical investigations will likely precede the development of comparably facile physiological assays. This is not harmful; genetic information on vertical distribution patterns should lay the groundwork for asking physiological questions that demand the smaller vertical scale resolution.

4. The coupling of molecular tagging with subsequent molecular assay techniques is a reasonable goal and will probably be achievable at least for some assays and organisms. Methods for assaying physiological parameters should be developed with such a coupling in mind, but should not be constrained by such a requirement.

5. Methods should be developed for molecular labeling of live and intact zooplankters.

6. Some sampling for biomolecular characters will have to be conducted beyond the boundaries of designated study areas in order to develop reliable genetic markers and understand possible shifts in genetic structure and physiology of local populations.

VI: LITERATURE CITED

Joint Oceanographic Institutions, Inc. 1990. Initiatives for the accelerated transfer of biotechnology to the ocean sciences. Report of a workshop held September 16-18, 1988, Tucson, AZ. 40 p.

Powers, D.A., F.W. Allendorf and T.T. Chen. 1990. Application of molecular techniques to the study of marine recruitment problems. Pp. 104-121 *In* Large marine ecosystems: Patterns, processes and yields (K. Sherman, L.M. Alexander and B.D. Gold, eds.). Amer. Assoc. Adv. Sci. Publ. No. 90-30S, Washington, D.C. 242 p.

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APPENDIX II: DRAFT RFP FOR PHYSIOLOGICAL RATES AND CONDITION

GLOBEC CALL FOR RESEARCH PROPOSALS CONCERNING THE EXPLOITATION OF BIOCHEMICAL AND MOLECULAR TECHNIQUES FOR GAUGING PHYSIOLOGICAL STATE OF MARINE ZOOPLANKTON

General Goals

GLOBEC seeks to encourage the development of biochemical and molecular methods which can be used in laboratory and shipboard analysis of the physiological state of marine zooplankton. Methods are sought for obtaining accurate estimates of rates of metabolism, growth, reproduction, and physiological status--locomotory ability, dietary state, life stage, reproductive condition and morbidity--that can be applied to both invertebrates and fishes. The methods should be capable of assessing how changes in the environment, for example in seawater temperature, impact secondary production in different marine systems.

Specific Objectives

The rapid development of biochemical and molecular methods provides biological oceanographers with a rich potential for using new procedures to address long-standing questions about fundamental marine processes. Included among these are rates of secondary production and the characteristics of the organisms responsible for this production. Biochemical and molecular methods show potential for rapidly advancing our understanding of each of the following areas.

1. Indices of rate processes. The rates of metabolism, growth, and reproduction of zooplankton are amenable to analysis by simple biochemical and molecular methods, *e.g.*, enzymatic indices. Enzymatic indicators appropriate to these rate processes need to be developed for major classes of zooplankters. These enzymatic indicators should be developed for shipboard analysis of large numbers of samples. Consideration should be given to the development of indices that can be applied to analysis of individual organisms, including very small species, and to unsorted collections of organisms.

2. Indices of physiological state. Characterizing the physiological state of marine zooplankton is critical for analyzing the factors governing secondary production. Biochemical and molecular approaches to indexing such key physiological characteristics as growth rate, reproductive state, developmental stage, and morbidity are necessary. The regulatory mechanisms and environmental factors governing diapause and quiescence in copepods need to be understood. Among the potential methods for addressing these diverse questions are: (i) procedures for quantifying nucleic acids, *e.g.*, RNA:DNA ratios; (ii) enzymatic techniques for quantifying nucleic acid synthesis, *e.g.*, DNA polymerase activity; (iii) antibody techniques for identifying and quantifying the egg populations in seawater samples; (iv) high resolution gel electrophoretic techniques, *e.g.*, 2-dimensional gels, for characterizing the taxonomic compositions of populations and plankters, (v) enzymatic, immunochemical, molecular genetic, flow cytometric approaches and other highly sensitive chemical methods for assessing nutritional state and dietary composition; (vi) methods for quantifying the rate of chitin synthesis; and (vii) high sensitivity chemical techniques for estimating such growth- and reproduction-related chemicals as molting hormones and egg-releasing factors.

Target Species

There is a dearth of biochemical and molecular data on most marine invertebrate zooplankters. The development of biochemical and molecular indicator methodologies must entail substantial initial characterization of the biochemistries and molecular biology of key species of invertebrate zooplankters like copepods. Study species should include organisms for which one or more life stages is apt to be strongly impacted by global warming.

Training Components of Research Proposals and Cross-Calibration Among Laboratories

The successful design and implementation of biochemical and molecular indicator methodologies for use with marine zooplankton will require training regimens for biological oceanographers not familiar with these methods. Investigators submitting proposals should address the issue of necessary training in their submissions. Because it is desirable that different workers employ biochemical and molecular methods that will allow cross-calibration of data sets, investigators are encouraged to consult with colleagues who plan to use similar approaches, perhaps with different species or in different marine environments. Proper design *(e.g.*, using physiologically realistic in vitro measurement conditions) of protocols which can be standardized among laboratories will greatly facilitate the exploitation of biochemical and molecular indicator methods for addressing major questions concerning secondary production and the impact of environmental change on this production.

APPENDIX III: DRAFT RFP FOR POPULATION GENETICS

GLOBEC CALL FOR RESEARCH PROPOSALS CONCERNING GENETIC IDENTIFICATION AND CHARACTERIZATION OF ZOOPLANKTON POPULATIONS

Context and Need for Proposals

The GLOBEC initiative seeks fundamental information about basic mechanisms that determine the abundance and distribution of zooplankton populations (including holoplankton, meroplankton and ichthyoplankton) and, most importantly, the variability of these populations about their average values. Obtaining this fundamental information depends critically on the ability to characterize zooplankton field samples according to taxonomic and population genetic criteria. Ideally such characterization would be rapid and accurate enough to allow real-time automated sorting of live zooplankton field samples. In reality, current methods of molecular genetics cannot simply be coupled to plankton pumps and flow cytometers to achieve this ideal. New molecular technology must be developed for this task. Existing molecular techniques can, however, be applied immediately to studies of zooplankton systematics and population genetics in order to provide a foundation for the development of new technology and data on the coupling of oceanographic and population processes. This is a call for proposals that, in the context of the GLOBEC program, address the development and application of technology for improved resolution of zooplankton taxa and populations through all life stages from egg to adult.

Research Areas of Interest

Three research areas of interest have been identified:

(1) application of existing methods of molecular biology and genetics to the identification and characterization of zooplankton species and populations;
(2) development of new technology, amenable to shipboard practice, for the rapid and accurate identification of preserved, intact or live zooplankton;
(3) investigations into the genetic bases of phenotypic variation that might allow adaptation of zooplankton populations to global climate change.

A range of existing molecular techniques -- allozyme electrophoresis, RFLP analysis, sequence-specific oligonucleotide probes, DNA fingerprinting, and immunofluorescent probes --are available to discriminate morphologically similar species and to analyze population genetic structure. Proposals that apply these techniques to zooplankton species are solicited. Characterizing species-specific markers through studies of molecular systematics will provide a foundation for unambiguous identification and enumeration of zooplankton species in GLOBEC field studies. Assessing intraspecific variation in molecular markers will allow sampling of conspecific zooplankton populations on spatial/temporal scales appropriate to physical oceanographic features or processes that may influence population mixing or recruitment success. There is a basic need to assess which molecular markers are most suitable, in terms of informational content and sampling efficiency, to which conceptual problems.

Proposals to develop new technology that could be applied to the problem of identifying and sorting zooplankton field samples should address one or more of the following aspects of the problem: 1) reducing existing or new biotechnology to shipboard practice; 2) extracting molecular

information from preserved zooplankton samples, allowing rapid preservation of field samples for genetic analysis as well as retrospective studies of historical zooplankton collections; 3) labeling of intact zooplankton at the species level; 4) labeling of live zooplankton at the species level; 5) automating zooplankton identification, sorting, and enumeration through molecular or physical methods such as optical image analysis or a combination of these.

Proposals that assess the genetic bases of variation in phenotypes likely to determine responses of zooplankton populations to global climate change are solicited. Such phenotypes include: 1) characters important in recruitment success *(e.g.,* duration of the pelagic larval phase, competency for metamorphosis, response to environmental cues for settlement); or 2) physiological, behavioral or life-history traits likely to be influenced directly by global warming.

Selection of Target Species or Model Systems

Zooplankton species selected for study under this call for proposals should meet one of the following criteria: 1) they are important in marine ecosystems likely to be targets of GLOBEC field studies; 2) they are particularly suited to the development of new technology for zooplankton identification; 3) they are amenable to study of fundamental mechanisms governing abundance and distribution. These guidelines are intended to give investigators broad latitude in choosing appropriate species yet serve the overall goals of the GLOBEC program.

Training Component of Research Proposals

A major impediment to accomplishing the GLOBEC agenda is the need for cross-disciplinary training of marine biologists at all professional levels in molecular and population genetics. Proposals incorporating funding for collaborative research and for graduate students, post-doctoral scholars or sabbatical-leave faculty are encouraged.

APPENDIX IV: MEETING AGENDA AND WORKING GROUP ASSIGNMENTS

November 13, Tuesday

Afternoon/Evening

Out-of-town participants arrive at MIA, take cab or shuttle from baggage claim area (lower concourse) to Sheraton Royal Biscayne (20-30 min drive and cost will be about *$25* for a single cab). Enclosed is an annotated list of restaurants on Key Biscayne near the hotel if the hotel restaurants don't appeal to your tastes. We'll leave a message at the desk if there is any social "event" planned; most likely we'll just congregate in the hotel bar.

November 14, Wednesday

0800 Depart Hotel Lobby (We will have a rented multi-passenger van and personal cars to take you the 5 minute drive to RSMAS)

0830 RSMAS Library Chart Room

Opening comments by: Bruce Rosendahl, RSMAS Dean
Don Olson, Member GLOBEC Steering Committee
Dan Morse, Editor, Biotech/Ocean Sci. Initiative
Lew Incze and Pat Walsh, Workshop Organizers

0930 Break into Working Groups I and II (see attached lists): Group I to Library Conference Room, Group II in Library Chart Room.

COFFEE AND DOUGHNUTS WILL BE BROUGHT IN AT MID-MORNING

1130 Break for Lunch at RSMAS Cafeteria (you'll need to pay your own way and keep receipt)

1300 Reconvene Working Groups I and II

1445 Break for Coffee at RSMAS Cafeteria (closes at *1515)*

1515 Reassemble in Chart Room for a) plenary discussion of how groups are progressing; what problems they are encountering; what they see as things which will need addressing; discussion of group objectives and assignments for the following day; b) any further discussion that individual groups wish to have.

1730 Adjourn to RSMAS Bar for R and R. We will arrange for a group dinner at a local eatery (Cuban, Thai, etc.)-we hope all of you can make it. We'll coordinate transportation back to the hotel and then to dinner. We'll try to leave time for those who want to run or swim before dinner.

November 15, Thursday

0800 Depart Hotel Lobby

0830 General remarks, objectives and agenda for the day.

0900 Split into Working Groups III and IV (see lists). Note: Day 2 working groups are tentative,

with changes subject to Day 1 discussions.

COFFEE AND DOUGHNUTS WILL BE BROUGHT IN AT MID-MORNING

1130 Break for Lunch at RSMAS Cafeteria
1245 Reconvene Working Groups III and IV

1445 Break for Coffee at RSMAS Cafeteria (closes at 1515)

1515 Working Group Reports (ca. 15 mins ea.); plenary discussions; any further discussions that individual groups wish to have.

1700 Closing remarks; wrap-up details; writing/reviewing deadlines for workshop report; where do we go from here?

1730 Adjourn for socializing. For the time being no specific plans are made regarding a trip to the bar, dinner, etc. If people want, we can organize something; we'll bring this up on Thursday morning.

Overview and Objectives

Before we proceed to working group discussions, it is useful to consider the framework in which the deliberations of each group should take place.

GLOBEC wants, ultimately, to understand how marine populations respond to physical variability of the environment. Thus, we must have the ability to assess certain critical physiological parameters (rates and condition) with sufficient detail to resolve differences among animals on spatial and temporal scales typical of the ocean's variability. Concurrently, this will require a much better ability to sort animals, involving speed and accuracy and no change in critical proteins or molecules. Ideally, we would be able to sort samples and make physiological assessments rapidly enough and efficiently enough (in terms of space and personnel) that they both can be done at sea with reasonable turn-around times. Sorting probably would be based on specific genetic, biochemical or molecular markers and should be adaptable to increased levels of automation. In this way, the biotechnology eventually could become an interactive part of research cruises. This should provide many spin-off benefits to shore-based research efforts as well.

We do not expect to design final solutions to these goals as an outcome of this workshop; this would be a tall order under the best of circumstances. Rather, the workshop objective is to summarize results by drafting a "call for proposals" with supporting comments for each of the two goals, that is, development of techniques for 1) physiological measurements and 2) rapid sample sorting (and genetic identification). These drafts will reflect the expertise of this group of scientists, your deliberations here at this workshop, and your consensus of what is feasible, what is desirable, and what is the most progressive way to proceed in terms of research. GLOBEC is strongly committed to supporting the necessary biotechnology development and biotech/oceanographic collaborations and seeks the guidance of this group in developing its strategies. If our recommendations are accepted, biotechnology RFP's will be issued through the proper agencies in the near future, and we expect that many individuals from this group will be applying for portions

of the available funds.

We have asked two individuals from each group, one each from the oceanography and the biochemistry/molecular biology "sides", to lead the discussions and prepare a brief report of results from each working group. The results should include highlights from the discussions and a draft of how you would want to see an RFP on the topic focused. Leaders are free to solicit additional help as they see fit for note-taking, report-writing, etc. The end product will be a report to the GLOBEC Steering Committee for their action.

Working Group I-Physiological Condition and Rates

Jose Torres & George Somero Co-Discussion Leaders

Charlie Miller	Gary Kleppel	Elizabeth Clarke
Roger Mann	Michelle Wood	Gail Theilacker
Steve Hand	Bruce Sidell	

The assessment of physiological condition, age and growth and developmental rates of individual zooplankters, and the variability of these parameters, is an important part of understanding the influences of physical processes on population dynamics. This working group is charged with identifying techniques, which are currently available or which show promise for rapid development, to assess the age, physiological rates and condition of zooplankters. In order to guide the discussion somewhat, we've posed several questions below that should be answered regarding the identified methods.

1. What biochemical/molecular techniques are available to assess physiological condition and rates in the following (or additional) categories?
   a. general performance capabilities (e.g., locomotion);
   b. morbidity/low physiological capability (e.g., point of no return);
   c. egg production rates;
   d. "diapause" state;
   e. growth rates;
   f. developmental stage;
   g. age.

2. Are there particular taxa/species which should be targeted, either because the information gathered is most important to the underlying scientific question(s), or because the methods will be particularly adaptable?

3. Are the methods adaptable to individual zooplankters, or must individuals be pooled to obtain enough tissue? How many?

4. Are the methods adaptable to ship-board use? If so, how much time is required to obtain results? If not, what degree of sample sorting and mode of preservation (e.g., freezing, ethanol, etc.) are suitable or unsuitable?

5. What equipment needed? Specialized or off the shelf? How expensive? How time consuming is the process? Can the methods be mechanized or automated?

6. Is laboratory development of the method necessary, and if so, how much spin-up time is

anticipated before field samples can be meaningfully analyzed?

7. How easy/difficult will it be to train technical personnel to use the method routinely?

Working Group Il-Population Genetics and Genetic Markers

Michael Lynch & Dennis Hedgecock- Co-Discussion Leaders

Mark Huntley	Peter Ortner	Dan Morse
Jason Hofman	Diane Stoecker	Doug Crawford
Dave Hillis	Steven Fain	Kelly Thomas

An important part of understanding the influences of physical processes on population dynamics is knowing what species and what relative abundances of species are present at various points in time and space, and what physical factors influence gene flow. Traditional (e.g., morphological) methods have shown to be inadequate in resolving some particular species identification problems. Also, traditional batch preservation of plankton tows, with subsequent sorting and enumeration are tedious and time consuming. Typically many months transpire before biological oceanographers can match their data to the corresponding physical data that were collected and examined in real time. One ultimate, perhaps presently unrealistic, goal is to enable biologists to collect real time data on distribution, abundances, and species/genetic composition of zooplankters. The charge of this working group is to assess the applicability of recent developments in molecular biology and genetics to these problems and to determine the feasibility of achieving the above stated goals. In order to guide the discussion somewhat, we've posed several questions below that should be answered regarding the identified methods.

1. What biochemical/molecular techniques are available to define genetic structure in zooplankton populations, and to quantitatively assess species composition.

Questions 2-7 as for Group I

Working Group Ill-Feeding Rates and Dietary Components

Gary Kleppel & Steve Hand- Co-Discussion Leaders

Elizabeth Clarke	Gail Theilacker	Mark Huntley
Diane Stoecker	José Torres	Steven Fain
Dave Hillis	Kelly Thomas	

Feeding rates and composition of the diets of organisms are two variables that might change over moderate spatial scales or might be quickly altered by physical forcing. These, in turn, probably affect condition, growth, egg production and other important aspects of individual physiology. To understand linkages between physical forcing and population dynamics requires advances in our ability to assess feeding and diet. In order to guide the discussion somewhat, we've posed several questions below that should be answered regarding the identified methods. They follow the same themes used on Day 1.

1. What molecular/biochemical techniques would enable rapid and sensitive assessments of feeding rates? What about quantifying dietary composition?

Questions 2-7 as before.

Working Group IV-Sampling

Peter Ortner & Bruce Sidell- Co-Discussion Leaders

Roger Mann	Michelle Wood	Michael Lynch
Dan Morse	Jason Hofman	George Somero
Doug Crawford	Dennis Hedgecock	Charlie Miller

It is imperative that the molecular/biochemical techniques that we have discussed in other groups be combined effectively with sampling methods. Sampling must be of a scale (e.g., depth resolution) and speed to satisfy field questions, must handle organisms in acceptable ways, and must deliver these organisms in a manner that can be handled by the shipboard (biotech) procedures. To resolve the necessary spatial and temporal scales of interest to GLOBEC, all of this will have to be done often, probably several days running at a time. New procedures may have to be invented to make the linkage feasible. There are individuals in this group who can make reasonable guesses about the demands of sampling to answer typical questions of interest in oceanic ecology; the charge to the group is to consider what it would take to put the biotechnology and field sampling needs together effectively. There are at least two distinct but related needs. First, rapid identification of the plankton community (or selected parts of it: Working Group II). Second, delivery of desired individuals (e.g., species, stage) to the apparatus of physiological assessment (Working Groups I and III). Questions that might be asked are:

1. How many depths might be sampled at a single station?

2. How quickly will samples be delivered to the surface; by what methods and in what quantities should they be collected?

3. How many replicates should be run from each depth/sample for a single species for a single parameter (e.g., enzyme system)? You should consider here, and elsewhere when appropriate, the different demands of a small meroplanktonic larva, such as a bivalve; a copepod or copepodid; and a larval fish.

4. What would the transfer time be like once the sample is on deck until it is being processed by the appropriate method? Is this a problem?

5. How much space and how many people would the necessary biotechnology set-ups require to keep pace with the desired sample delivery rates, especially if 3 different taxa were being investigated? Is this doable on a ship? Can this be improved?

6. Is significant automation feasible within the next several years? Measure its potential impact in terms of a) speed and b) space and personnel requirements (questions 4 and 5).

7. What taxa and techniques would you recommend be attempted first? Why? What would it take?