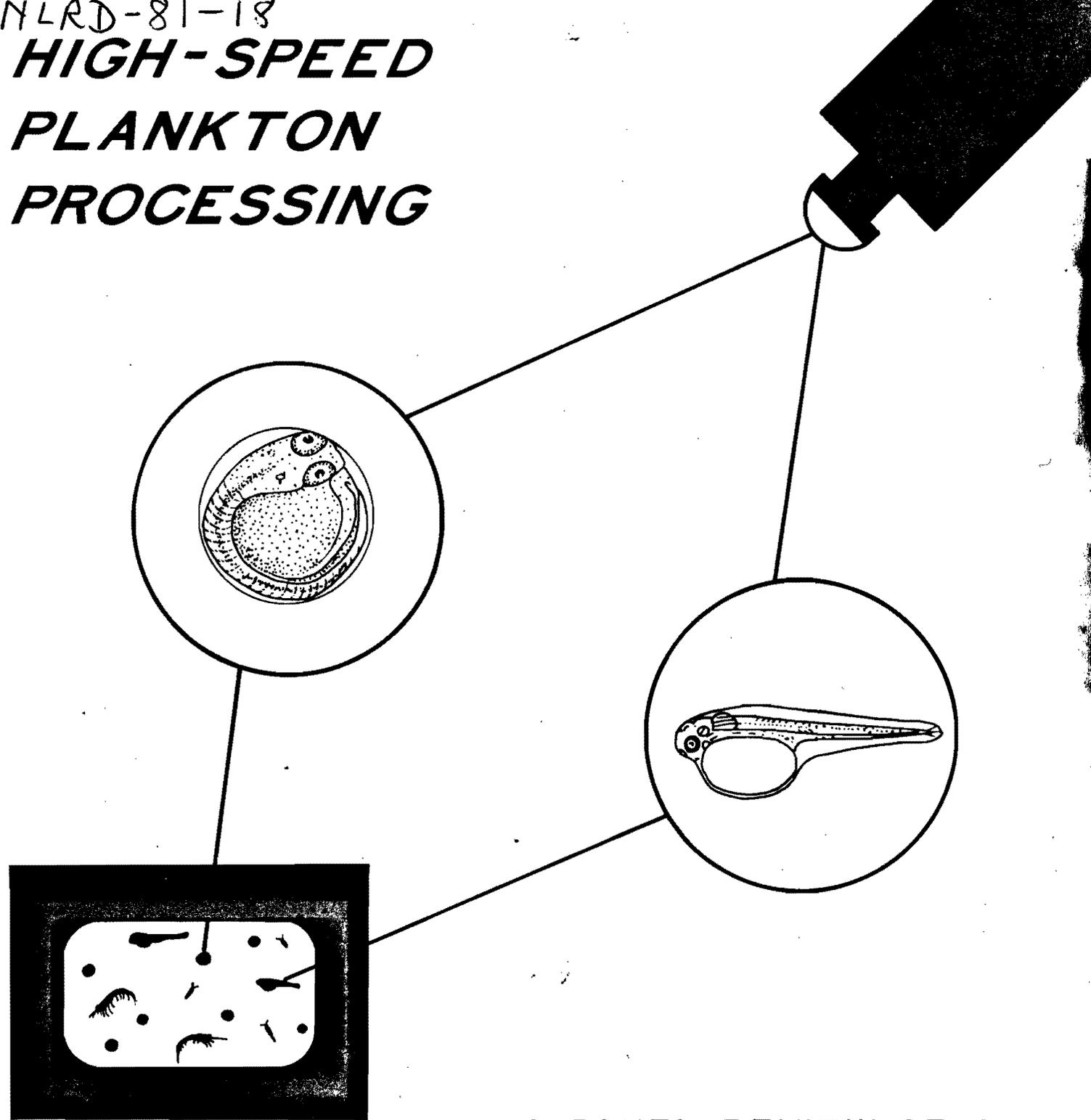


NLRD-81-18
**HIGH-SPEED
PLANKTON
PROCESSING**



**A PANEL REVIEW OF A
HIGH-SPEED PLANKTON
SORTING AND
IDENTIFICATION SYSTEM**

Department of Commerce
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NMFS
Woods Hole, Massachusetts



U.S. DEPARTMENT OF COMMERCE, NATIONAL OCEANIC & ATMOSPHERIC ADMINISTRATION
OFFICE OF TECHNICAL & ENGINEERING SERVICES
NATIONAL MARINE FISHERIES SERVICE

A Panel Review
of
The High-Speed Plankton Sorting
and Identification System

National Marine Fisheries Service
Northeast Fisheries Center
Narragansett Laboratory
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Narragansett Laboratory Reference No. 81-18

May 22, 1981

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1. Introduction

1.1 The Panel

A panel was convened by NOAA's Office of Technical and Engineering Services to review progress on the joint URI-NOAA development of a high-speed plankton sorting and identification system and offer recommendations for future directions in development of the system. The review was held 17-18 February at the National Marine Fisheries Service Laboratory, Narragansett, Rhode Island, by a panel representing expertise in pattern recognition and biological oceanography:

Panel Members

Dr. H. Ackefors	University of Stockholm, Stockholm, Sweden
Dr. G. Grice	Woods Hole Oceanographic Institution, Woods Hole, Mass.
Dr. R. Kirsch	Bureau of Standards, Washington, D. C.
Dr. T. Pavlidis	Bell Laboratories, New Jersey
Dr. M. Reeve	University of Miami, Miami, Florida

Discussants

Dr. M. Berman	University of Rhode Island Graduate School of Oceanography
Mr. L. Bivins	Office of Technical and Engineering Services NOAA, Rockville, Maryland
Dr. P. Jeffries	University of Rhode Island Graduate School of Oceanography
Mr. C. Katsinis	University of Rhode Island Department of Electrical Engineering
Mr. R. Maurer	National Marine Fisheries Service Narragansett, R. I.
Dr. A. Poularikas	University of Rhode Island Department of Electrical Engineering
Dr. K. Sherman	National Marine Fisheries Service Narragansett, R. I.

1.2 Global Problem

The growth and survival of all marine fish stocks is dependent on plankton. While in the larval stage, ecological factors act to control the year-class strength of marine fish stocks through the "match" or "mismatch" of larval fish populations with their planktonic food. A better understanding of the entire process of fish-stock recruitment, from larvae to adults is needed to improve the forecasting of abundance levels of fishery resources. The serious gaps in knowledge of the

recruitment process in the sea have been identified by the marine science community as a critical issue that needs to be addressed. The National Academy of Sciences lists the recruitment process foremost among the problems in fisheries ecology that require additional attention (Steele, 1980). The problem has also been targeted as a major issue to be addressed during the 1980s by the International Council for the Exploration of the Sea (Thurow, 1980) and the Food and Agricultural Organization of the United Nations (Austin and Gulland, 1980).

2. Constraints to Understanding Very Large Marine Ecosystems (VLE's)

2.1. Logistics Problems in Studies of VLE's

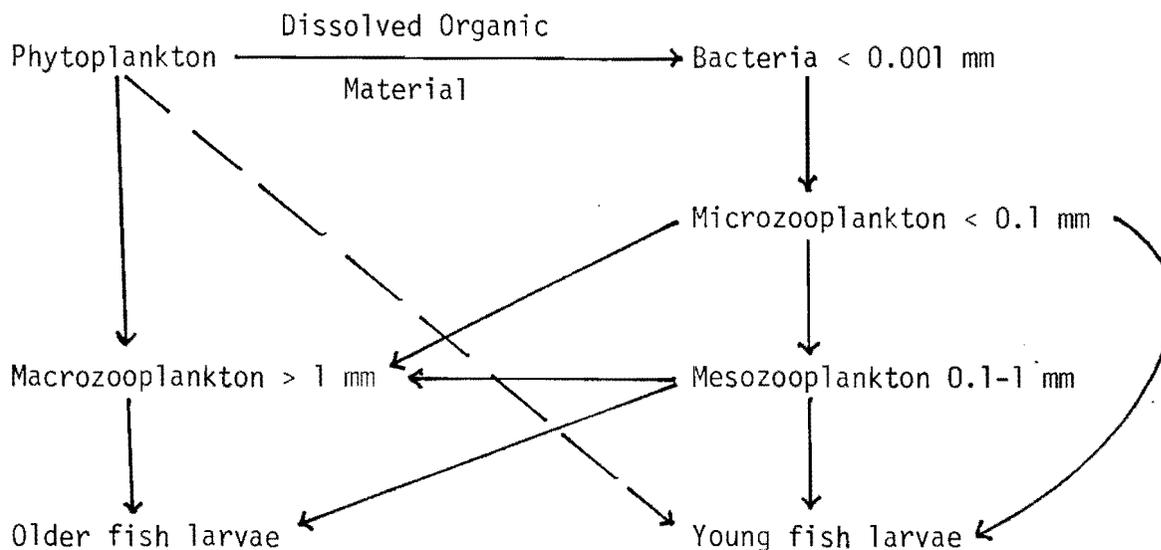
The principal constraint to understanding fish-stock recruitment is not lack of intellectual awareness of the problem, but rather the enormous logistic effort required to succeed in an all-out attack on the problem. The most critical inhibitor to getting on with the job is the large number of zooplankton samples required to examine predator-prey relations between zooplankton and ichthyoplankton over a wide-range of space and time scales. The recruitment problem exists throughout the 2.2 million square miles of the newly established marine Fisheries Management Zone (FMZ) of the United States. The National Marine Fisheries Service of NOAA is conducting studies of the recruitment process on an unprecedented scale in the Very Large Ecosystems (VLE's) extending for thousands of kilometers within the FMZ of the East Bering Sea, the Gulf of Alaska, the California Current, the Gulf of Mexico, the Florida Current, and the region of the continental shelf off the northeast coast extending from Cape Hatteras to the Nova Scotian shelf. For example on the northeast continental shelf an area of 260,000 km² is under study. In addition to zooplankton and ichthyoplankton samples collected for recruitment studies, collections are made over the shelf at 180 locations for measuring changes in key structural components of the ecosystem including primary production (¹⁴C), chlorophyll a, (phaeophytin), nutrients (NO₂, NO₃, SiO₃, NH₄, PO₄), fish, seabirds, water-column temperature, salinity, and circulation.

For each of the components measured operational efficiencies have been achieved to reduce time required for processing the samples. At the high end of the food chain, fish are examined at sea, weighed, samples of body tissue taken, and the fish remains discarded at sea. Examination of samples collected from the lower end of the food chain have been successfully automated. Chlorophyll and primary productivity samples are processed at sea. Autoanalyzers allow for nutrient analyses at sea. The oceanographers have developed sensors that provide printouts of temperature, salinity, and current flow. However, zooplankton and ichthyoplankton samples are collected with nets and preserved aboard ship for land-based sorting, identification, measurement, and population analyses. The methods used in collecting and processing of these samples are archaic, time-consuming, and costly. A full day is required to sort and identify an aliquot of 500 organisms. Several thousand samples are required annually to obtain sufficient quantitative data for: 1) monitoring

changes in the ecosystem due to natural events and increasing pollution of ocean waters, 2) estimating the size of the spawning biomass of adult fish stocks from samples of fish eggs and larvae, and 3) for improving forecasts of the abundance of resource populations based on a better understanding of the recruitment process. The time required to process zooplankton-ichthyoplankton samples from a single survey collection is of course dependent on the availability of staff for processing. A staff of approximately 18 people is required to sort and identify 4,500 samples collected annually in the MARMAP fisheries ecosystem study of NMFS off the northeast coast (Sherman, 1980). In recognition of the labor intensive nature of plankton processing, NOAA's Office of Technical and Engineering Services provided funds to the University of Rhode Island in 1978 to initiate a research project for developing a "High-Speed Plankton Processing System" in cooperation with the NMFS Laboratory located at the URI Bay Campus at Narragansett, Rhode Island. Progress in the development of the system has been reported by Jeffries and Poularikas (1980), Berman et al. (1980), and Jeffries et al. (1980).

2.2 Linkages between Size Fractions of Zooplankton in Relation to Fish Production in VLE's

The knowledge of the plankton community structure including all levels from phytoplankton to fish larvae is of utmost importance in order to understand the basis for fish production. The different size fractions and the most important species within these fractions must be sampled both from the temporal and spatial viewpoint. A brief outline of the plankton community gives us the following predator-prey patterns with two parallel food chains and their connecting links.



In order to study the different processes involved in such a system it is necessary to sample on different scales ranging from 10 m to 100 km. The macrozooplankton and fish larvae have to be sampled on the macroscale (10-100 km), and the mesozooplankton and the microzooplankton on the

microscale (10-100 m) to estimate patchiness and average biomass. The key factor regulating the successful survival of fish larvae resulting in poor, medium or strong year-classes of fish species seems to be the smaller zooplankton fractions. If the dynamic problem of sorting out the functional relationships between microzooplankton and mesozooplankton, and their relationship to the younger fish larvae in the food web, is to be accomplished it will be necessary to overcome the present inefficiencies in sample processing, as large numbers of samples will be required.

3. Panel Comments

3.1 Advances in Counting and Sizing Zooplankton

The computer-pattern-recognition system under development at URI and NMFS, Narragansett, uses preserved samples to provide counts and size frequencies of selected taxonomic groups. In this way samples can be processed at a rate apparently 4 1/2 times that of a person using traditional and time-consuming sorting procedures. Moreover, data acquired in this system readily permit standard computer analyses using statistical techniques and graphical presentations. Currently the system is being used for MARMAP samples and is being applied to other programs of trophodynamic research.

Considerable progress has been made on the identification of several common zooplankton taxa using computer implemented pattern recognition techniques. This system uses preserved specimens or silhouette photographs. Effort is currently being directed at reducing the processing time. The interfacing of a new computer configuration will permit the automated operation of zooplankton identification and enumeration including the acquisition of size frequency data. This is an exciting development from the point of view of the biologist. We urge accelerated development of this system so that a prototype instrument can be available without delay.

3.2 Preconditioning of Samples

Computer recognition of zooplankton-ichthyoplankton organisms can be enhanced through techniques of sample precondition. Continued efforts in developing preconditioning techniques, including staining and organism orientation procedures, will result in higher confidence of machine recognition. Silhouette photography is one new methodology which offers representation of live samples. This technique is also being modified to obtain representations of plankton spatial distribution in the ocean.

3.3 Taxonomic Acuity: A Hierarchical Approach

There are a series of levels of information which can be derived from preserved zooplankton samples. The simplest of these are gross counts and biomass determinations. Data on size distribution and area may be obtained using automated techniques similar to those routinely performed for phytoplankton for many years using a Coulter counter. Although instrumentation such as the Bausch and Lomb image analyzer could theoretically accomplish this for zooplankton it has not been

applied previously. Such an approach can be made more useful by separating the sample into major taxonomic groups with the aid of the human observer, and represents a second hierarchical order of information. This can be automated by computer pattern recognition technology and is already well developed toward a working prototype. A third level of detail which is likely to be of major importance in the understanding of functional interactions is a breakdown of the largest group into approximately 6 major constituents. This is not a formal attempt to separately identify the hundreds of species of copepods in the biological sense, but is limited to the separation of major functional groups which may or may not happen to be recognizable species. A typical dominant copepod community might be composed of local species of Calanus, Pseudocalanus, Acartia, Paracalanus, Oithona and Corycaeus. This level of analysis might be equivalent to categorization of a human community by nature of employment.

The most detailed level of information is that of life history stage. The analysis of population structure to this level of intricacy can only at this time be expected to be a distant goal in automated analysis. It is equivalent to a complete population census in human terms.

4. Panel Recommendations for Future Development

4.1 Status of Technology Development at URI

The original goals and objectives of the technology development effort were to conduct research into the application of image processing and pattern recognition to count, size and identify 6-12 major taxonomic groups of zooplankton and demonstrate the system's applicability to NMFS's need for high speed processing. The feasibility of using pattern recognition has been demonstrated. The general problem of recognizing major taxonomic groups has been effectively reduced by the NOAA/URI effort to distinguish among a small number of key zooplankton groups having biological and economical significance. The analysis techniques have appropriately addressed this small number of groups. The panel felt confident that techniques tested thus far can successfully be adopted in any operating automated zooplankton processing unit as long as the number of groups of interest is not significantly increased.

There exists a temptation to promote a technology of this type beyond its area of appropriate application. To avoid this difficulty, further application of these tools should be directed more by the biology than by exploratory investigation of the technology. To carry this out, biologists will have to learn more about computer processing of information pertinent to pattern classification.

The tools that are being exploited for automated zooplankton processing have been developed entirely outside the context of marine biology, e.g., the field of metalography pioneered the development of quantitative microscopy. It is encouraging that the combined NOAA/URI effort has succeeded in extending these tools into the area of quantitative biology. Further applications of such extensions to the species level

of identification would require significant innovations in computer science and engineering. Tools for the expanded goal of species identification are not presently available and a considerable increase in the level of research effort specifically directed at this problem will be required if progress is to be made in that direction.

Within the context of the original goals the group had the following comments concerning the existing NOAA/URI activity:

- Simple discriminating methods which are easy to implement have been selected among many that have been investigated.
- The importance of sample preparation to reduce processing workload has been identified.
- The URI engineering team has done a commendable job of learning marine biological problems.
- The effort has appropriately addressed work on measuring, i.e., calibrating and evaluating machine performance.
- Silhouette image presentation techniques developed by Ortner et al. (1979) have been tested and successfully used by URI to provide high resolution high contrast images.

4.2 Future Plans

Immediate plans would be to exploit the discriminating techniques already tested and leave other techniques such as syntactical approaches to expanded goals should they be adopted. The group agreed that the building blocks are available and future effort should be directed toward integration of these building blocks into an experimental unit with which to test the accuracy and throughput capability required by NMFS. This approach is wholly consistent with the existing Program Development Plan for the System.

5. References

- Austin, T. S. and J. A. Gulland. 1980. Meeting of Experts on Ocean Sciences in Relation to Living Resources (OSLR). Intergovernmental Oceanographic Commission of Unesco IOC/INF-438. 26 p.
- Berman, M. S., H. P. Jeffries, R. Maurer. 1980. A preliminary report on the use of an image analysis system for the characterization of zooplankton communities. Report OC-EE-6-80. University of Rhode Island, Kingston, RI. 13 p.
- Jeffries, H. P. and A. D. Poularikas. 1980. High speed processing of zooplankton samples by electronic image analysis. Progress Report submitted to National Oceanic and Atmospheric Administration Office of Ocean Engineering Dec. 1, 1980. Grad. School of Oceanography, University of Rhode Island, Kingston, RI.

- Jeffries, H. P., K. Sherman, R. Maurer, and C. Katsinis. 1980. Computer-processing of zooplankton samples. pp. 303-316. In V. C. Kennedy (ed.), *Estuarine Perspectives*. Academic Press, Inc.
- Ortner, P., S. R. Cumings, R. P. Aftring, and H. E. Edgerton, 1979. Silhouette photography of ocean zooplankton. *Nature*, 277:50-51.
- Sherman, K. 1980. MARMAP, a fisheries ecosystem study in the NW Atlantic: Fluctuations in ichthyoplankton-zooplankton components and their potential for impact on the system. pp. 9-37. In F. P. Diemer, F. J. Vernberg, and D. Z. Mirkes (ed.), *Advanced concepts in ocean measurements for marine biology*. Belle W. Baruch Institute for Marine Biology and Coastal Research. University of South Carolina Press.
- Steele, J. (Chairman). 1980. Report on Fisheries ecology, some constraints that impede advances in our understanding. National Academy of Sciences, Wash., D. C. 16 p.
- Thurow, F. 1980. Evaluation of the work of the Consultative Committee (C.C.) 1978-80 and some suggestions for future changes in procedures. *Cons. int. Explor. Mer* C.M.1980/A:3.