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WORKSHOP on Closed System Ecology

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WORKSHOP ON CLOSED SYSTEM ECOLOGY

Summary Report

July 15, 1982

NASA
Jet Propulsion Laboratory
California Institute of Technology
Pasadena, California
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ABSTRACT

Self-maintaining laboratory-scale ecological systems completely isolated from exchanges of matter with external systems have now been demonstrated. Far from being mere curiosities, these new research tools are expected to become unique resources for understanding: (1) global ecological material and energy balances, (2) the dynamics of stability and instability in ecosystems, (3) the effects of man-made substances and structures on ecosystems, and (4) the precise requirements for dynamic control of Controlled Ecology (human) Life Support Systems (CELSS).

Very likely, a wide variety of materially closed ecosystems is possible. The most urgent challenge now is to find non-invasive methods for accurately monitoring the thermodynamics, chemodynamics, and biodynamics of closed ecosystems and to perform the key experiments that will develop a science of closed ecology.

This report is a summary of the Workshop on Closed System Ecology held at the California Institute of Technology on January 18-22, 1982, the Workshop was arranged by the Jet Propulsion Laboratory, and was sponsored by the National Aeronautics and Space Administration.
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INTRODUCTION

The term, "materially closed ecosystem," frequently shortened to closed ecosystem (CES), is applied to biological systems that contain a variety of plant, animal, and microbiological species which persist for long time periods (years) within and upon gas, liquid, and solid substrates even though they are isolated from exchanges of matter with other systems. Driven primarily by photosynthetically active region (PAR) radiant energy, such systems appear to recycle the chemicals necessary for the maintenance of the life they contain. Only four types of materially closed ecosystems are definitely known to have persisted for years so far: Those of Clair Folsome (University of Hawaii), Bassett Maguire, Jr. (University of Texas), Joe A. Hanson (Jet Propulsion Laboratory), and the Earth itself.

Until the independent results of Folsome, Hanson, and Maguire were recognized quite recently, persistent materially closed ecosystems were generally considered improbable at best. But, with the recognition that a number of significantly different closed ecosystems had survived for years, the possibility of innovative research in closed system ecology demanded acknowledgment. This acknowledgment was afforded by the Life Sciences Division of the National Aeronautics and Space Administration's (NASA) Office of Space Sciences (OSS, now OSSA) when it sponsored an invitational workshop on Closed Ecosystem Research at the California Institute of Technology, January 18-22, 1982. Participants in the planning and conduct of this Workshop were:

Dr. Melvin M. Averner
Complex Systems Research Center, University of New Hampshire

Dr. Daniel B. Botkin
Chairman, Environmental Studies, University of California at Santa Barbara

Dr. James H. Bredt
NASA/Life Sciences Division

Dr. Clair E. Folsome
Professor of Microbiology, University of Hawaii

Mr. Joe A. Hanson
Jet Propulsion Laboratory, Pasadena, California

Dr. Bassett Maguire, Jr.
Professor of Zoology, University of Texas

Dr. Harold J. Morowitz
Professor of Molecular Biophysics and Biochemistry, Yale University

Dr. Lawrence B. Slobodkin
Professor, Department of Ecology and Evolution, State University of New York

Dr. Frieda B. Taub
Professor, School of Fisheries, University of Washington

Dr. Mark V. Wilson
Environmental Studies, University of California at Santa Barbara
WORKSHOP OBJECTIVES

The Workshop on Materially Closed Ecosystem Research had two basic objectives:

1) To assess the value of materially closed ecosystems as resources for basic and applied ecological science.

2) To recommend initial closed ecology research priorities.

THE WORKSHOP PROCESS

Both the planning and conduct of the Workshop were cooperative. Two reviews of the agenda were performed prior to the Workshop and through this process all participants provided inputs to the Workshop's form and substance. The final agenda is given below.

January 18  Descriptions of CES work using physical examples by Folsome, Hanson, Maguire, and Taub, followed by discussion of agenda details.

January 19 a.m.  Material Closure in Ecological Research, chaired by Basset Maguire, Jr.

p.m.  Toward Defining Materially Closed Ecosystem Homeostasis, chaired by Clair Folsome.

January 20 a.m.  Theoretical Bases for Closed Ecosystem Research, chaired by Harold Morowitz.

p.m.  Defining Closed Ecosystem Monitoring Problems, chaired by Joe Hanson.

January 21 a.m.  Technological Options for Monitoring Closed Ecosystems, chaired by Joe Hanson.

p.m.  Recommendations for Closed Ecosystem Research Priorities.

January 22  Review, Summary and Conclusions, presented to James Breit.

Although each of the participants did give informal presentations on work in progress, the majority of the time was spent in give-and-take examinations of the issues at hand. As this Workshop was the first time that independent CES experimentalists and theorists had come together for intensive, organized examination of closed ecology research, the level of cross-fertilization was high, and a number of significant new insights emerged. Descriptions by JPL specialists of modern and developmental technologies having possible applications for CES monitoring added further substance.

SUMMARY OF WORKSHOP RESULTS

THE VALUE OF CES RESEARCH

With respect to the first objective of the Workshop, the participants reached unanimous agreement that:

1) It may be much easier to achieve persistent materially closed ecosystems than had been believed in the past.

2) CES research promises to become a significant resource for the resolution of global ecology problems which have thus far been experimentally inaccessible because

(a) Global parameters of whole ecosystems can be monitored under controlled, replicable conditions.

(b) Boundary conditions such as chemical, biological, and physical starting values and post closure energy fluxes can be varied experimentally.

(c) Global energetics of whole ecosystems can be measured and experimentally manipulated.

3) For the reasons just cited, closed ecology research may very well prove an invaluable resource for predicting the probable ecological consequences of anthropogenic materials on regional ecosystems.

4) CES research is an empirical resource for validating and calibrating general and special mathematical models of ecosystem structure dynamics and stability characteristics.

5) CES research may become pivotal in discovering the basic laws to which Controlled Ecology Life Support Systems (CELSS) must conform and in establishing the foundation for a CELSS control theory.

*A "global parameter" in this context, is one that is representative of the entire system, e.g., O2 or CO2.
CLOSED ECOLOGY RESEARCH ISSUES AND PRIORITIES

These results were divided by the participants into two categories: (1) key research issues, and (2) research priorities.

Key Closed Ecology Research Issues

- What are the parameters which constitute the best indicators of system state? For example,
  
  1. Index of biological activity (energy storage and release rates) as measured by microcalorimetry or infrared emittance.
  2. Index of stability as measured by temporal energetic, chemical, and biological patterns.
  3. Species list persistence, direction of change, rate of change, etc.

- If one materially closes any heretofore untested but logical assemblage of autotrophic and heterotrophic organisms, what is the probability that it will maintain itself?

- What are the minimum gas, liquid, and solid volumes or masses per unit biomass that will permit CESs to persist? These values may be expected to vary significantly as functions of species lists, physical characteristics, chemical composition of the inorganic phases, and energetic environments of the systems.

- What are the minimum sets of species required for viability of CESs; what are the ecological characteristics of the species which make up these sets, and are there critical patterns of interaction among such species sets?

- What are the experimental methods that will permit us to monitor the important biological, chemical, and physical parameters of CESs with adequate repetition rates and without unacceptable violations of closure?

- What are the earliest, most sensitive and most accurate indicators of impending instability in closed ecosystems?

Recommended Closed Ecology Research Priorities

- Disseminate present knowledge concerning the protocols for closing and maintaining closed ecosystems and thereby encourage a large number of independent investigators to attempt a wide variety of CESs and to report their results. Dr. Slobodkin volunteered to initiate this action.

- In conjunction with the above, established CES investigators should expand the variety of CESs employed in their work.

- Establish a cooperative program among established CES investigators for the development of non-invasive CES monitoring techniques and protocols for their employment. This thrust must be accompanied by the establishment of a single highly qualified central facility dedicated to developing applications of advanced approaches such as ultrasensitive microcalorimetry, Mass Spectrometry-Electro-Optical Ion Detection (MS-EOID), Nuclear Magnetic Resonance (NMR), etc.

- As the availability of monitoring methods permits, perform extensive studies on the energetic and material fluxes within selected CESs. This should proceed together with the development of the necessary methods. At present, it appears that O₂, CO₂, energy charge, and microcalorimetry measurements will be among the parameters of first choice.

- Expand research into the behavior of representative CESs as a function of varying such boundary conditions as initial gas, liquid, and solid volumes or masses per unit biomass, photosynthetically active region (PAR) irradiation levels and times, and degree of closure.

- Initiate one or more mathematical modeling activities that will investigate the general dynamic characteristics of CESs and that will employ closed ecology research in the usual theoretical/experimental verification procedure.

- Initiate ongoing analytical efforts for the purpose of further specifying the unique contributions of closed ecology research to global ecology, CELSS, the ecological consequences of human actions, and basic ecological research.

PERSPECTIVES OF INDIVIDUAL WORKSHOP PARTICIPANTS

Except for the organizer (Joe Hanson), each of the participants has provided short summaries of his or her individual observations on the Workshop process and on closed ecology research. These are given here in alphabetical order.
The NASA Workshop on materially closed ecosystems was productive and stimulating. During the Workshop it became clear that microcosms can be a practical tool for ecological research. Demonstrations by Hanson, Folsome, and Maguire showed that many materially closed microcosms exhibit surprising persistence, although little is known about which properties of these ecosystems permit persistence after closure.

Several research topics were identified that can be effectively investigated by using materially closed ecosystems. Closure to chemical energy exchange permits complete system energy budgeting; a necessary step in tracing and understanding ecosystem dynamics. The constraints of closure and the relative simplicity of microcosms may allow the construction of powerful dynamic models useful in tying biochemical generalities into ecological theory. In addition, the relatively low cost of microcosms makes high replication levels practical; an unusual opportunity in ecological research.

"Several research topics were identified that can be effectively investigated by using materially closed ecosystems. Closure to chemical energy exchange permits complete system energy budgeting; a necessary step in tracing and understanding ecosystem dynamics. The constraints of closure and the relative simplicity of microcosms may allow the construction of powerful dynamic models useful in tying biochemical generalities into ecological theory. In addition, the relatively low cost of microcosms makes high replication levels practical; an unusual opportunity in ecological research.

The number of closed microcosms now being studied is small. A broader data base is necessary for a fuller understanding of closed microcosms. Microcosms from a wide range of natural ecosystems should be initiated, and should include different taxonomic and trophic structures, substrate types (e.g., carbonate and non-carbonate sand), and biomass:atmosphere:water:solid ratios. Additional synthetic microcosms should also be attempted. Special monitoring techniques should continue to be developed, especially for the measurement of energy flow and biomass, and for taxonomic identifications.

Theory in closed microcosm research should proceed on several fronts. The constraints inherent in closure, including mass balance, energy capture, and trophic efficiency, should be explored. Simulation models should be constructed to prod our ideas about how microcosms function. For example, at Santa Barbara we are developing a dynamic plankton simulation model, based on Taub's standard microcosm biota, in which the physiological attributes of feeding, reproduction and behavior drive the ecosystem.
Through models such as this one, general ecosystem characteristics of microcosms can be delineated and more persistent and resilient microcosms designed.

"Theory and observations on existing microcosms will suggest experimental manipulation appropriate for the study of the internal control processes of closed ecosystems. Useful experiments would include removing biomass from the microcosms at regular intervals as a simulation of harvesting, and subjecting microcosms to perturbation, such as high light or no light."

Dr. Clair Folsome, Exobiology Laboratory, University of Hawaii

"The recent Workshop on materially closed ecosystems was the most intellectually stimulating and profitable meeting I’ve attended. Within the short span of a week there developed a surprising agreement that small materially closed ecosystems have the potential to serve as experimental tools which effectively and perhaps uniquely can answer with precision a variety of fundamental biological questions. In particular, this approach seems to provide the only quantitative link between thermal physics and biology, and promises to be as useful a tool to the field of ecology as E. coli is to the field of molecular genetics. I list below those major questions which can be approached using materially closed ecosystems.

"Global variables of an ecology can be quantified. Primary production and consumption can be measured with a precision, simplicity and speed unobtainable by any other means. This implies that numbers defining the "aliveness" and the stability of many kinds of ecologies can be obtained. Experiments affecting these variables obviously can not be performed on the terrestrial global level. Such microecology experiments promise to yield relatively simple and temporally foreshortened information. It should be possible to test by direct experiment the effects of environmental perturbations upon various materially closed ecosystems and to obtain precise data upon primary production capabilities and stability.

"Before this meeting, materially closed ecosystems were mainly considered a laboratory curiosity; afterwards, I think all participants were convinced that these ecosystems represent a fundamentally new and most promising experimental tool.

"To my way of thinking, our leading research priority now is to make experimental determinations of primary production, consumption and apparent quantum efficiency for a variety of different (marine, fresh water, etc.) materially closed ecosystems. To date, we have only made sufficient measurements to be assured that it is possible to do so. Current data do show that closed ecosystems appear to have primary productivity and quantum efficiencies similar to terrestrial values. To measure with precision and speed fundamental values of model ecologies would provide the foundations for a research tool of general applicability. This tool would link biology with thermal physics and would serve as an experimental vehicle for future experiments which are impossible if limited to our terrestrial ecology.

"Note that these measurements use standard laboratory methods (gas chromatography, calorimetry, isotope labeling) which have been successfully applied to materially closed ecosystems. It is known that these data can be obtained and it is only a matter of finding modest funding support to begin such a study of fundamentals.

"Materially closed ecosystems offer an infinity of miniature worlds which can closely model or can depart from that one world which is our terrestrial ecology. As a direct consequence, the variety of research topics which can be based upon the concept of materially closed systems is enormous and of potential value to almost any field of science, pure and applied. Knowledge of these key system parameters which define system "aliveness", stability, productivity, and activity, is pivotal to all these applications."

Dr. Bassett Maguire, Jr., Department of Zoology, University of Texas, Austin

"The closed ecosystem Workshop meeting held January 18-22, 1982, at JPL, was exceedingly valuable from the standpoint of progress in the investigation of the ecology of closed ecosystems. Most valuable of all was the interaction which occurred within the small
group mixture of experimentalists and theoreticians; the short but effective discussion of state of the art of chemical- and activity-level analysis was also helpful and stimulating.

"In addition to the above, the opportunity for interaction between Folsome and Maguire, the only scientists currently making simultaneous biological and chemical observations of closed ecosystems, was especially valuable. The similarity of some of the responses of their different systems suggests that similar ecological processes may be occurring in the rather dissimilar systems; those instances in which the response was different provide important suggestions concerning the effects of the different chemical and biological characteristics of the different systems. These similarities and differences of response, especially when taken together, provide for considerable insight into the mechanisms which operate in the closed ecosystems and make possible a considerable improvement in the design of the next set of experimental questions which need to be asked concerning their dynamics.

"From the current experimental work of Folsome, Hanson, and Maguire, it is clear that closed ecosystems, in which the biota is derived from the larger co-evolved communities of the world, not only can persist for considerable time, but also have dynamics — the study of which is important to the development of ecological theory, to our better understanding of global ecology, and to the more practical needs of NASA to develop CELSS. It is becoming clear (and this was reinforced by the IPL meeting) that the use of closed ecosystems as tools to study ecological dynamics provides for important and effective approaches, some of which are not otherwise available, to some of the more difficult ecological problems.

The kinds of support which will be most valuable towards the increase of our understanding of the dynamics of closed ecosystems (and the development of understanding of the larger world) is an integrated mix of the following:

a. Support of individual, well conceived research projects on closed ecosystems which are both aquatic and terrestrial (initially separate, later together).

b. Support for substantial interaction between those who are active experimentalists. In some instances work trips to each other's labs would be most valuable in providing for the best interaction and for the best development of cooperative and interlinked research projects. (Maguire working in Folsome's lab for 2 to 3 weeks, and vice-versa, for example.)

c. Support for a series of work meetings of experimentalists mixed with theoreticians (similar to the recent IPL meeting).

d. Support for the use of already developed but state-of-the-art instrumentation which permits analysis of state or change within experimentally closed ecosystems (especially that which requires no breach of closure).

e. Support for development of some kinds of instruments which appear to have special promise and importance in closed ecosystem analysis.

f. Support for continued development of the theory of closed ecosystems (and thereby of theory of the larger world of which they are models).

"In some respects, most important of all with regard to support of closed system research (which is not yet recognized by the general scientific community and which is relatively long term in nature), is the need for continuity of funding for an integrated approach such has been briefly outlined above."

Dr. Harold Morowitz, Professor of Molecular Biophysics and Biochemistry, Yale University

"This meeting brought together for the first time experimentalists who had achieved long-term closed systems, theorists who studied the importance of closed system work in ecological theory, and instrumentation specialists who possess a wide knowledge on non-destructive, non-invasive measuring techniques. The meeting was very valuable in establishing that the field of closed system ecology exists; that is, the experimental results make it clear that such systems can be achieved with relative ease. We no longer need doubt the possibility of a laboratory centered system ecology; it is rather a matter of deciding what experiments are to be done."
"From a theoretical point of view, closed system ecology opens the way to certain global variables--which characterize the system. Three seem most important:

a. Total Energy. In a thermodynamic sense, energy in and out can be measured by radiometry and calorimetry so that complete energy bookkeeping is possible.

b. Energy Charge. External NMR measurements can determine, ATP, ADP, AMP, and P, thus allowing determination of a variety of ratios of importance in ecological considerations.

c. Species Lists. By setting up replicate systems, a complete initial and final species list can be determined by destructive procedures on some aliquots.

"Closed system ecology really opens up a whole new field of biological research. It should be of great value in:

a. Planning closed system space missions.

b. Understanding open system ecology (in the same way that closed system thermodynamics aids in the understanding of more general systems).

c. Partially closed agricultural systems.

"It should be stressed that this is a genuinely innovative approach to biology and it is therefore difficult to predict all the ramifications in basic understanding."

"First, I suggest that a much broader spectrum of trials should be done. How many classes, genera, and species of ecological assemblages will maintain themselves under conditions of material closure? Another critical question has to do with final states. The evidence we heard and saw suggested trends toward decreases in diversity and numbers of the eucaryotes and increases in procaryotes following closure. Will the final states of all material closure be dominated by blue-green algae or is there a wide spectrum of final states possible? Finally, the Workshop was effective in disclosing that there are numerous non-invasive technologies available or under development that may be valuable for monitoring the thermodynamics, chemodynamics, and biodynamics of materially closed ecosystems. These technologies must be explored and evaluated as a prerequisite to extensive closed ecosystem research."

Dr. Frieda Taub, School of Fisheries, University of Washington

'The 'Materially Closed Ecosystem Research' meeting was certainly an eye-opener. Each of us thought that our own ability to sustain life in 'closed' systems was a unique success. Together, we represented four researchers, each of whom has numerous living systems in some stage of multi-year closure. Given our combined experience, it appears that many designs will work; two of the systems were freshwater, two were marine. Maguire's and Folsome's systems were derived from natural communities, mine were synthesized from laboratory reared organisms, and Hanson's were a combination. Systems with absolute closure, such as Hanson's and Folsome's, answered the skeptics who feel that success in less rigorously sealed systems, such as mine, must be due to leakage. Folsome's and Hanson's systems allow sampling and an approach to complete closure.

'The reduction in species diversity documented by Maguire is not unique to closed systems, and does not indicate that they will eventually die out (in my
opinion). Species diversity of the algal community is reduced in my open microcosms during the 63-day standard run, in spite of weekly reinoculations. The reduction in species diversity seems to be related to nutrient depletion and competition among the algae for the recycled nutrients. In spite of the reduction in the number of algal species in my open systems, the algal community remains functional. The ability of my microcosms to support living Daphnia populations in sealed systems for over a year, even after the reduction in algal diversity, suggests that systems are not losing essential functional abilities if they simplify their taxonomic diversity over time. Nutrient recycling and continued photosynthesis must be occurring to provide a continuing source of food and oxygen. Given that many ecosystem processes are carried on by microorganisms, and that none of us are measuring microbial taxonomy to the level of species or genotype, we really don't have any information on loss of diversity. This should be a research topic, not an assumed fact.

"The next immediate task should be to document these and similar studies so that the greater scientific community can be made aware that materially closed systems are possible. Of course, much of the problem is concerned with the Catch-22 that most of the work on materially closed systems has not been supported as research, and many of the studies are not ready for full publication as mature research efforts. Without publications, the work is not taken seriously enough to obtain research funding. Hopefully, those of us who have early and moderately mature studies will have an opportunity to bring our studies to a higher level of maturity and through to publication in widely distributed scientific journals. With the publication of those researches, it would then be appropriate to open research funding to the general scientific community with less of a risk of each new investigator reinventing all of our earlier studies, many of which have extended over 15 years.

"It will also be important to identify important ecological problems that can best be approached by materially closed ecological systems. In my opinion, these systems are the most appropriate way to study: (1) the potential biological control of oxygen concentration in the atmosphere; (2) the potential biological control of nitrogen fixation and denitrification; (3) microbial community development and evolution with and without the introduction of new genetic material; and (4) the minimal biological complexity (in terms of trophic levels, taxonomic complexity on each trophic level, and physiological and genotype complexity within each species) that can be self-sustaining through the continued production and recycling of carbon, nitrogen, etc. All of these biological subjects can be studied along with the measurement of the thermodynamics of steady-state systems."

"My own past research has tended to examine the minimal biological system that is capable of regenerative behavior. The published studies do not include those closed to the atmosphere, but I have unpublished results on systems that included only an algal culture and a bacterial culture separated by a gas bridge. We also had an almost closed system with one species each of an alga, protozoan, and bacterium, which remained biologically active for months. As part of that study, I found that even without the protozoa and bacteria, the algal culture (Chlamydomonas) remained photosynthetically active for several months.

"It will also be important to develop instrumentation that will allow measurements to be taken on materially closed systems without destroying the material closure. The potential of measuring the heat output has intriguing possibilities."

"The Workshop was an exciting beginning. I hope that it will serve as a means to get materially closed ecosystem research beyond the anecdotal stage and into making a real scientific contribution."

SUMMARY OF WORKSHOP TOPICS

This section contains abbreviated reviews of each topic addressed by the workshop. Because of time constraints, these summaries are brief and, thereby, somewhat unjust to the individuals who discussed their work, as well as to the quality of the intellectual collaboration that characterized every session. They are offered here in the hope that they will suffice until a more thorough account can be published. Additional information on research to date can be obtained through the bibliography.

MATERIAL CLOSURE IN ECOLOGICAL RESEARCH TO DATE

Table 1, condensed from the five reviews presented early in the Workshop, attempts to illuminate, on a single page, past work and work in progress as it relates to the six major CES research issues specified by the Workshop (see Key Closed Ecology Research Issues, above).
### Table 1. Summary of Past CES Investigations

<table>
<thead>
<tr>
<th>Research Issues</th>
<th>Long-Term Material Closure (years)</th>
<th>Short-Term or Incomplete Closure</th>
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<tbody>
<tr>
<td></td>
<td>Folseme</td>
<td>Hanson</td>
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<td>Global Parameters</td>
<td>$\Delta$CO$_2$ and $\Delta$O$_2$</td>
<td>O$_2$ and CO$_2$ rate changes</td>
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<td></td>
<td>Preliminary quantum efficiency measurements</td>
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<tr>
<td>Stability</td>
<td>O$_2$ monitoring</td>
<td>Near 2 yr persistence</td>
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<td></td>
<td>Up to 15 yr persistence</td>
<td>4 yr persistence of some systems</td>
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<tr>
<td>Species Lists</td>
<td>Procaroyte/eucaryote ratios show early approach to new persistent state</td>
<td>Long-term decreases in algae following closure. Some macrophyte blooms in intern</td>
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<tr>
<td></td>
<td>Marine carbonate sand algaete</td>
<td>Large (14 mm) coralline in synthetic brackish water with a variety of algae</td>
</tr>
<tr>
<td></td>
<td>System size varied from 50 to 2000 ml</td>
<td>Varied initial H. rubra inclusion from 3 to 16 shrimp in 1000 ml systems with 860 ml synthetic brackish water at 11% salinity. No inorganic solids included; early results suggest carrying capacity of these to be 8 to 9 shrimp.</td>
</tr>
<tr>
<td></td>
<td>Systems average 10000 procaroyte/1 eucaryote. Max ratio = 1:1</td>
<td>Apparent reductions in species diversity</td>
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<tr>
<td>Lab Tools and Protocols</td>
<td>Calorimetry</td>
<td>Controlled illumination</td>
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<td>O$_2$ probes</td>
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<td>Sampling</td>
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<td></td>
<td>PAR radiometry</td>
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</table>

Early Indicators of Instability

Lack of young Diaphanosoma
It is important to recognize here that the work of Schwartskopf, et al., at NASA Ames Research Center, is designed to study control characteristics in materially closed ecosystems rather than persistence under material closure. Similarly, the work of Taub, et al., at the University of Washington has focused recently on the development of replicable controlled bioassay ecosystems rather than on material closure per se.

TOWARD THEORETICAL FOUNDATIONS FOR CLOSED ECOLOGY RESEARCH

The following perspectives are offered as initial steps toward the eventual development of a coherent theoretical foundation for closed system ecology.

Toward Defining Degrees of Closure in Ecological Research (from adiabatic closure to chain-link fences)

Harold Morowitz proposed and the group discussed the following hierarchy of closures:

Adiabatic. The system would be closed to the flows of both energy and matter. At present, long-term adiabatic closures of living systems appear uninteresting. Short-term removal of light from CESs, however, is useful as an experimental variable (Kearns and Folsome, 1982).

Material Closure. The system is, for all practical purposes, closed to the flow of all matter but is energetically open. This degree of closure was the primary focus of the Workshop.

Biological Closure with Chemical Buffering. The system is biologically and chemically closed except that it may be in contact with selected external chemical sources/sinks employed to maintain such parameters as oxygen, carbon dioxide, and pH within specified ranges of variability.

Biological Closure. The system would be biologically closed but open to uncontrolled chemical exchanges with the external universe.

Selectively Biologically Open. Such a theoretical system would be chemically open but, from viruses to larger vertebrates, entry and exit of organisms would be selective.

It was recognized that the foregoing is as yet imprecise and very likely incomplete. In particular, the issue of non-selective sampling (for example, microsampling of materially closed systems) is not yet accommodated adequately. However, we believe that this is a beginning toward a rigorous hierarchy of system closure that will become useful in ecological research.

Toward Defining the Closed System Ecology Phenomenon

From material closure to selective biological closure, autonomous and semi-autonomous ecosystems, may function for years, decades, or centuries if a viable dynamic balance is established. Such systems are characterized by immensely complex feedback networks. Terms such as "stability," "steady state," and "homeostasis" seem inadequate references to this phenomenon, both because they imply something less and because they have other more or less rigorous meanings. Moreover, not all closed ecosystems achieve "stability," "steady state," or "homeostasis." On the contrary, in CES research, failure (or instability) phenomena will prove to be as interesting and important as their alternatives.

The Workshop was unsuccessful in its attempts to coin and define a term for the CES phenomenon; however, it did contribute the following observations:

(1) Such a system theoretically can be characterized by reduced state vectors such as: (1) species list, (2) "niche spaces" (as a possible alternative to species lists), (3) chemical cycling patterns, and (4) energetic patterns.

(2) State vectors will contain endogenously derived set points around which measured values will oscillate if the endogenous control network results in a persistent ecosystem.

(3) Non-catastrophic CES perturbations will result in damped oscillations leading to a return of state vectors to near the pre-perturbation set point mean values while more extreme perturbations will result in significant state vector set point mean value changes.

Toward a General Definition of Closed System Ecology

The following observations are offered in the belief that at least some of them will prove to be useful stepping stones toward a coherent definition of a science of closed system ecology.

Materially Closed Ecosystem Macrostructure. Bounded physically by barriers which allow flows of PAR radiation and heat but are closed to matter,
materially closed ecosystems are made of gases, liquids, and solids in and upon which function multispecific biological communities.

**General Biostructure.** Experimental biological communities could theoretically be made up entirely of procaryotes. Typically, however, they will be combinations of procaryotes and eucaryotes, and even purely eucaryotic systems may be conceivable. In all cases, however, autotrophic and heterotrophic functions must be performed such that the critical material cycling pathways are maintained at mutually complementary rates, otherwise state vectors will shift.

**General Chemical Structure.** The chemical constituents of CESs may be expected to be similar in all critical aspects to the Earth's biosphere, although many CESs will be more simplified and their chemical ratios very likely will differ from Earth's. Essentially, all energy input will be through the chlorophyll centers, and most energy storage and transfer will be chemical. Irreversible chemical reactions, if any, will proceed slowly; if not, established state vectors will not persist.

**General Thermodynamic Structure.** Captured radiant energy in the photosynthetic wavelengths is reduced to chemical energy potential which is stored and oxidized with the concomitant release of thermal energy as well as bioactivity. The foregoing appears to be the key energetic pathway. Other energetics may become important in the development of experimental protocols. It is expected that it eventually will be possible to characterize, in juxtaposition, energetic, chemical and biological state vectors. CESs permit, for the first time, testable ecological energy balance experiments.

**General Mathematical Structure.** Given the very general, and in practice, insoluble expression:

\[
\frac{dN_i}{dt} = f(N_i, t, B)
\]

where the ith variables of some bounded system are some function of the ith variables, time and the boundary conditions, the fact of material closure may constrain the range of possible solutions to the point that a mathematical characterization of a CES could become impossible. The following expression may be a beginning:

\[
\sum \alpha_{ij} N_i = \bar{N}_i
\]

where

\[
\bar{N}_i = \text{total number of atoms of the } i\text{th type.}
\]

\[
\alpha_{ij} = \text{fraction of } i\text{th atoms in the } j\text{th compartment.}
\]

\[
\frac{\alpha_{ij}}{\alpha_{ik}} = \text{ratio of atoms or molecules in compartments,}
\]

which because of biological constraints must always fall within some known or knowable range for each type of compartment (i.e., Redfield ratios).

Therefore,

\[
C_{ijk} < \frac{\alpha_{ij}}{\alpha_{ik}} < A_{ijk}
\]

represents the constraint expression, with \(C_{ijk}\) and \(A_{ijk}\) as upper and lower bounds.

**Closed Ecosystems as Analogs.** It was the unanimous judgment of the Workshop that a science of closed ecology is certain to result in valuable insights to the significant structure and thermodynamics, chemodynamics, and biodynamics of: (1) the Earth's global ecology; (2) ecological subsystems of the global ecosystem; and (3) Controlled Ecology Life Support Systems. The global ecosystem is far too large and complex to study adequately with today's monitoring resources, and one can scarcely perform controlled, replicate experiments with the biosphere: varying the boundary conditions as one goes. Moreover, there is no present or predicted method for studying empirically the complex, globally important interactions between procaryotes and eucaryotes. Beyond that, what is and is not important to study remains a matter of debate, and the debate shows every promise of continuing until a science of closed ecology can begin to narrow down the options. By experimentally varying boundary conditions and understanding how different classes of CESs respond to internal and external perturbations, an empirically derived global model can be postulated. A model such as this should constitute sufficient grounds to justify the development of tools to monitor the model-predicted significant variables of Earth's global ecology.

With respect to ecological subsystems of the global ecology, ecosystems studied thus far have been whatever the investigators decide they should be, and this has been equally true of laboratory and field work. Microbial ecologists have examined dynamics in procaryotic communities essentially isolated from eucaryotic dynamics. Other laboratory ecologists, for the most part, have worked with eucaryotic communities either combined with undefined procaryotic communities or, sometimes, gnotobiotically. In field work, as a matter of practical necessity, the ecologist must select some very small number of parameters to monitor. The science of closed ecology, here too, can illuminate the key parameters and, thereby, guide open ecology scientists in determining how closely the systems of interest to them mimic the dynamics of CESs. If these similarities and differences can be
understood, it should pave the way toward understanding the important boundary parameters of open ecosystems.

NASA's Controlled Ecology Life-Support Systems (CELSS) Program has as its goal the development of the knowledge and technology required to maintain humans in extraterrestrial habitats for long periods of time. Its underlying rationale is that, for missions of long duration or large numbers of people, regenerative techniques including biologically based processes will be more cost effective than either storage or resupply of consumable life-support materials. Although we do not yet know adequately what a CELSS is, we recognize that, with humans as the top heterotrophs, it must perform all of the key functions of a CES. Thereby, a CELSS must somehow conform to essentially the same control laws. Thus, if we are able to discern the key feedback loops and stability characteristics of CESs, this knowledge must surely transfer directly to the design and control of CELSSs.

THE PROBLEM OF MONITORING INTERNAL PARAMETERS OF MATERIALLY CLOSED ECOSYSTEMS

Internal parameters of CES are divided here into four rather obvious categories: (1) physical; (2) chemical; (3) biological, and (4) cutting across the first three, repetition rates and data management. With respect to the first three, it must be recognized that measured values (i.e., dissolved oxygen) may be representative of the entire system; or conversely, may only be representative of some subset. Clearly, the site within a system at which a measurement is taken frequently will determine whether it is a global or local value. Moreover, a given parameter (i.e., temperature) may be a boundary condition if its value is set by the experimenter. Also, many chemical (i.e., N\textsubscript{2}/O\textsubscript{2}) and biological (i.e., species list) parameters that are boundary conditions at closure may become post-closure experimental variables.

When one recognizes the need to: (1) violate closure minimally, (2) avoid perturbing CESs energetically, (3) monitor a variety of parameters, and (4) continue experiments for months or years, it becomes apparent that the monitoring problem is far from trivial. Each of the four parametric categories is discussed briefly here.

Physical Parameters

Recognizing that closed ecology research will vary such boundary conditions as pre-closure gas, liquid, and solid volumes or masses, and shapes and composition of containers, etc., that pre-closure biomass will be varied in its make up and relative proportions, that post-closure temperature will be varied; and that post-closure intensity, spectral composition, and periodicity of input light will be varied; what physical experimental variables must be measured? The basic answer is temperature and pressure. Additionally, it may become very interesting to know infrared and shorter wavelength spectral emissions of some CESs under both illumination and dark conditions.

Chemical Parameters

The chemical parameters of interest to closed ecology that were listed by the workshop participants are:

- O\textsubscript{2}
- CO\textsubscript{2}
- Argon
- Volatile organics
- CH\textsubscript{4}
- Dissolved organics
- N\textsubscript{2}
- Redox state of transition metals
- NO\textsubscript{x}
- pH (Mn, Mg, Md)
- H\textsubscript{2}S
- ATP/GTP/ADP/AMD
- SO\textsubscript{x}
- Chlorophyll
- NH\textsubscript{3}
- Exited chlorophyll

Biological Parameters

The biological parameters which appear to be of most interest are:

- Species Lists
- Eucaryotic
- Prokaryotic
- Total Biomass
- Dead
- Living
- Prokaryote/eucaryote ratios (both mass and cell count ratios)
- Dominant autotrophs
- Dominant heterotrophs
- Reproductive States (when observable)

Repetition Rates and Data Management

At this juncture, we have yet to verify what physical, chemical, and biological parameters will be key in determining the important reduced state vectors of CESs, let alone to understand the minimum repetition rates for data taking that will be acceptable. Consequently, erring on the side of too much data is clearly advisable in the early stages. Therefore, for purposes of making a rough estimate of the magnitude of the problem, let us say that we wish to measure 12 param-
ederly, 12 times/day, over 120 replicates, for 1 year. The product is 6,307,200 data points for this single hypothetical experiment. Attempting whichever of the foregoing assumptions one wishes, it will still be difficult to reduce the product below several hundred thousand.

During the early exploratory phases of CES research, when a small number of replicates may be acceptable, the number of measurements per unit time may be small enough to handle manually. However, as closed ecology research becomes more rigorous and one's results must have high statistical confidence, automated measurements, front-end data reduction, and sophisticated automatic data indexing and retrieval must come into play. It seems worthy of mention that the electron data systems and technologies that will be evolved for CES experiments are very likely themselves to provide direct analogs for CELSS-monitoring subsystems, eventual global-monitoring systems, and eventual systems for monitoring the health of regional ecosystems.

TECHNOLOGIES FOR MONITORING CLOSED ECOSYSTEMS

As is true of much of this short report, there is insufficient space to afford any of the potential monitoring approaches more than superficial treatment. Here we attempt only to suggest what is and is not known thus far about each recognized approach with respect to what parameters it can measure, its sensitivity, its long-term reliability, the degree to which it is likely to perturb the CES it measures, and its cost and compatibility with automation (EDP).

Macroscopic Observations

For the CESs studied thus far, unmagnified visual observations provide an integrated global index of the health and relative eucaryotic living and dead biomass concentrations of the systems. For the parameters they measure, macroscopic observations are comparatively insensitive, and as reliable over the long term as is the objectivity of the observer, they usually perturb the CES not at all unless they are made during dark hours, they are moderately expensive in man-hours, and they are incompatible with EDP unless a human interface is provided.

Microscopic Observations

Except for Maquire's work, the only microscopic observations made thus far have involved microsampling of ongoing systems, preclosure analysis of biota to be included, or destructive analysis. We are confident that non intrusive designs for visual microscopic monitoring of living systems can be developed, but few have yet been tested. Adequate visual microscopy should provide a great deal of information on masses and species of procaryotic and microscopic eucaryotic communities. Its sensitivity for these parameters may be quite high if acceptable vital staining methods can be evolved. Because of possible biofouling, its long-term reliability may be a design problem and, because it may create a physical niche not present in systems not so monitored, its perturbation effects require study. Primarily, because of man-hour requirements, it will be relatively expensive and, barring very sophisticated image processing technology, it must be interfaced with EDP through a human being.

Immobilised Chemical Indicators

Some complex polymers such as Du Pont's "Nafion" can act as immobilization substrates for chemical indicators. Thus, it may be possible to calibrate imbedded color responses of chemical indicators and then imbed them in CESs such that changes can be measured photometrically. Presumably, a variety of organic and inorganic chemical parameters could be measured but the theoretical specifics have not yet been cataloged. The degree of sensitivity of the approach remains to be investigated and certainly will vary by parameter. Biofouling and chemical activity may affect its long-term reliability, and these two factors would be related to its perturbation effects. Cost and EDP compatibility are subjects to be addressed if this approach is found to be technically feasible.

Ion-Sensing Electrodes

In general, state-of-the-art electrode probes require frequent cleaning and recalibration. CES research requires probes that can be left in place for months or years and monitored automatically. So far, the only (seemingly) attractive probes found measure only O2. If long-term in-situ reliability could be achieved, the probe approach could be very attractive for a variety of inorganic measurements because it would be relatively inexpensive and highly EDP-compatible. One likely problem is that probes necessarily use up the chemical they measure and, thus, might involve some system perturbation.

Microsampling

Triple septum sampling ports suitable for employment in closed ecology research are commercially available and have been used successfully. This approach permits both global and local sampling, and permits highly sensitive and detailed biological, biomolecular, and chemical analyses as long as the analyses are performed immediately after sampling.

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the CES is tailored to accommodate microsampling, system perturbations so introduced should be insignificant. Long-term reliability should be excellent and the cost for sampling per se should be moderate. The sensitivity, cost, and EDP-compatibility would depend on the analysis approach used on the microsamples.

Gas Chromatography, Mass Spectrometry Electro-Optical Ion Detection (GCMS-EOID)

Ongoing work at the Jet Propulsion Laboratory is developing ultra-sensitivity instrumentation which may prove valuable in analyzing CES microsamples. The analysis of a CES atmosphere can be accomplished by direct injection of a small aliquot, say 100 microliters, in a GCMS/EOID system. GC and GCMS procedures for the analysis of gases and vapors are now routinely performed. The MS/EOID now extends such procedures to the pico and even to the femtogram levels. Gases in solution as well as volatile organics in the aqueous matrix can be analyzed by the well known headspace technique or the purge-and-trap approach, both using GCMS/EOID. Involatile organics such as amino acids, small peptides, carbohydrates, lipids, and other nutrients or metabolites can be sequentially extracted, derivatized, and analyzed by GCMS/EOID. Involatile, non-extractable organics and biologicals such as fiber, cell debris, and others can be analyzed by pyrolysis-GCMS/EOID.

The long-term reliability of this technique (versus the number of samples required for each measurement) is yet to be calibrated. Since the instruments involved are still developmental, the cost per measurement is likely to be high until a single combined production instrument can be dedicated to a closed ecology laboratory, making many measurements over tens of years. Since outputs are relatively easy to digitize, EDP compatibility should be quite good.

Nuclear Magnetic Resonance

Nuclear Magnetic Resonance (NMR) appears attractive for monitoring pH and, in fact, precise knowledge of pH is required for many other NMR measurements because numerous organic compounds have NMR lines that shift with pH. Some other compounds for which NMR has been demonstrated are

ATP, ADP, and inorganic phosphate. Such measurements are of particular interest to closed system ecology since they are non-invasive and indicative of the "energy charge" of a CES. NMR sensitivity in living CESs remains to be investigated, as does its cost, long-term reliability, type and degree of CES perturbation and EDP compatibility. It appears that NMR can be employed in a non-invasive mode and, because it is routinely employed on living tissue without ill effects, its prospects for employment in closed ecological science seem good.

Microcalorimetry

Microcalorimetry can provide an integrated index of biological activity for CESs. The integrated heterotrophic metabolic rates of CESs under dark conditions should closely approximate the same rates under light and, if the system is assumed to be balanced, the integrated heterotrophic metabolism rates should be balanced by the integrated photosynthetic rates during lighted periods.

Alternatively, it may be possible to build microcalorimetry devices in which a small diameter, measured light beam entering an otherwise closed Dewar could be focused to cover a Dewar-contained CES, thus providing calorimetric data during photosynthetic activity.

Initial inquiries suggest that there are likely to be no commercial calorimeters that will meet closed ecology research needs. However, it appears likely at this juncture that they can be developed with available technology. However, their sensitivity, long-term reliability, perturbation impacts on CESs, and costs remain as topics for study. Compatibility with EDP, on the other hand, should be quite good.

Other Approaches

The Workshop touched upon other possible approaches to CES monitoring such as Raman spectroscopy, laser-induced IR reflectance spectroscopy, electron spin resonance, etc. Further theoretical investigations of these advanced technologies would be advisable.
The following citations have been submitted by the Workshop participants as recommended reading for investigators interested in the emergent science of closed system ecology.


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