Self-Sustained Closed Ecological Systems

University of Washington, Seattle, WA

Authors: David Barbee

Research Team: Emily Augustein, David Barbee, Zach Frazier, Mike Guerquin, Hillary Marshal, Yuichi "Eugene" Saito, Emily Thompson

Faculty Advisor: Dr. Frieda Taub, University of Washington, School of Aquatic and Fishery Sciences

Abstract

The focus of this project is the development of closed ecological systems (CES) to sustain a population of aquatic invertebrates for more than 30 days. Permanent habitation of space will require the development of CES capable of meeting the biological needs of human inhabitants. Such a system must provide water, oxygen, and food while removing waste products and recycling the mass of the system. Ideally, the ecological system will recycle wastes into requirements and function with minimal mass exchange. Current NASA research has been directed toward the optimization (in terms of volume, energy, mass) of the components of closed systems (i.e. crop production in space). This project explores the creation of small (75ml) aquatic CES testing different chemical media, temperature, light cycles, and physical conditions. Experimental CES consisted of freshwater algae and the grazer *Daphnia magna*. Computerized image analysis techniques were developed and implemented for abundance estimation and modeling population dynamics. Rotation of the containers, to vary the direction of gravity, did not alter survival or reproduction. Increased inorganic nutrients (Nitrate and Phosphate) were tested to determine if inorganic limitations prevented additional generations. Most CES conditions resulted in the survival of *D. magna* population including the F1 (offspring of original animals) generation. Ongoing work focuses on conditions that will permit an F2 generation (Grandchildren of original animals) and beyond.

Introduction

Problem statement: Create an ecological community including all of the chemical requirements for photosynthetic organisms to produce food and oxygen and animals to use the food and oxygen and supply carbon dioxide and ammonia as nutrients for the photosynthetic organisms. The goal is to sustain the animal population for at least 30 days.

Establishing a balanced ecosystem appears to be difficult initially, but biological systems tend to self-organize. For example, as animals in an aquatic ecosystem feed, they reduce the abundance of the algae and the food shortage causes reduced animal reproduction and survival. As animal feeding is reduced, the algal population can increase. Evidence of this relationship can be seen in any nearby lake if one were to look closely. If the system was closed and the animals ate all of the algae, they would also eliminate their oxygen source, however this has not been observed to occur. Creating a biological community is painfully simple; every time one closes a bottle, a living ecosystem is created. Historically, space programs have neglected the effects of complex assemblages of organisms on space travel. We must deal with the fact that every human is a community of organisms. The evidence of this neglected viewpoint can be seen inside any space station. The effects of biology have not been addressed during design; MIR suffered repeated equipment failures from the acids produced by fungi. The offending fungi thrive on the sloughed tissues, lipid, and sweat from industrious astronauts. The glorious achievements of the 60's space race and the current ventures into space have been the biological equivalent of a fish jumping out of the water. A permanent human presence in space requires that we understand and can create a biological community to process our wastes into requirements for life in space.

The planet earth can be considered a giant space vessel with a functional ecosystem where processes occur on a vast scale in volume, time, and complexity to sustain its various astronauts (all life). Earth is essentially closed to mass exchange with its surroundings. Energy is input to the earth as sunlight, which drives physical and biological processes that power nutrient cycling. Life continues by the recycling of carbon, nitrogen, oxygen and other elements through the ecosystem in a self-sustaining manner that passes energy on until it is lost as heat. To create our own functional support systems for space exploration, testable hypotheses must be tried on an ecosystem-wide scale. The volumetric and time considerations of influencing the variables of our planet-scale ecosystem preclude any such experimentation or replication of experiments. Extreme variation can occur on a planet-wide scale that may obscure the true cause of some relationships. Thus, smaller self-sustaining ecosystems have been setup with light as the only quantifiable input, with the aim of studying the behavior of an ecosystem over time and to enable the trial of testable hypotheses. These systems would receive light energy from outside but would recycle everything needed for species survival without any external input (Beyers and Odum 1993). Small closed ecological systems allow perturbations to an ecosystem to be studied at a scale that humans can effectively observe. Using CES to study patterns and effects of elements within an ecosystem allows one to not only observe specific interactions, but also to replicate and reproduce experiments and results [Taub 1980]. Scientists can make the system as complex or as simple as needed in order to study as few or as many aspects of the ecosystem as desired [Odum 1989]. Eventually, a self-sustaining system for the support of humans can be engineered from the data gleaned from the study of closed ecological systems (referred to as CES, or microcosms).

Experimental approach: Background

A common theme in the scientific pursuit of knowledge is that one question asked usually results in many subsequent questions. In the creation of a self-sustaining CES, we addressed several resultant issues that were facets of the overall design goal. To set up a CES, we must construct a food web that cycles nutrients to sustain itself.

An aquatic ecosystem was selected as the base for study of CES dynamics. Aquatic environments facilitate matter transport in a time-scale conducive to lab study. Nutrients are transported by mixing, diffusion, and transport as a result of zooplankton movement. The celerity of matter transport in aquatic systems approximates facilitated transport that would occur in an anthropogenic system with pumps, etc. The physical characteristics of aquatic ecosystems need to be considered when constructing a self-sustaining CES. Natural aquatic systems are in constant motion, with wave action and temperature differentials driving mixing and facilitating nutrient transport over vast distances. Spacecraft are subject to non-uniform orientation of gravity. During maneuvers, the orientation of gravity may change substantially for long periods of time. Volumetric scales available in a lab scenario are limited by logistics, but what

scale is appropriate for CES study? Taking samples during experimentation is a necessity. However, small-volume (<100ml) systems can experience large variations in volume if samples of only a few milliliters are taken and replaced. To avoid taking samples from small ecosystems, we used visual observation. Animals require oxygen; their persistence indicates the presence of oxygen. The intensity of greenness in CES was used to estimate algal density. The number and size of animals is indicative of reproduction and growth. Our team conducted several studies to evaluate the physical and chemical variables of aquatic ecosystems for CES study.

A liquid's chemical composition defines the aquatic ecosystem. The media used for traditional open ecosystem studies may be inadequate to sustain a closed ecosystem. Liquid media must contain the correct balance of pH, nutrients, and salts to support a food web consisting of algae and an invertebrate grazer. T82-LoSi (Low silicate) medium is chemically defined mixture (22 elements) designed for the growth of algae. It does not contain an inorganic carbon source because most algae are grown in open containers where the atmosphere supplies carbon dioxide. The limiting nutrient for algal growth in T82-LoSi is 0.5 mM nitrate and all other elements except carbon are in excess. Therefore, in closed systems an inorganic carbon source is necessary. Kent media is a commercial mixture of salts that supplies inorganic carbon as sodium bicarbonate; it does not supply nitrate or phosphate. Thus, T82-LoSi and Kent media together could provide an adequate collection of elements to support a CES. We conducted two studies to explore the characteristics of different media in CES conditions.

The grazing invertebrate *Daphnia magna* was selected as the research animal due to availability and in-depth background knowledge of biology. *Daphnia magna* are easy to see with the unaided eye, are hardy, and reproduce quickly. *Daphnia* are used commonly in studies of toxicology and freshwater ecology. We have a large knowledge base accumulated on varied aspects of *Daphnia* ecology. *Daphnia* will graze algae and use oxygen during respiration, and excrete ammonia and carbon dioxide as byproducts. Algae in the CES occupy the role of primary producer, and will fix inorganic nutrients into lipid, protein, and carbohydrate that feed *Daphnia*.

The population of *Daphnia magna* was used for hypothesis testing to determine the significance of a treatment. *Daphnia magna* are the largest *Daphnia* species available for study, but their biology poses another problem for hypothesis testing. Counting by eye is limited by detection and availability biases to very small populations (< 20 individuals) that are frequently reached in the smallest volumes. Detection bias is introduced when large populations occur and individual *Daphnia* become difficult to track. Availability bias is introduced when clumps of algae and crowding from other individuals obstructs *Daphnia* enumeration. Since *Daphnia* population is the variable that we observe to determine significance, we developed new computer-aided methods for population enumeration to improve the resolution of our data. Computerized methods enable large populations and volumes to be accurately evaluated; where in the past populations would be measured in "dozens" now exact counts are feasible. Additionally, data gathered with a computer can be archived for future study.

Figure 1. Tissue culture flasks

Freshwater CES were housed in tissue culture flasks of various dimensions. Tissue culture flasks are useful for CES study due to their universal availability, impermeability, low procurement cost, and optical characteristics. Tissue culture flasks are optically flat and transparent, allowing systems to be visually sampled without introducing distortion from rounded surfaces. Figure 1. shows and example of CES in three different sizes of tissue culture flasks.

Experimental approach: methods

Techniques common among studies were culture setup and population enumeration. Typically, a culture flask was filled with media and two types of algae—*Ankistrodesmu*s and *Scenedesmu*s were introduced. The flask was allowed to sit open for a five-day setup period, letting unknown organics oxidize and to let algal growth begin. After the setup period, six *Daphnia* were introduced to the flasks and then the systems were sealed. CES replicates were then placed into an incubator where temperature and light cycle were controlled. Observation commenced for thirty days at intervals of at most every three days. In CES with large populations, computer-aided enumeration was utilized. Freshwater closed ecological systems for various studies followed the same initial set-up and composition: Kent water and T82-LoSi served as the liquid environment and initial source of nutrients for the algae. A small amount of media (<2ml) was added with zooplankton introduction. Figure 2. shows a typical experimental setup in an incubator. Computer enumeration techniques allowed large populations to be accurately evaluated, enabling the volume studies performed by our team. In all cases, care was taken to devise a technique that samples the entirety of a CES simultaneously with our sub-sampling. Sub-sampling would introduce a large availability bias to abundance assessment because zooplankton exhibit mild flocking behavior. Several complimentary sampling methods were used.

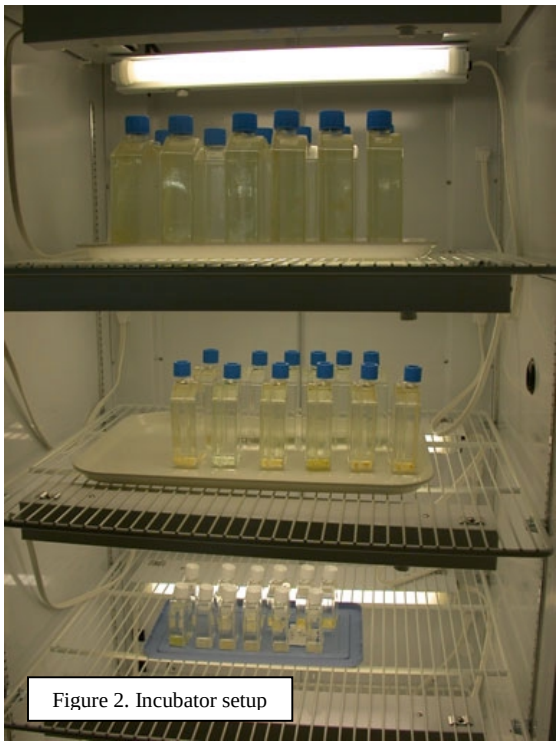


Figure 2. Incubator setup

A digital camcorder was used to observe CES replicates. Video of CES replicates was re-played to manually enumerate *Daphnia* from recorded movement.

A program to track groups of pixels was implemented in MATLAB using the image processing toolbox. The program inputs series of pre-formatted TIFF files and outputs track length, object size and velocities.

Adobe Photoshop 6.0 was used to enumerate *Daphnia* from high-resolution (3.2Mpixel CCD, image size 2048X1536 pixels) digital still images. A custom script was implemented to improve contrast between *Daphnia* and the surrounding media, so that manual enumeration could result. Figure 3. shows the input and output of the contrast enhancement script. Observers were instructed to recognize *Daphnia* by the crooked gut, defined carapace, and eyes. Aspect differences prevent automated image recognition of *Daphnia*, but Photoshop enabled large populations to be enumerated.

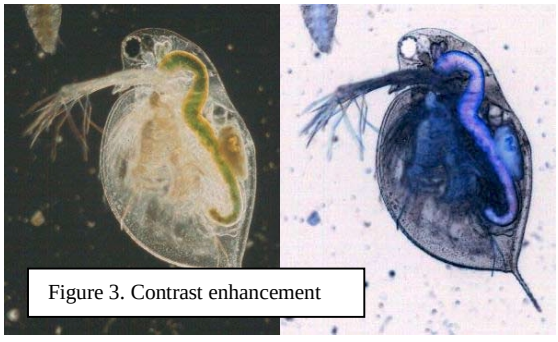


Figure 3. Contrast enhancement

Physical CES studies

Natural systems have wave action to aid in nutrient cycling. To determine if motion affects the sustainability of CES, six replicates of CES were attached to a Cell culture roller drum (New Brunswick Scientific Inc.) that completed one full revolution every five minutes. As a control, six replicates of CES and six replicates of open ecosystems were left stationary under similar light conditions.

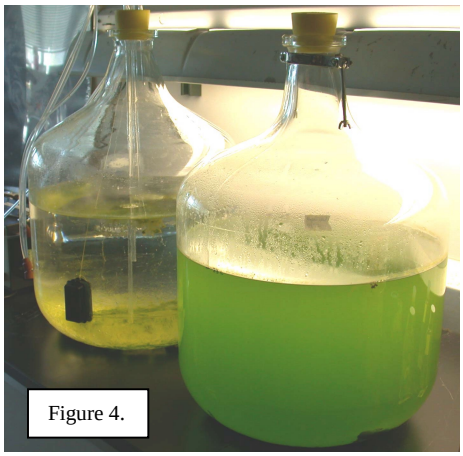


Figure 4.

Difficulty of counting large zooplankton population has restricted our previous research to using small-volume ecosystems. The small (75-ml) ecosystems that we have been using for CES study may not be the optimal scale for our work. Taking a 1-ml sample for chemical analysis represents a large mass exchange for small systems. Computer-aided enumeration has enabled large volume CES to be accurately assessed. Two studies were conducted on a range of flask sizes. One study used three (75, 250, 750-ml) volumes of tissue culture flasks with the same media to explore the relationship between volume scaling and *Daphnia* abundance. Another study setup a 33-Liter salt-water CES in a 60-Liter carboy and measured carbon dioxide and zooplankton density to determine the dynamics of a large CES. Figure 4. to the left shows two 60-Liter sized CES.

Chemical CES studies

Two studies were conducted to explore the viability of the chemically defined media that we use in CES work. A mixture of T82-LoSi and Kent water was used as the chemically defined media. The first study evaluated the sustainability of chemically defined freshwater media versus natural media. In long-term culture, *Daphnia* populations often grow better if lake water is occasionally used, suggesting that some trace elements may be lacking (Taub, 2002, personal communication). It is conceivable that synthetic media are not supplying the necessary nutrients to algae for long-term survival and reproduction of *Daphnia*. The defined media was compared to a natural media, in this case autoclaved water from Lake Washington. Open and closed ecological systems with both types of media were also compared. The second study augmented the nitrate (N) and phosphate (P) loading of defined media to explore the effects of nutrient enrichment on *Daphnia* population dynamics.

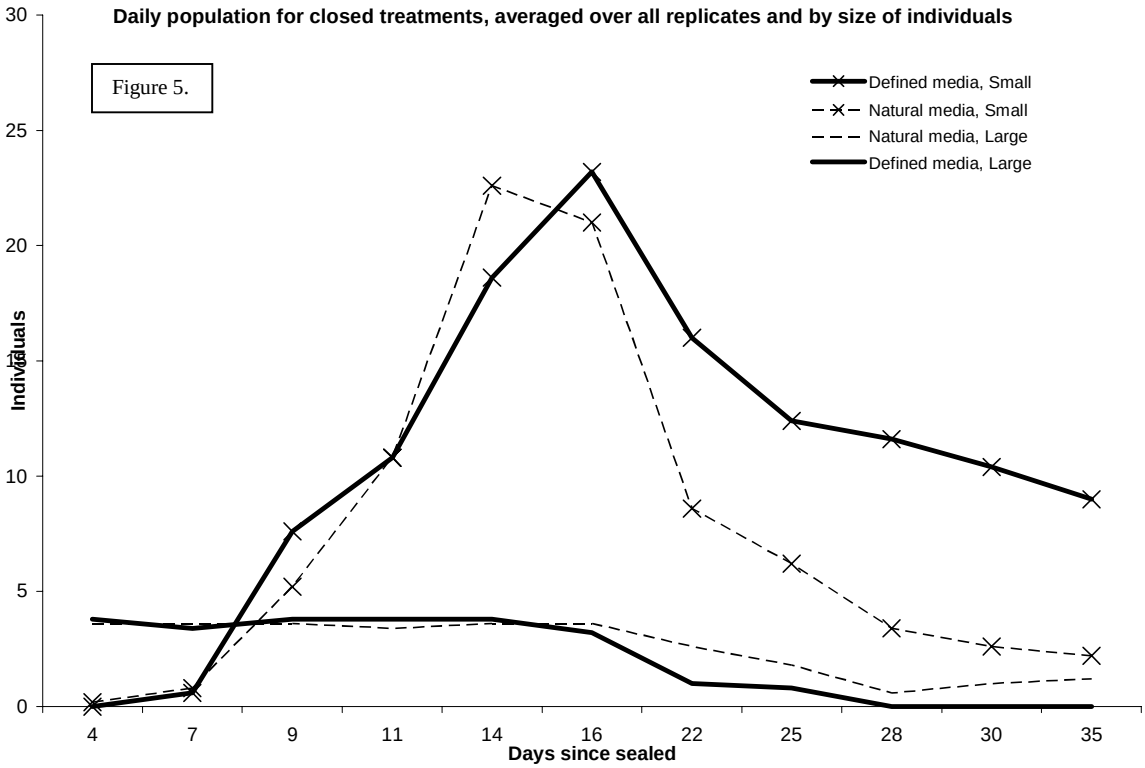
Results: Physical CES studies

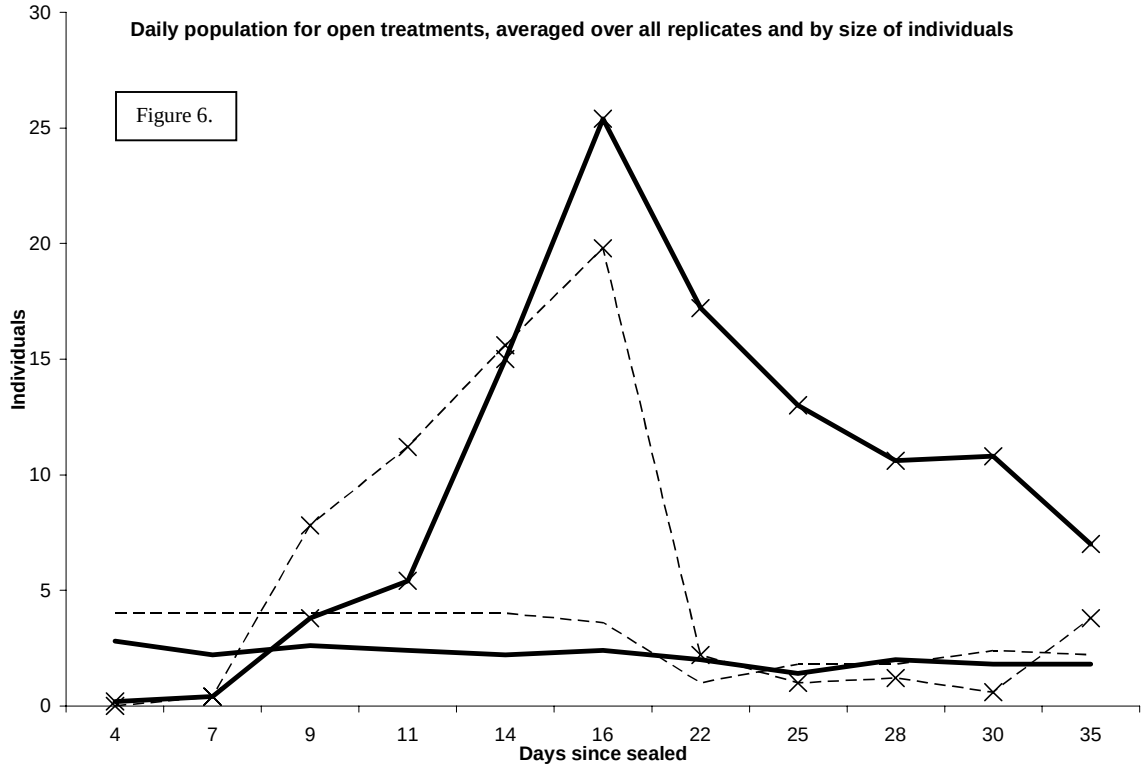
Daphnia density was inversely related to microcosm volume, while smaller volume microcosms (75-ml) experience higher extinction rates. More animals were found in larger volumes, but their density was not as high as that of smaller systems. Computer-aided enumeration allowed accurate counts of *Daphnia* population. Populations of up to 200 individuals were observed only in flask sizes of only 750-ml. Orientation was not observed to affect *Daphnia* populations.

60-liter CES studies are feasible for long-term study and allow sampling with a low percentage of total mass to be exchanged. Atmospheric carbon dioxide was observed to decline precipitously within the first week of observation. The copepod *Tigriopus califonicus* (Monk, 1941) became dominant in the salt-water CES.

Results: Chemical CES studies

T82-LoSi+Kent media supported higher *Daphnia* populations than autoclaved lake water, suggesting that it does supply necessary elements for survival. Figures 5 and 6 show population dynamics for closed and open systems of natural and defined media, averaged over six replicates and categorized by size of individual *Daphnia*. The small animals are the offspring of the initially introduced animals. It was noted that small animals peaked around day fifteen and then declined without maturing and reproducing.


Daily population for closed treatments, averaged over all replicates and by size of individuals
Figure 5.
Defined media, Small
Natural media, Small
Natural media, Large
Defined media, Large
Individuals
0
5
10
15
20
25
30
4
7
9
11
14
16
22
25
28
30
35
Days since sealed


Daily population for open treatments, averaged over all replicates and by size of individuals
Figure 6.
Individuals
0
5
10
15
20
25
30
4
7
9
11
14
16
22
25
28
30
35
Days since sealed

T82 has been used as an algal culture media and nitrogen and phosphorous than natural media. Natural media would be expected to have less algal carrying capacity compared to T82 and would support less *Daphnia*. It was observed that the large animals declined in all replicates in all treatments. Offspring (F_1 generation) of the animals introduced initially were not observed to grow to maturity and reproduce. It may be that T82-LoSi+Kent media, while more suited for CES study than natural media, still lacks necessary ingredients for the maturation of *Daphnia magna*. Open systems did not support significantly different *Daphnia* populations, suggesting that both carbon dioxide and oxygen are not limiting. Nutrient loading (N, P) increase has an inverse effect on peak *Daphnia* population, possibly due to toxic effects of nitrate at high concentrations, also indicates that N, P are not limiting in CES studies. Figure 7. shows the peak population of *Daphnia* at each nutrient loading, averaged over six replicates per treatment. Figure 8. illustrates the variability of replicates in each treatment, and show dynamics of *Daphnia* population at different nutrient loading.