Designing a Closed Ecological System to Support Animal Populations for Greater than Thirty Days: Palm Sized Ecosystems

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Abstract

The study of closed ecological systems (CES) has wide implications for study of ecological interactions both on earth and in space. Our design problem was to create closed ecological systems that were able to support animal grazer populations for greater than thirty days. CES were developed for freshwater and marine systems, in 75 mL Tissue culture flasks. Systems were studied under various influences, such as different light levels, and after the introduction nutrients into the systems. Results from saltwater systems observed under varying light levels suggest that six hours of light per day was adequate and longer light periods were not beneficial. Results from the nutrient introduction experiment indicate that nitrogen was a limiting factor in the health and survival of Daphnia in freshwater CES, but phosphorus was not.

Design problem

To design closed ecological systems that sustain themselves for thirty days or greater.

Introduction

Systems that have no or very little mass exchange with their surroundings are considered to be "closed" to the environment. The planet earth can be described as such—a biosphere in which very little is exchanged to space. Life is sustained by the cycling of carbon, nitrogen, oxygen and other elements throughout the planet in a way that is self-sustaining. In other words, the life on this planet has interacted in ways that continue this cycling of nutrients. This process is one of the many intriguing aspects of our planet's ecology. Organisms have evolved to not only consume each other but are able to recycle the waste products of each other as well. Plants, by photosynthesis use light energy to convert CO_2 and H_2O into organic material:

$$6CO_2 + 12\ H_2O + \text{light energy} \rightarrow C_6H_{12}O_6 + 6O_2 + 6H_2O$$

The opposite reaction is respiration:

$$C_6H_{12}O_6 + 6O_2 + 6H_2O \rightarrow 6CO_2 + 12\ H_2O + \text{light energy}$$

The CO_2 is a waste product of respiration of organisms including grazers that in turn use the byproduct of photosynthesis, oxygen, to survive. These grazers are eaten by higher-level predators which contribute to the cycle with their waste products. This cycle is continuous and vital to aerobic organism survival. Species interactions can also be seen in the cycling of nitrogen throughout the planet. While this cycle is more complex, with many chemical transformations, the basic concept is the same: plants (primary producers) need nitrogen in the form of nitrate or ammonia. nitrogen is the fourth most abundant element on the planet but very little is in a form usable by plants. This makes nitrogen one of the most limiting factors in plant growth. nitrogen is fixed into nitrate by a number of ways including plant/microbe symbiosis, prokaryotes and lightening. Grazers and predators secrete waste in the form of urea, which contains ammonia, and this can be transformed into nitrate by soil bacteria. All of the steps involved require an intimate coordination with other organisms.

Studying ecology on a planetary level has many drawbacks. The scale of our planet allows for a wide variety of climatic and ecological effects with very little idea of what is driving them. There is also a long lag period before many effects are seen at the planet-wide level. This is clearly seen in the development of our atmosphere. The oxygen content of our atmosphere did not develop the day after cyanobacteria started producing oxygen as a waste product. It has taken millions of years and many organism permutations to get to the atmosphere content we have today. The atmosphere most likely went through many cycles of aerobic and anaerobic conditions to reach the state it is in. This lag time would make it hard to see the effects of a chemical that has become off balance within the Earth's environment. Planetary size also effects how smaller ecosystems within the larger biosphere react to stresses. The condition of the planet may not affect every community or ecosystem the same way due to distinct differences in species diversity. This makes it hard to determine what effect a stress may have on more than one ecosystem. Many organisms travel through large tracts of the planet, such as whales and birds, while other organisms, especially plants, are non-motile. It would be very impractical to just release a stress onto the planet and "see" what happens. Further complicating the picture is the conditions that organisms face at the local level. These conditions are often dynamic and not optimal. This makes producing things like computer models difficult because one must account for all the changes an ecosystem may experience and optimal conditions are very seldom seen in nature.

With all of these limitations on ecological study, how can we even hope to discover all the secrets of the planet? One way the scientists have developed is laboratory ecosystems. These are often small-scale models of systems seen in nature that can be as complex or simple as needed. These systems can be used to study species interactions and such but since most of them are open to and rely on inputs from scientists, effects may be based on many factors . One way to eliminate this problem is to develop systems that are "closed" to the surrounding environment. These systems

would receive light energy from outside but would recycle everything needed for species survival without any inputs from the outside [Beyers and Odum 1993]. This is essentially what happens on Earth. We receive light energy from the sun and release energy in the form of heat into space, but we must recycle everything else from within the boundaries of our atmosphere. With closed ecological systems (CES) research, 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]. These systems are easier to monitor and reduce the problems that large-scale systems have as described above. Using CES to study patterns and effects of elements within an ecosystem allows one to not only observe specific interactions, but replicate and reproduce experiments and results [Taub 1980].

Experimental Background

Closed ecological systems have been over looked as research tools for some time. Research began in the field in the early 1960's and has evolved from small glass bottles to the Biosphere II project and the Closed Ecological Life Support System (CELSS) project funded by NASA [Straight et. al. 1994]. The two latter projects were extremely costly, which may be one reason that research is not pursued in this area. The data that have been gathered from these experiments, however, are extremely useful and continue to be used to develop new methods of researching CES. One application of this data is thought to be sustaining organisms in space for long periods of time. By developing a system that is self-regenerating, astronauts would be able to spend longer periods of time in space while utilizing a more efficient source of oxygen. This research is also important on a species level. By looking at invertebrates, for example, in a small CES, information can be gathered on tolerance to differing levels of nutrients, light or oxygen, all of which help to describe the limits of the species. By knowing when a specific species may be killed by a pollutant, one may be able to extrapolate the health of an ecosystem based on the condition of these species. Data gathered from CES research could be utilized in many different ways, from understanding what upsets balances in lakes to create algal blooms to allowing long-term space flight an option to combat bacterial blooms that may disturb oxygen and other balances vital to the survival of the vehicle occupants. At a seminar for closed ecological systems in 1982, it was found that CES "promise to become a significant resource for the resolution of global ecology problems which have thus far been experimentally inaccessible…" [NASA 1982] due to the reasons stated previously. For our closed ecological systems, we looked at what environmental factors affect the ability of a system to sustain itself for greater than 30 days. Using both freshwater and marine systems, we explored how differing both day length and levels of nitrogen and phosphorus affect algal and invertebrate populations.

Methods

All of our CES were constructed using 75mL tissue culture flasks. These are inexpensive, sealable containers typically used to culture tissue cells. The benefit of

using these containers versus glass culture tubes or other types of containers is they have an optically flat surface. This allows easier observation of the grazers as well as the opportunity to place the entire flask on a dissecting or inverted microscope to observe algal cells. Each flask, both marine and freshwater, was filled with water, media and algae and allowed to sit open for 5 days. This allowed for gas exchange to take place and for any unexpected organics to oxidize. The invertebrates were then added and the flasks were placed in their perspective incubators.

Freshwater Systems:

Freshwater closed ecological systems for various studies followed the same initial set-up and composition: Kent water and T82-LoSi (an algal medium) [Appendix I] served as the liquid environment and initial source of nutrients for the algae. Three types of algae—*Ankistrodesmus*, *Scenedesmus*, and *Chlamydomonas*—were introduced, along with 6 *Daphnia magna*, a freshwater invertebrate. They were added in the following composition:

45 mL Kent water

15 mL T82-LoSi

0.8 mL *Ankistrodesmus*

0.8 mL *Scenedesmus*

0.8 mL *Chlamydomonas*

Systems were allowed to sit open to the atmosphere for five days and on day five 6 *Daphnia magna,* generally 3 adults and 3 juveniles, were added and the systems were sealed.

Two studies were performed with freshwater closed ecological systems:

Light Exposure Length Experiment:

The first study looked at the effect of different light exposures on the health of closed systems. Systems were placed under varying light cycles—0, 6, 12, and 18 hours of light—while kept at constant temperature and (except for the 0-hour set) constant light transmittance. Each set was observed for algae content, *Daphnia* (adult and juveniles), number of carapaces or carcasses present, and the overall state of the system.

Nitrogen and phosphorus Introduction Experiment:

In the second study, we asked whether the viability of closed ecological systems was limited by the uptake of nutrients in the form of *Daphnia* carapaces, carcasses or in other forms. It is thought that nutrients, especially nitrogen, are removed from the systems by being held in these durable forms. Slightly open systems, into which nutrients were added, were used to gain an understanding of the limitations on entirely closed ones.

Nutrients were introduced into the systems by means of syringes; a second syringe was used to relieve the increased pressure by taking up an amount of atmosphere from the flask equal to the nutrients added. While technically not *closed* systems, we were able to limit the openness to a single variable. The first nutrient introduced was a Nitrate (NO_3) solution to test whether nitrogen is taken out of the systems and becomes a limiting factor. To examine the possibility of phosphorus becoming a limiting factor after nitrogen is introduced, an NO_3/PO_4 solution was injected into the second set. The third set of flasks was injected with Kent water, acting as the control.

Nutrients (and the control) were injected two weeks after the systems were sealed. The amount of NO_3 added was equal to the amount originally introduced in the system with the T82-LoSi, effectively doubling the amount available to algae and *Daphnia*. Introduction of the NO_3/PO_4 solution doubled both the NO_3 and the PO_4 available in the system. Control sets were injected with equal amounts, but of Kent water, which contains no nitrates or phosphates.

Marine Systems:

Marine closed ecological systems employed many of the same techniques as freshwater. Three different species of algae were employed: *Nanochloropsis, Isochrysis* and *Tetraselmus*. The salt water used was obtained at the Seattle Aquarium, Seattle, Washington. It was pumped from Puget Sound through a sand filter process to eliminate contaminants. It was not autoclaved so bacteria and other microorganisms were allowed to become part of the system. The elements of each flask were added as follows:

45mL of filtered salt water

15mL of f/2 media [Appendix I]

0.8mL of *Nanochloropsis*

0.8mL of *Isochrysis*

0.8mL of *Tetraselmus*

Each system was allowed to sit for 5 days, after which four *Tigriopus californicus* were added to 24 of the flasks and sealed. We then sealed 24 more flasks that did not receive any invertebrates. Six flasks of each treatment were then placed in four light treatments. Three incubators were set up at a temperature of 20ºC each with a different light treatment: 18 hours light, 12 hours light, 6 hours light and 0 hours light based on a 24 hour day. These treatments are generally expressed as 18L:6D or 18 hours light to 6 hours dark and so forth. The 0L:24D flasks were wrapped in tin foil and placed in a dark box within the 6L:18D incubator in order to minimize any light leaks.

Daphnia and *Tigriopus* (juveniles and adults) were counted and observed using the naked eye as well as the aid of a dissecting microscope. Trials were done to observe animal populations using a video camera, but this method is still being perfected. Algal growth was determined using a Plant Stress Meter (PSM), which measures the in vivo fluorescence of the algae. This method was successful until the algae began to fall out of

suspension and clump together. At this point the PSM was unable to get a reading. In the experiments performed, algal growth continued throughout the duration of the experiment. Naked eye observations were also conducted on the overall health of the systems.

Results

Freshwater Systems:

Results of the light exposure length experiment indicate that balances are achieved between the plant and animal populations in the 12- and 18-hour light-cycle sets. Populations of *Daphnia* remained fairly stable over the 2-week time period. In the 0-hour light cycle set, *Daphnia* persisted for longer than expected. While adults died off quickly, juveniles lived beyond the point when any visible algae could be detected. The 6-hour set also showed a drop-off in adults, but a rise in juveniles.

Introduction of nitrogen into the system, in comparison to the control sets of Kent water, showed a relatively higher population of *Daphnia* (both adult and juvenile) and, over the first 4 weeks, a higher PSM level—indicating more algae. Systems into which nitrogen and phosphorus were added showed similarly high levels of *Daphnia* and algae, but in two systems the *Daphnia* died off completely (Fig. 3). Overall, *Daphnia* in the nitrogen and nitrogen/phosphorus sets were more populous, produced more young, and appeared healthier (their guts were dark, indicating high food consumption, while *Daphnia* in the Kent sets appeared quite pale after the first three weeks, as visible algae diminished). Only the nitrogen and nitrogen/phosphorus sets showed, after 74 days, an average population of *Daphnia* about that number originally introduced.
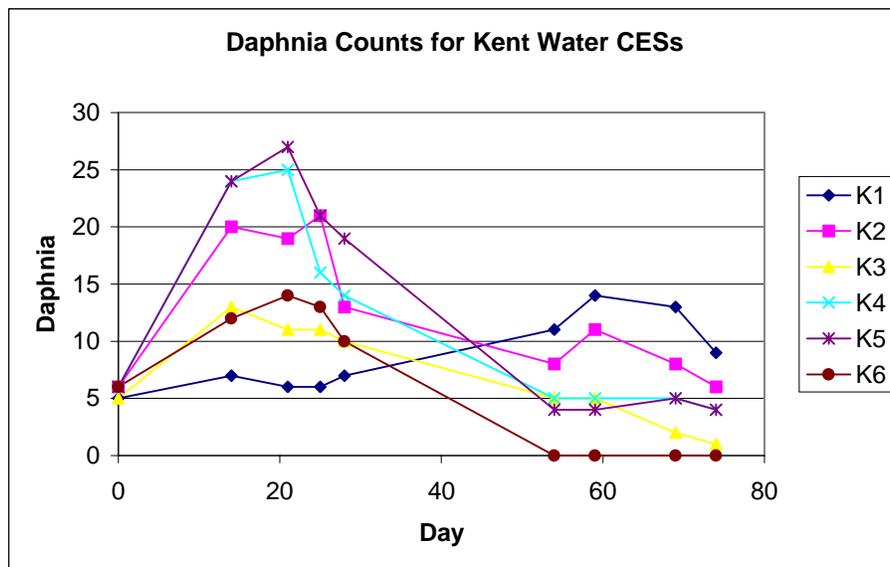
Fig. 1 – Daphnia Populations for Kent Water
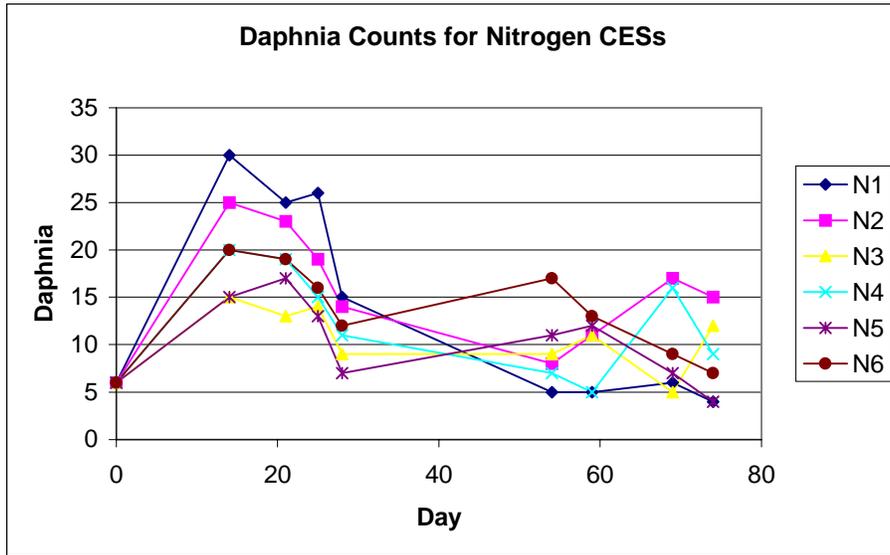

Fig. 2 – Daphnia Populations for Nitrogen Added

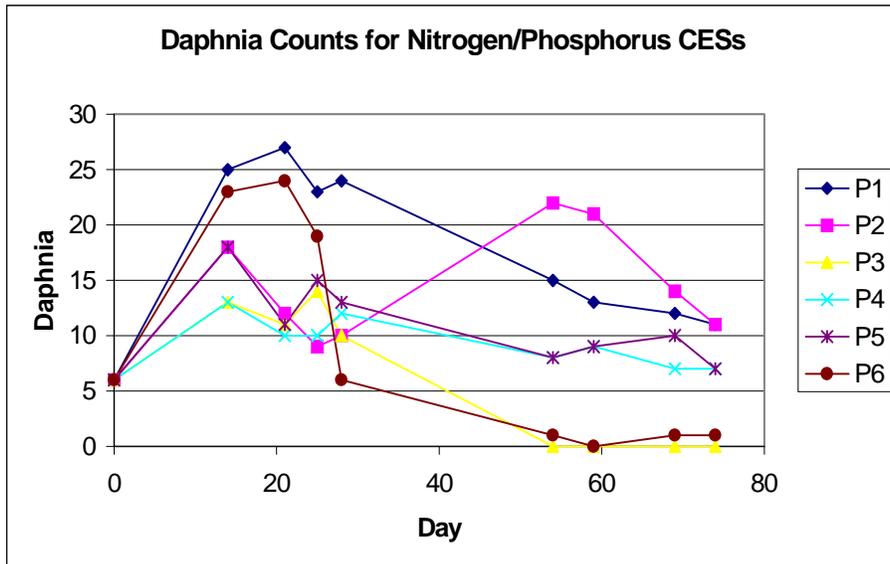Fig. 3 – Daphnia Populations for Nitrogen and Phosphorus added

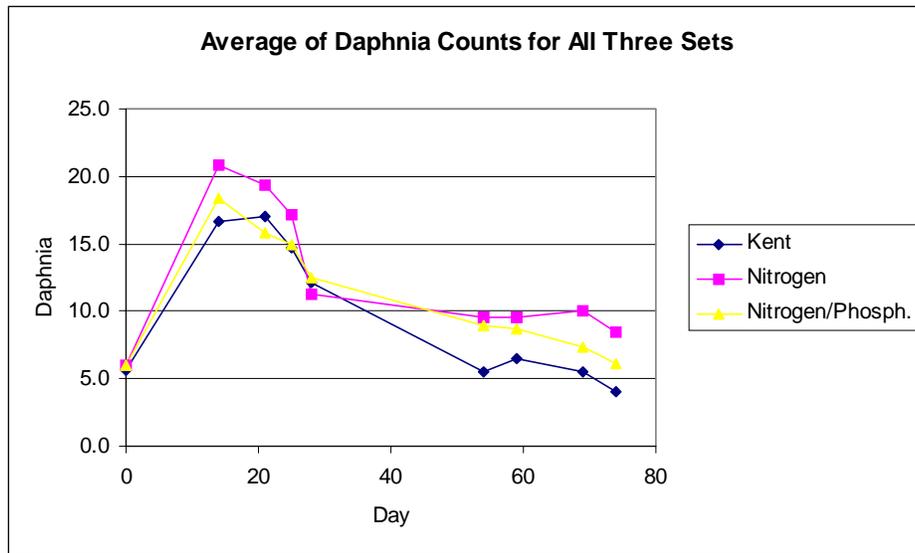Fig. 4 – Daphnia Populations Averages

Average of Daphnia Counts for All Three Sets

Marine Systems:

Effects on *T. californicus:*

In the flasks containing both algae and grazers, the results show that *T. californicus* has a considerable range of survival. At the end of week three, all replicates showed egg hatching at least once, with many replicates still containing egg-bearing females. This suggests that to this point the day length had very little direct effect on the *T. californicus.* The replicates in the 0L:24D and 6L:18D had eggs hatch earlier than the replicates in 12L:12D and 18L:6D. The number of *T. californicus* did increase in all replicates until the end of week three, at which point the 0L:24D began to expire. The other three light treatments showed a slight decrease and leveling off at the same time.

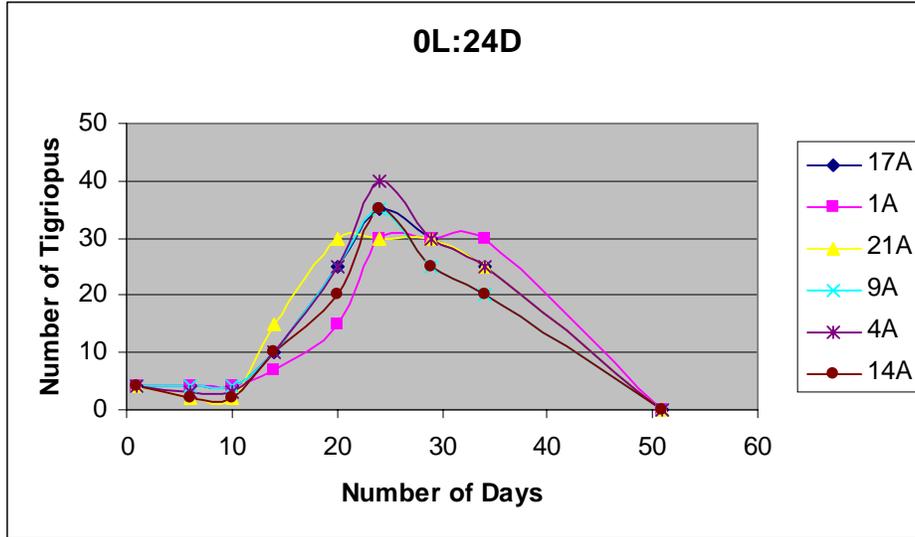Fig. 5 – *Tigriopus* populations at zero hours light.

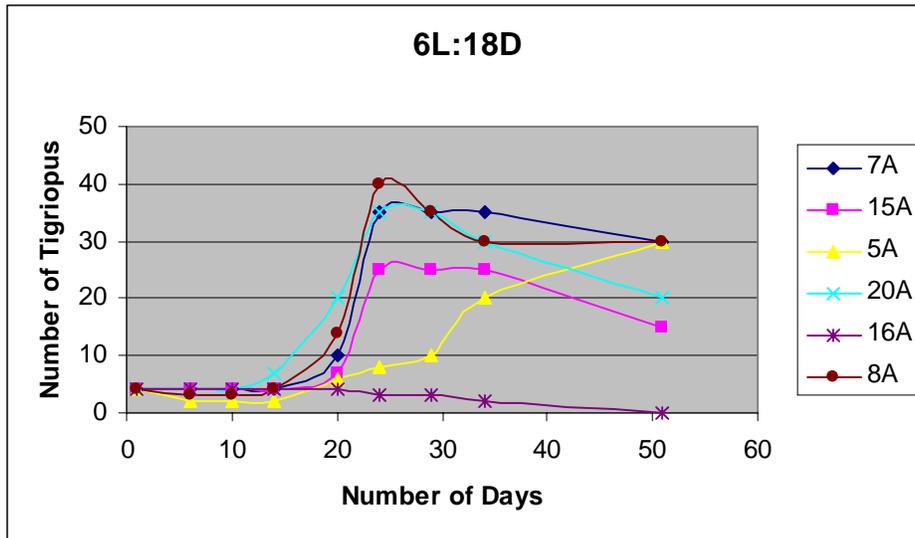Fig. 6 – *Tigriopus* populations at six hours light.

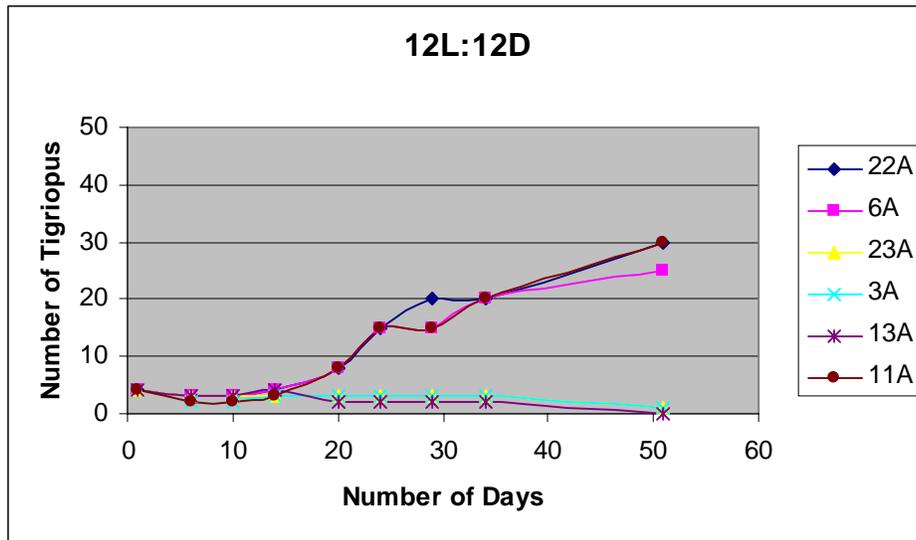Fig. 7 – *Tigriopus* populations at twelve hours light.

12L:12D

Legend: 22A, 6A, 23A, 3A, 13A, 11A

X-axis: Number of Days
Y-axis: Number of Tigriopus

Fig. 8 – *Tigriopus* populations at eighteen hours light.

18:L6D

Legend: 24A, 2A, 19A, 12A, 10A, 18A

X-axis: Number of Days
Y-axis: Number of Tigriopus
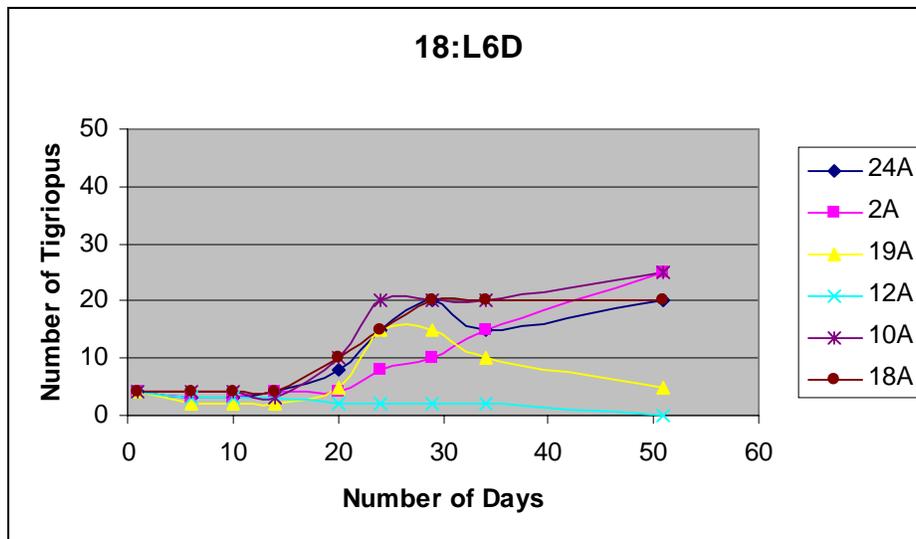
Effects on Algae:

    The algae in the 6L:18D without grazers shows a much higher amount of growth than the other three light treatments. The overall difference between the algal growth in the flasks without animals and those with animals is expected. The flasks without animals (Flasks numbered with a 'W') showed a fairly consistent growth. The 0L:24D

did begin to see a decrease in algal cells around day 11 and visual observations did not show an increase in clumping, which could account for the decrease in PSM readings. This would mean that the algal cells were dying off from the lack of light. The flasks that contained animals showed a sharp decrease in PSM readings but visual observations showed an increase in cell clumping at the bottom of the flasks.

Fig. 9 – Chlorophyll concentrations over time in CES with and without *Tigriopus.*
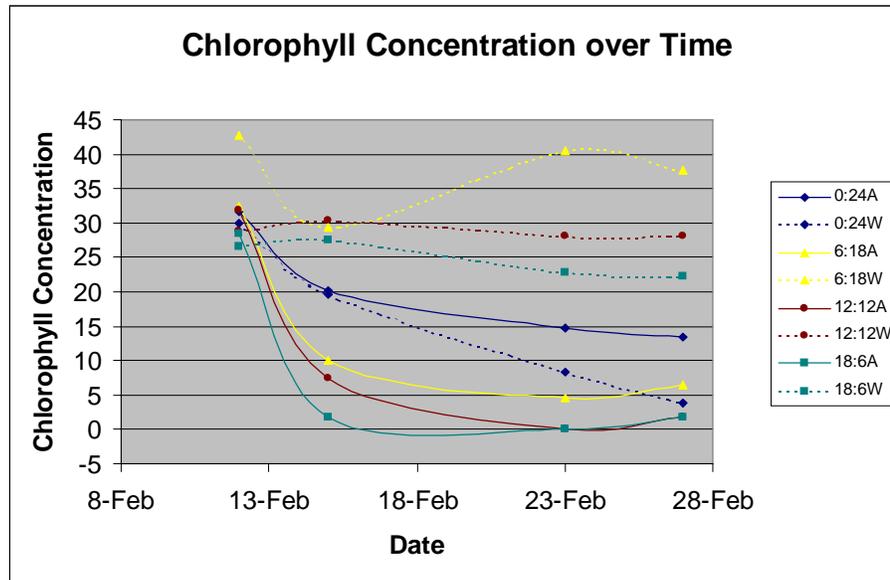
Conclusions

All replicates indicated survival past the thirty day mark. The *Daphnia* did not hatch an F2 generation but this has been seen with past research in *Daphnia* studies. In the marine systems, *Tigriopus* did hatch eggs past the F2 generation and were still producing eggs at the end of the experiment.

Freshwater Systems:

     Data from the experiment introducing nitrogen and phosphorus into the otherwise closed system suggest that nitrogen is a limiting factor in closed ecological systems because the health of algae was significantly higher in systems where NO_3 was introduced, and average the *Daphnia* population was slightly higher (Fig. 4). There were no indications that phosphorus was a limiting factor, and in fact systems with nitrogen and phosphorus performed, on average, worse than those with only nitrogen.

Marine Systems:

     When there are no grazers present, algal abundance was much greater than those systems where the algae is being grazed upon. In grazed systems, the algae tend to clump together, which may be a defense mechanism against predation. The amount of light

needed to sustain an invertebrate population past 30 days seems to be quite variable. The 0L:24D systems did show a much quicker growth increase than those in the 12- and 18-hour light treatments. There seemed to be very little immediate advantage to increasing the day length over six hours. This may have been due to the intensity of the light which was measured at 32 μE m^{-2} s^{-1}. Longer day lengths may increase the amount of time needed to hatch out eggs as all the flasks reached about the same animal density, just at different times. It took the 0L:24D treatments approximately 24 days to start showing any decline in *Tigriopus*. Once these systems began to decline, they did so very rapidly.

Overall Results and Implications for the Future:

Our small, closed ecological systems are a great stepping stone for further research in the field. Future investigations include creating larger systems with slightly more complexity and varying trophic levels. What we have learned from these simple systems will help us to know what the starting point is for larger systems and what limitations these organisms have in any system. We are also working on more efficient ways to record the growth of animal populations using a video camera. Documenting animal populations on video will not only allow us to get a more accurate count of animals present but will also allow us to do visual analysis of swimming patterns and other behaviors of both *T. californicus* and *Daphnia magna*. We would also like to develop ways to determine the exact O_2 and CO_2 amounts within the systems so that we can get actual amounts and not relative amounts based on the health of the invertebrates. This will also allow us to get a better picture of what they can tolerate. The data gathered in these experiments as well as those done by pervious groups will allow us to pursue the goal of creating more complex systems with the ability to sustain large organisms for many years.

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Appendix I – Media contents

Freshwater Algal Media (T82)   **(Taub, 1993)**

Compound	Element	
$NaNO_3$	N	7.0 mg/L
$MgSO_4 \bullet 7H_2O$	Mg	2.43 mg/L
KH_2PO_4	P	1.23 mg/L
NaOH	Na	2.27 mg/L
$CaCl_2 \bullet 2H_2O$	Ca	40.0 mg/L
NaCl	Na	34.5 mg/L
$Al_2(SO_4)_3 \bullet 18H_2O$	Al	0.26 mg/L
$Na_2SiO_3 \bullet 9H_2O$	Na	36.8 mg/L
	Si	22.4 mg/L
$FeSO_4 \bullet 7H_2O$	Fe	.00625 mg/L
EDTA	EDTA	0.4145 mg/L
H_3BO_3	B	0.008 mg/L
$ZnSO_4 \bullet 7H_2O$	Zn	0.0015 mg/L
$MnCl_2 \bullet 4H_2O$	Mn	0.0135 mg/L
$Na_2MoO_4 \bullet 5H_2O$	Mo	0.0024 mg/L
$CuSO_4 \bullet 5H_2O$	Cu	0.00032 mg/L
$Co(NO_3)_2 \bullet 6H_2O$	Co	0.00015 mg/L

Table 2
Salt water Algal Medium (f/2)   **(McLachlin, 1973)**

$NaNO_3$	0.075 g/L
$NaH_2PO_4 \bullet H_2O$	0.005 g/L
$CuSO_4 \bullet 5H_2O$	0.25 ml/L
$ZnSO_4 \bullet 7H_2O$	0.25 ml/L
$CoCl_2 \bullet 6H_2O$	0.25 ml/L
$MnCl_2 \bullet 4H_2O$	0.25 ml/L
$Na_2MoO_4 \bullet 2H_2O$	0.25 ml/L
O3 Stock A	0.76 ml/L
f/2 vitamins	0.5 ml/L
TRIS	5.0 ml/L

Table 3
Kent Water   **(Kent Marine, Marietta, GA)**
A combination of carbonates, sulfates and chlorides of sodium, magnesium, calcium and potassium with all necessary minor and trace metals necessary for cichlid fish. Contains no phosphates, nitrates or organics.