ANALYSIS OF *HALOCARIDINA RUBRA* IN AN ENDOGENOUSLY CONTROLLED CLOSED ECOSYSTEM

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ABSTRACT

The biota from Hawaiian anchialine ponds have been known to survive for long periods of time in sealed containers. *Halocaridina rubra (H. rubra)*, a keystone species, was used in a laboratory model that examined how density of shrimp affects a closed ecosystem. Multiple, small systems simulated closed anchialine environments in order to study the survival of the small endemic shrimp *H. rubra*. *H. rubra* were stocked at different densities of 4, 8, 11, 13, and 16 individuals per 1-liter container which was sealed. Optimum density was found to be 3.2 shrimp per liter of semi-sealed environment with evidence of a negative, cumulative boom and bust cycle of 8 shrimp per liter causing an ecological crash. The biology of *H. rubra* was also examined by a salinity tolerance LC50 study which found the shrimp to have a 5-40 ppt salinity tolerance. Subsequent starvation trails showed *H. rubra* to survive 42 days without food. Natural anchialine habitats were explored by visiting several pond complexes to understand how the natural system works and to aid in creating better artificial closed ecosystems. A list of environmental parameters and biota from field observations useful for the creation of closed ecosystem was created.

INTRODUCTION

The Islands of Hawaii contain unique environments and ecosystems. Particularly the island of Hawaii because of its unusual abundance of anchialine ponds. Anchialine ponds are isolated saline pools generally restricted to porous substrates such as lava flows. The ponds are unusual because they have a tidally influenced subsurface connection with the water table yet have no surface connection to the ocean. Because of the extremely sparse rainfall combined with extreme isolation from nearby vegetation, some anchialine ponds are ultra-oligotrophic. These ponds have developed an ecosystem with extremely high rates of nutrient recycling making them ideal for closed ecosystem research. A closed ecosystem, also known as a microcosm, is a small sealed environment with biota that survives for an extended period of time through recycling nutrients between organisms. The shrimp *Halocaridina rubra* have been used in several studies of closed ecosystems including a two-year microgravity environment study on the International Space Station.

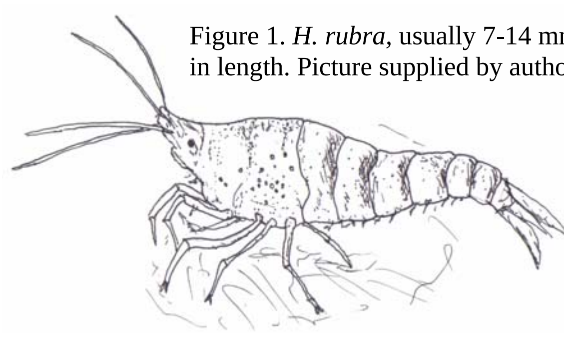


Figure 1. *H. rubra,* usually 7-14 mm in length. Picture supplied by author.

Halocaridina rubra (Figure 1), is an important inhabitant of anchialine ponds and is endemic to the main Hawaiian Island chain. *H. rubra* is a small (7-14mm), red omnivorous shrimp that can be found in pristine ponds with densities approaching 100 per square foot. They have extremely flexible nutritional requirements and have a lifespan of at least 10 years. *H. rubra* shrimp often spend much of their time in the underground hypogeal environment of porous rock around and underneath anchialine ponds. *H. rubra* come to the surface of the anchialine ponds to feed but retreat underground for safety and to breed (Maciolek, 1983).

In order to better understand the anchialine biota for closed ecosystems it was necessary to explore three areas of study. First, a laboratory model examined how density of shrimp affects the closed ecosystem. Secondly, the biology of *H. rubra* was explored though salinity tolerance and starvation trails. Third, the natural anchialine habitat was explored by visiting several pond complexes. Numerous readings were taken in the field such as maximum density of shrimp, survey of various biota, and water quality measurements. This data will help to understand how the natural system works and could perhaps aid in artificially creating more complex closed ecosystems for experimentation.

METHODS OF DENSITY IN 1-LITER LAB MICROCOSMS

Laboratory microcosms consisted of one liter sealed glass Erlenmeyer flasks that contained the following: 900 mL of sea water (35 ppt salinity), 125 g “seeded” gravel, 1 inch square porous sponges used as substrate for development of algae and phytoplankton, a varying quantity of the shrimp *Halocaridina rubra*, and 2 g of photosynthetic *Melosira* sp. algae. The differing numbers of shrimp are 4, 8, 11, 13, 16 individuals with 3 replicates of each density. Each flask was given a number and letter for identification. The first number corresponds to number of initial shrimp and letter (A, B, or C) the specific replicate. A maturation period of 7 days was applied before the shrimp were added to the flasks. The microcosms were subjected to a natural light cycle of 12 hours of light, and 12 hours of darkness. Lighting was from a 30” 64 watt Orbit artificial marine aquarium light consisting of 1 dual actinic [420 nm + 460 nm] and 1 dual daylight [6,700K + 10,000] bulbs. Once the experiment began, after the shrimp were added, nothing was removed or added to the flasks. The only time they were unsealed was for taking water quality measurements with a YSI probe. Measurements of salinity, temperature, dissolved oxygen, percent oxygen saturation specific conductivity and number of living shrimp was taken every other day and recorded on a data sheet.

METHODS OF SHRIMP LC50 AND STARVATION TRAILS

Lethal Concentration (LC) values refer to the concentration of a chemical in air or water. The concentration of the chemical in air/water that kills 50% of the test animals in a given time is the LC50 value. To accomplish this study, 50 mL plastic sample vials were filled with water at 8 different salinity concentrations of 0, 5, 10, 35, 40, 45, 50, and 55 ppt. Three replicates for each concentration and one shrimp was placed in each vial. Deaths were recorded every 60 minutes for 72 hours after loading. Surviving shrimp were left without food to see how long they can survive without food in subsequent starvation trial. These shrimp were left in the open containers and checked daily and all were deaths recorded.

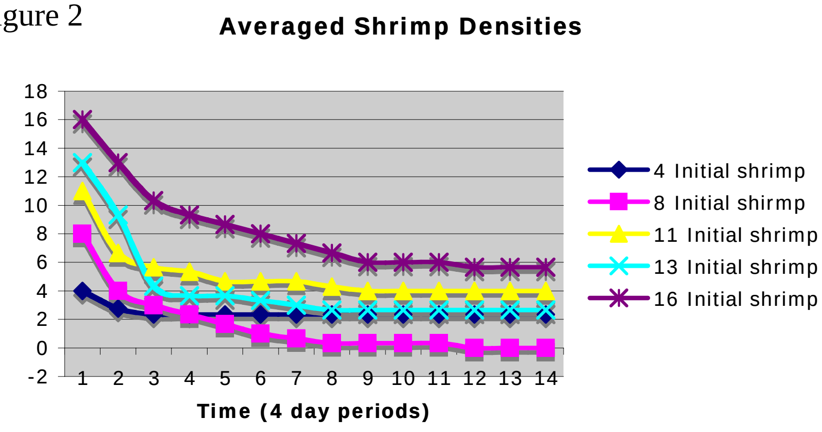
METHODS OF FIELD SURVYING

Sampling was conducted at 27 ponds in the Makalawena and Awakee coastal area. Also, brief surveys and collections were done at Hilton Waikoloa anchialine pond complex, Lua O Palahemo, and Leleiwi. This data was then compared to the survey performed in May 1988. At each pond several different types of data was taken. First, the presence/absence of *Halocaridina rubra* was noted at each pond site. This was conducted by running visual transects with snorkel gear if the pool was large enough to dive in. Ponds that were too small to dive in, thorough observations were made by walking in and around the pool to search for the shrimp. If the shrimp density was considerably high, maximum density of the shrimp was found by taking 5 random photo quadrates with an underwater digital camera. Readings of temperature and salinity were taken within the top 10 cm of the water column. Measurements were taken using a portable refractometer and a Hanna Instruments portable pH, EC, TTD, and C^{o} water tester.

RESULTS AND DISCUSSION OF DENSITY IN 1 LITER LAB MICROCOSMS

After laboratory experimentation, optimum or stable density for the *H. rubra* in a semi-closed microcosm appears to be 3.2 individuals per liter of environment. Success of each system seems somewhat dependent on the initial number of shrimp in the system. The average number of living shrimp after 56 days was as follows, 2.33 for replicates with 4 initial shrimp, 0.0 for 8 initial shrimp, 4 for 11 initial shrimp, 2.66 for 13 initial shrimp, and 5.66 for 16 initial shrimp (Figure 2). The microcosms starting with 4, 11, and 13 initial shrimp dropped down to 2-4 shrimp within 32 days and seem to have stabilized at 2-4 per flask. The flasks contained 16 shrimp initially lost an amount of shrimp similar to the other flasks. However, the death rate slowed at a higher number then seemed to level off shrimp. The one density that exhibited an unusual pattern was flasks that contained 8 shrimp initially. These microcosms exhibited an ecological unbalancing with the resulting death of nearly all shrimp within 24 days. In all densities except for those with 4 initial shrimp, there was a loss of 6 shrimp on average within 8 days. After 8 days the death rate slowed in all densities.

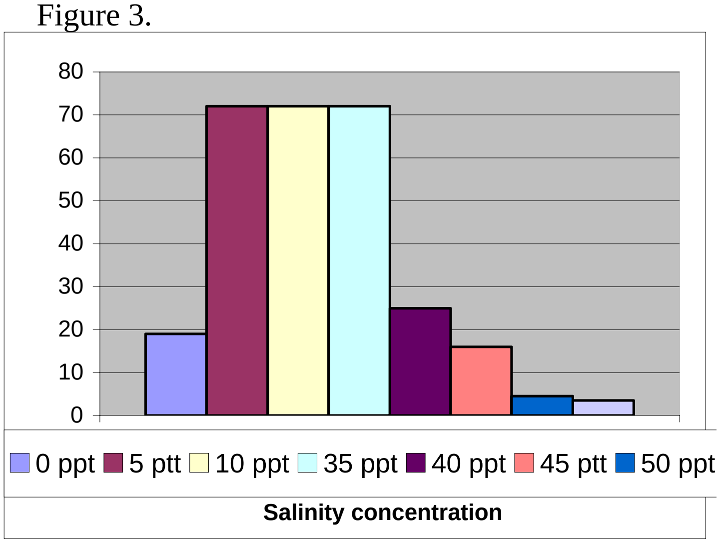
Figure 2



All replicates with 8 initial shrimp experienced rapid death of all shrimp while densities both higher and lower stayed relatively stable was unusual. In previous work, Straphanopoulos and Liepmann, 1985, also mention this phenomenon though provided no explanation. I believe this may be explained by an unstable boom and bust cycle or a series of cyclic growth and decline between the shrimp and algae, the shrimp's main food. The microcosms had a minimum of seeded substrate containing aerobic bacteria therefore the primary producer of CO_2 was most likely the shrimp. While the higher densities of shrimp offer a greater CO_2 output from respiration and the breakdown of their waste for a faster production of algae, though the grazing pressure is also much higher. At a lower density of shrimp there would be a much smaller grazing pressure on the algae combined with less CO_2 production and less algae growth. Both higher and lower densities experienced a boom and bust cycles that eventually stabilized into a constant of a nearly equal rate of grazing versus algae growth. At a density of 8 shrimp, all replicates first experienced a typical cycle however it slowly and cumulatively deteriorated until the ecological system crashed. This may have been due to the grazing rate being very slightly higher then the growth rate of algae as it oscillated back and forth. Once this negative feedback cycle was started, it continued even as shrimp died off. The continuation, even with less shrimp, was probably caused by lower production rates of CO_2 and the grazing rate still exceeding the rate of algae growth. As algae levels got smaller, a dip in O_2 was noticed once all shrimp had died, a sharp spike in O_2 followed by a reversal, ending in very high levels of CO_2 as the ecological systems crashed.

RESULTS OF LC50 STUDY AND STARVATION TRIAL

Figure 3.



The lowest salinity concentration the shrimp, *H. rubra*, could survive in was 5 ppt and a maximum of 35 ppt salinity for the duration of 72 hours (Figure 2). The shrimp that survived in the 5, 10, and 35ppt replicates were then kept without food to see how long they will survive. These shrimp being put through subsequent starvation survived for a maximum of 42 days proving their extremely flexible nutritional requirements.

RESULTS FROM FIELD SURVEYING

Surveying several field sites gave a much better idea of the complexity of an anchialine habitat. Some ponds at Awakee may be half a mile from the ocean and any vegetation in a rugged `a`a lava flow; yet will be teaming with shrimp and only 10 cm deep. These are both important species in the anchialine environment, which may be useful for creating larger more complex environments. Ideally it would be good to get large amounts of environmental biota collected from natural ponds for anchialine microcosm research. Also, using data from the actual anchialine pond collections are made from may increase success. By modeling water quality parameters, and collecting biota straight from a pond gave a noticeably smaller initial die off of biota. If natural collection is not available, Table 1 gives recommended environmental parameters found in the natural environment that should be mimicked in order to accurately recreate an anchialine system. Table 2 gives recommendations for biota that was commonly found in natural, oligotrophic anchialine ponds and can be used in closed ecosystem creation.

Table 1

Chemical parameter	Recommendation
Water Salinity	20-35ppt
Water pH	pH 8.2 ± 1.5
Temperature	20-28 ^{0}C
Lighting	Light levels are particularly harsh in the western part of the Hawaii island. Also, the shallow water in the pools filters little light so a broad light range (6,700-10,000K) is needed to mimic natural sunlight.

Table 2

Biota	Recommendation	Notes
Shrimp	*Halocaridina rubra*	Microcosms should be loaded with an initial density of 3.2 shrimp per liter of environment plus an additional 20 percent of the total number to account for initial die offs.
Algae	*Milosara spp.*	Best if collected directly from natural environment.
Encrusting Cyanobacteria	Species unknown	Environmentally collected, orange/yellow/brown in color.
Snails	*Assiminea nitida* *Thiara granifera*	Small, herbivorous snails common throughout anchialine ponds good for larger microcosms.
Maturation period	Shrimp should be added 2 weeks after all other biota added to allow the bacteria and algae to establish and mature.	Little or no maturation time may cause systems to ecologically crash within 1 week.

CONCLUSION

The density of the H. rubra appears to fluctuate toward equilibrium for optimum stability in these small closed ecosystems and not spatially for individual shrimp. The density levels would most certainly change with different biota such as faster growing algae, longer periods of sunlight or greater amount of substrate. The phenomenal length of time that these microcosms may persist is astounding and may be attributed to *H. rubra* acting as a buffer organism. The shrimp may act as a “buffer” in a closed system to keep other species from over-growing enough to cause a major shift in nutrients and species succession to crash the system. *H. rubra* probably acts as an endogenous control to regulate the levels in the rest of the system. The shrimp’s long life span, extremely flexible diet of both macro and micro biota, it not gaining in mass to a sizable amount and not breeding without suitable habitat makes them an extremely effective control mechanism. It acts almost as a simple, autonomous machine to keep all levels of the food chain in check. In designing other closed ecosystems, a buffer organism similar to *H. rubra* with these traits would be highly desirable. *H. rubra* is a chemically tolerant organism with extremely sparse nutritional requirements make it idle for the fluctuating conditions found in a microcosm. Also, when designing a closed ecosystem it is important to take into consideration oscillations and negative feedback like that which occurred at a medium density such as 8 shrimp per liter. Many small anchialine microcosms sold commercially or homemade often only last 2-3 years. This slow deterioration is probably due to a very slow version of what happened to all replicates containing 8 initial shrimp. It has been proven that a microcosm may last 25 years or more and long-term success is very possible.

The microcosms based off existing anchialine ponds provide a cheap, small scale and very robust system for research. During the course of fieldwork, it was very difficult to find pristine anchialine ponds with high levels of shrimp. The majority of anchialine habitat has already been destroyed and much of what is left may soon be developed. Some ponds such as Lua O Palahemo, a rare deep lava tube pond sampled, contains a shrimp species found only in that pond and no were else on earth. Yet has no protection from collection or dumping of refuse into the pond exists and even less for most other anchialine ecosystems. Conservation measures are greatly needed for the fragile anchialine habitat.

ACKNOWLEDGMENTS

Without the unyielding support and guidance of my mentor Dr. Jason Turner of the Marine Science Department at University of Hawaii, Hilo, this project would never become a reality. Thanks also to Dr. Patrick Hart whose advice and help with data analysis and statistical tests made everything clear. Kevin Donmoyer, for hours compiling data on the computer and Chris, Margret, Sara, and Lisa for help doing water quality tests. Finally, thanks to the Hawaii Space Grant Consortium and the NASA Space Grant Undergraduate Fellowship Program for funding and the amazing opportunity.

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