Session 1408

A Living Laboratory: The Maryland Crayfish Project

Paul D. Schreuders, Andrea Lomander
University of Maryland, College Park

Abstract

Biological engineers differ from other engineers in that they must consider not only the abiotic components of a system but the biotic components as well. While this relationship may appear to be obvious, it is the implications of this relationship that defines the field. Successful biological engineering can only be achieved if the students develop an understanding of their designs as systems. These systems respond significantly differently from the isolated parts. The understanding is achieved through knowledge in three areas, (1) the behavior of the abiotic components, (2) the behavior of the biotic components, and (3) the relationships between the biotic and abiotic components, and the resulting dynamic responses of the system. In our program this is achieved through a combination of laboratory experiences and classroom instruction. In one of these laboratories, the emphasis is on the experimental design and practical implementation of systems with living components. The premise of the exercise is that, if students truly understand the requirements of living creatures, they can keep those animals alive and healthy. Over the last few years, this laboratory has involved the design of an ecosystem to support crayfish in a sealed (airtight, watertight, but light admitting) system. Early in this laboratory, the students are asked to identify critical variables for supporting these animals, and to decide if, when, and how they should be measured. This decision-making process moves from a thought experiment to a physical process and the students are asked to design and make measurements on the crayfish and their environment. Next, the students set up their ecological microcosms and try to stabilize them. During this phase of the laboratory, the students become aware of the complexity of their challenge. As the self-organization proceeds, environmental variables within the microcosm become inextricably linked. Finally, the students seal the tanks for several periods. Requiring that the tanks remain sealed for progressively increasing periods requires the students to examine the microcosm's response to the perturbation, identify the problems and their respective source(s), and redesign the system. This iterative failure and redesign cycle results in stronger designs and increases the student's confidence in their design abilities.

Introduction

This project was planned as an integrative experience for seniors in biological engineering. The project supports the following programmatic objectives (EAC, 2000):

- Applies a knowledge of mathematics, science, and engineering
- Requires students to design, conduct, and analyze experiments, and
- Requires students to design systems to meet a desired need.

In addition to these programmatic issues, the project had several specific educational goals (described below). In today's rapidly changing world, the public is becoming increasingly interested in, and aware of, the relationship between organisms and their environment. The role of ecology and ecosystem studies has a proven place in an engineering student's education, particularly in the emerging discipline of ecological engineering. However, students tend to look at organisms as isolated elements, rather than as systems. This project was designed to highlight the links between organisms and develop a systems approach to ecological relationships. The relationships that are created and maintained in this micro-ecosystem (mesocosms) can be used as the basis for understanding how organisms, including humans, affect the environment.

System Conceptualization

Ecology is the study of the relationships between organisms and other components of the environment. In the ecosystem for crayfish that can be created in this project, these relationships can be broken down into three components. Students should observe all of these components and try to balance the system in such a way that all of the components remain healthy. It is important to be able to both recognize and quantify the trophic relationships of the organisms within the ecosystem, since these are the primary variables that can be controlled in the design process. The major trophic categories in an ecosystem include the primary producers, consumers, and decomposers. The organisms within these subgroups form the basis for the rates of material and energy transfer within the ecosystem. Therefore, the modeling effort was directed towards defining the three primary trophic level relationships in the ecosystem.

Overlaying onto these relationships were the physical-chemical pathways. On a global scale, these pathways are effectively closed and form the major material cycles involved in sustaining the global biosphere. In constructing the model ecosystem, attention must be paid to establishing these cycles — particularly the oxygen, carbon, and nitrogen cycles — at adequate rates to sustain the various biota in the ecosystem.

Primary Production

Primary production includes the organic matter that supports all biological activity in freshwater ecosystems. Primary production is the process by which inorganic carbon (as carbon dioxide) is fixed into organic forms in biomass, producing oxygen, which other organisms use for respiration. The energy and matter that is fixed in this biomass is transferred to various levels within the ecosystem by transfer routes initiated by herbivores that graze on live plant biomass, or by detritivores that consume waste of organic matter after the senescence and death of the producer (Valiela 1991). In this project, only anacharis was considered for this trophic level, although the modular nature of the design allows the incorporation of other producer species in future iterations. The choice of anacharis was based on its easy availability at pet stores, its applicability as a food source for crayfish, its easy experimental manipulation, and the significant literature that documents its behavior.

The Consumers

Since the overall goal of the project is to design a closed ecosystem to sustain crayfish, consumers in the system play a pivotal role in the model conceptualization. The rates of feeding, respiration, and waste production (respiratory and metabolic) for the crayfish must be considered in the mesocosms design. The choice of crayfish for this role was based on two features. First, crayfish are invertebrates. This minimizes the regulations concerning their use. The use of vertebrate animals requires extensive oversight and approvals (Council 1996). Second, they are practical animals for use by students. They are inexpensive, small, robust, and available (Huner 1999).

Crayfish require aquatic habitats but they are able to survive on dry land as long as their gills remain moist. In a favorable environment, crayfish have the potential to live three years or longer. Crayfish tend to hide during the day and scavenge for food at night. They are very aggressive in nature, which leads to frequent fights and even cannibalism. When molting, a crayfish forms a vulnerable new exoskeleton and instinctively seeks cover in rocks and other hiding places during these times. Their diet can consist of submerged aquatic vegetation, emergent plants, small fish, snails, insect larvae, worms, and (of course) other crayfish.

The Decomposers

Organisms in the sealed ecosystem generate waste as organic and nitrogen compounds, particularly ammonia (Brock et al. 1991). Microorganisms oxidize the organic wastes as part of their metabolism, consuming oxygen and producing carbon dioxide in the process. Balancing an ecosystem model must, therefore, rely on the quantification of the bacterial component of ecosystem production. Particular attention should be focused on the cycle of nitrogen within the ecosystem, following the processes of nitrification and denitrification. The oxidation of many nitrogenous compounds can consume large quantities of oxygen. It is recognized that ammonia is a potential source of oxygen depletion in water (Delwiche 1981). Also, microbial nutrient cycling may act as a material sink for many biomass constituents (Valiela 1991). This is of particular importance in the modeling of a closed ecosystem since there are no geological material sinks or transport processes that can replace lost material.

Project Description

The objective of the project was to design and build a sealed ecosystem capable of keeping a crayfish alive and healthy. The ecosystem under consideration consisted of a sealed (air-, water-, and food-tight) 10-gallon aquarium. Each group was issued three to four crayfish, a fish tank, and some assorted hardware. Students were asked to come up with a way to place an airtight seal on the tank that could be opened and resealed. The seal was required have a water sampling port that could be used without breaking the seal.

One person from each group was assigned the task of checking on the aquarium each day and measuring specific water quality parameters through the duration of the project. The water quality parameters that were of importance were selected by the class and updated as the student's understanding of the system grew. Ecosystems were designed by first portioning the sealed tanks into aquatic and terrestrial portions, and then by selecting among various plants and invertebrate species in appropriate proportions to balance the various biochemical cycles to maintain crayfish vitality.

The Laboratory

At the beginning of each semester, the students are asked to identify critical variables for supporting these animals, and to decide if, when, and how they will be measured. The decision-making process moves from a thought experiment to a laboratory experience, and the students are asked to design and make measurements on the crayfish and their environment. The laboratory took place in four major segments. In each segment, the students monitored the system, assessed its stability, and took corrective actions based on their assessment. The four stages were:

1. Develop a stable, open system. In this stage, the students learned about the needs of the crayfish, set up their tanks, developed proficiency with the water quality tests, and allowed the tanks to stabilize. During this period, the students became aware of the complexity of their challenge. As the self-organization proceeds, environmental variables within the microcosm became inextricably linked.

2. First iteration of the sealed system (one week). During this phase the students sealed the tanks and monitored the tank conditions. Because of the short duration of this phase, the mesocosms did not have time to break down. However, there was sufficient time for water quality trends to be identified and corrective to be action taken.

3. Second iteration of the sealed system (two weeks). During this phase, the students resealed the tanks and continued to monitor the tank conditions. During this phase, the students responded to longer-term changes in the system. For example, during this stage, food availability first became an issue.

4. Third iteration of the sealed system (four weeks). This was the final “acid-test” for the student’s design.

Because of its complexity the mesocosms were developed through an iterative sequence of conceptualization, implementation, analysis and redesign. Multiple iterations ensure that the students actively structure the processes occurring in their systems, since they have to re-perform the experiment. The systems often become unstable within a day or two in the first design iteration. The system crashes require that the students abort their experiment, restructure their ecosystem, and reseal. This iterative phase of the laboratory lasts until the end of the course. The iterative failure and redesign cycle results in stronger designs (Petroski 1992) and increases the student’s confidence in their design abilities. We have also found that the students thoroughly enjoy the opportunity to work in an open laboratory where they cans control the experimental structure.

Typical Class Schedule

Date	***Event***
Week 1	Animals into Tanks
Week 4	1st Sealing of the Tank
Week 5	1st Opening of the Tank / Full Design Proposal Due

Week 7	2nd Sealing of the Tank
Week 9	2nd Opening of the Tank
Week 10	Final Sealing of the Tank / Design Progress Report
Week 14	Final Opening of the Tank
Week 15	Final Design Report and Presentation Due

Materials Required for the Laboratory

The materials that should be obtained prior to setting up the tank are listed below. Approximate prices (in US dollars for the year 2000) follow each item

Item	*Cost*	*Source*
Ten-gallon glass aquarium tank	$20	Pet Store
Aquarium gravel (5 lb. bag)	$7	Pet Store
Crayfish (2 to 4)	$1/each	Carolina Biological Supply/ Pet Store
Dechlorination Chemicals	$5	Pet Store
Snail (1 to 6)	$1/each	Pet Store
Anacharis plants (~1 bundle per crayfish)	$2-3/bunch	Pet Store
PVC pipe (~1 1/2” diameter x 4-6” long) or Small plastic cups		Grocery Store
Aquarium dividers (optional)	$7/each	Pet Store
Bucket		Bring from home
Aquarium Thermometer	$2	Pet Store
Dishwashing scrubber pad	$1	Grocery Store

Project Deliverables and Schedule

In addition to the project itself, there were three written assignments and one oral presentation. These included a design proposal, a design progress report, and a final design report. The purpose of the design proposal was to develop a detailed design of the enclosure and its contents. The preparation of the proposal required that the students acquire the background information to successfully complete the laboratory. The second assignment, the design progress report, was short (< 3 pages). The report described the status of the project, where the project was with respect to the design goals, and the changes made to correct any identified problems. The final design report consisted of two parts, a 15-minute presentation to the class and a written final design. This design was based on the initial design plus all of the design changes that occurred during the semester.

Experimental Data Gathering

Once the tank was set up, water quality had to be tested on a regular basis to insure that the environment was safe and healthy for the organisms. One of the traits of ecological systems is that they will "self organize." That is to say, if properly set up, over time the system will change and move towards a steady state. After the ecosystem is established and the bacteria and algae are properly recycling nutrients and breaking down wastes, the testing of water quality parameters can be done on a less frequent basis.

Biomass Measurements

The anacharis and crayfish biomasses were determined by weighing the organisms. Weighing the gravel, removing the bacteria using multiple washes in an ultrasonic cleaner, and reweighing the clean gravel approximated the bacterial biomass. Measuring these biomasses allowed the students to predict the needs of the crayfish as they grew.

Water Quality Analysis

Additional consideration was given to water quality parameters that might influence the health and vitality of the biological constituents. The main parameters included temperature, dissolved oxygen concentration, pH, alkalinity, and ammonia concentration.

However, while the system is organizing itself, it is also in its most fragile state. It is during this time that testing should be done on a daily basis. After the ecosystem has established itself, testing can be done on a less regular basis is order to save money on expensive testing equipment. If the budget allows, more tests can be added and the students can examine different parameters that may contribute to crayfish health. Factors other than just the water should be recorded. Observing the crayfish can be an important indicator on how the ecosystem is doing. Such observations as feeding habits and behavior can indicate the levels of certain water quality parameters. Doing research on the behavior of crayfish can give students the opportunity to apply theoretical knowledge to an actual experiment. To observe these changes, a log sheet for each tank was prepared. The sheets that have been used included columns for time of day, pH, ammonia, nitrite, nitrate, alkalinity, temperature, and animal and plant behavior.

A wide range of water quality analyses is available. The ones that students chose to perform were limited only by their imagination and our budget. While our students had access to a water-testing laboratory, most of the important parameters can be measured using test strips. One good source for these strips is the Hach Company. They have an extensive array of tests in their Environmental Education Catalog. Some relevant kits are:

Parameter	*Catalog Number*	*Approximate Cost*
Ammonia	27553-25	$15
Nitrate/Nitrite	27454-25	$15
5 in 1 Test: Total Chlorine Free Chlorine Total Hardness Total Alkalinity pH	27552-50	$11

pH levels should be maintained within the range of 6.7 to 8.5. (Lee and Wickins 1992). The ammonia levels in the tank should remain below 1 mg/L. For a desirable living environment, the alkalinity should remain between 40-200 mg/L $CaCO_3$. If the alkalinity is low, baking soda (calcium bicarbonate) can be added to the tank to increase the alkalinity. Ammonia is the most toxic of the compounds in the nitrogen cycle. It should not exceed 1 mg/L

Conclusions

Another invaluable benefit is the hands on experience that can be had with a project such as this. Working with the chemicals and methods required for water testing and setting up the tank can be carried over into other projects and, eventually, the workforce. Students will have had the opportunity to approach complex projects in an organized scientific manner. Furthermore, the crayfish habitat provides a unique opportunity to combine several fields of science and engineering. Students applied chemistry, biology, and design. This integrated approach to examining a system gave students an opportunity combine science and engineering in a real world setting and was more complex and interesting than a purely classroom experience.

Bibliography

Brock, T. D., Madigan, M. T., and Martinko, J. M. (1991). *General Microbiology*, Prentice Hall, Englewood Cliffs, N.J.

Council, N. R. (1996). *Guide for the care and use of laboratory animals*, National Academy Press, Washington.

Delwiche, C. C. (1981). *Denitrification, nitrification, and atmospheric nitrous oxide*, Wiley, New York.

Engineering Accreditation Commission (2000). “Criteria for Accrediting Engineering Programs,” Accreditation Board for Engineering and Technology, Baltimore.

Huner, J. V. (1999). “Keeping Crawfish as Pets.” *Aquaculture Magazine*, 25(2), 54 - 62.

Frye, F. 1992. *Captive Invertebrates A guide to their Biology and Husbandry* Malabar, FL: Krieger Publishing Company.

Lee, D. O. C., and Wickins, J. F. (1992). *Crustacean farming*, Halstead Press, New York.

Petroski, H. (1992). *To engineer is human: the role of failure in successful design*, Vintage Books, New York.

Valiela, I. (1991). "Ecology of Water Columns." Fundamentals of aquatic ecology, R. S. K. Barnes and K. H. Mann, eds., Blackwell Scientific Publications, Oxford England; Boston, vii, 270.

Acknowledgements

The authors would like to thank the students of ENBE 484 and ENBE 688P for their assistance in the development of this project and Ms. Anne Bet for her support in the preparation of this manuscript.

PAUL SCHREUDERS
Paul Schreuders is an Assistant Professor of Biological Resources Engineering at the University of Maryland. He teaches Biological Systems Controls and Biological Responses to Environmental Stimuli to seniors and is part of a team developing a completely web based course. His research interests include cryopreservation and the structure and function of bacterial biofilms.

ANDREA LOMANDER
Andrea Lomander is a graduate student in the Department of Biological Resources Engineering at the University of Maryland. She was part of a team of graduate students that developed a virtual mesocosms based on this project. Her research interests include the image analysis and the evaluation of sanitizers on bacterial biofilms.