

Note: Dr. Harold Ornes is the editor of Ecology 101. Anyone wishing to contribute articles or reviews to this section should contact him at the Office of the Dean, College of Sciences, SB130A, Southern Utah University, Cedar City, UT 84720; phone (435) 586-7921; fax (435) 865-8550; e-mail ornes@suu.edu.

*Our first article is by Norman Slade, Natural History Museum and Department of Ecology and Evolutionary Biology, University of Kansas, Lawrence. For those of us who have problems with jackknifing, bootstrapping, and permutations of ecological data, Professor Slade offers a randomization technique that should be helpful in both undergraduate and graduate coursework.*

*The second article is by John F. Boyer, Department of Biological Sciences, Union College, Schenectady, NY. Although Professor Boyer obviously knows the value of knee-crawling quadrat sampling in ecology, he offers a computer program that allows students to manipulate quadrat size, number, and distribution without the full-scale field intensity required for a perfect set of data.*

*Our third contribution is by Lawrence B. Cahoon, Department of Biological Sciences, The University of North Carolina at Wilmington. Professor Cahoon has discovered a gold mine of ecological source material in arguably the last understudied ecosystem on the urban landscape. . . stormwater detention ponds. Although my personal favorite study sites are animal and human sewage lagoons, if you desire more information on stormwater detention ponds, further exercises related to this subject can be found at Professor Cahoon's web address. Enjoy.*

## **RANDOMIZATION TESTS USING STANDARD STATISTICAL SOFTWARE**

Ecologists routinely use standard statistical tests, both parametric and nonparametric. However, in some situations, particularly with small sample sizes or violation of distributional assumptions, standard analyses are problematic. Randomization methods have become an increasingly popular alternative in recent years (Solow 1990, Manly 1991, Potvin and Roff 1993, Legendre 1997, Roxburgh and Chesson 1998), and so might be included in exercises in ecological data analysis. However, software implementing randomization or permutation tests frequently is not available. In the absence of specialized software, many standard statistical packages offer options for resampling from data sets. The results of repeated resampling could be summarized and used to generate percentiles for test statistics that may follow nonstandard distributions. The methods are relatively simple but often involve using tricks to condense results into usable form. I discuss randomization and related computer-intensive analytical techniques in a graduate seminar on data analysis, and have found that students appreciate working through a real example.

To illustrate, consider the following problem. An investigator collected estimates of population size from six sites, with the number of replicates varying from 1 to 6 (2, 1, 2, 6, 5, 5) among the sites. The numbers of replicates from the first three sites were extremely small and the investigator was uncomfortable with the assumption of normally distributed measurement errors. The Kruskal-Wallis test seemed appropriate to test

for differences among population sizes at the sites (Sokal and Rohlf 1995), but a problem arose in assessing the significance of the resulting test statistic,  $H$ . The distribution of  $H$  would follow a chi-square distribution, as implemented in Testimate (Ellison 1996) or MINITAB (Minitab 1996), but only if the samples sizes were five or more within each group (site, in this example). A table of critical values was available for sample sizes less than five, but only for three groups and  $\alpha = 0.05$  (Conover 1971). The investigator needed to compare the sample value of  $H$  to the distribution of  $H$  when the 21 observations were assigned randomly to six groups of the appropriate sizes. Specialized software, such as StatXact (Ellison 1997), would perform this test efficiently, but the cost is high (>\$900) unless such problems are encountered quite frequently.

Our task was to generate 1000 random assignments of observations to groups, and calculate a value of  $H$  for each randomization. The  $P$  value of the test would be determined by placing the original value of  $H$  within the rank-ordered 1000 random values. At our university, MINITAB was readily available, so it was used in the demonstration problem, but many commonly available statistical packages for personal computers can perform similar operations. We used a PC with a 200-MHz Pentium processor and 32 Mb of RAM, running under Windows 95.

The data were entered into two columns—the first column contained the response variable, population size, and the second contained an integer specifying the site—and the Kruskal-Wallis statistic was determined to be 10.95 with a  $P$  value of 0.052 when compared to a chi-square

distribution with five degrees of freedom. The program also printed a message warning of small sample sizes in one or more groups.

We used a text editor to create an executable file (an exec file in MINITAB jargon), rankw.mtb, which consisted of two lines of code,

```
sample 21 c2 c3  
kruskal c1 c3
```

and saved it as simple text.

The first command randomized the group codes for 21 observations in column 2 into column 3 without replacement. If your software does not have a feature for sampling from original data, generate a column of  $N$  random numbers, where  $N$  is the total number of observations in all groups. Then sort the values for the response variable by the values of the random numbers. Because the group codes remain constant, the values of the response variable are randomly assigned to groups. In our module, the second line of the file executed the Kruskal-Wallis test for the original data in column 1, but assigned observations to the groups indicated by the codes in column 3. We then executed this module 1000 times ("exec 'rankw' 1000" was the MINITAB command) and the 1000 values of  $H$  were embedded in 18 lines of output generated for each randomization. We saved this output to a text file. (Actually, 18,000 lines of output exceeded the size of the buffer, so we saved the output in two files, as prompted by the program, and then merged with a text editor.) Each value of the Kruskal-Wallis test statistic was printed as  $H = XX.XX$ , and we needed to rank-order the 1000 values to identify the rank of our observed value. Because the merged output file was simple text, we sorted it using the DOS sort command. Its size (800+ K) would require a different sort strategy with Microsoft oper-

ating systems predating Windows 95. We then marked the column of values of  $H$  (depress the ALT key while marking the block), pasted them into a column of the MINITAB worksheet, and sorted them. Our observed value of 10.95 was 981st of 1001 values, so we concluded that population sizes differed among sites at the  $P \sim 0.02$  level. The entire analysis took less than 30 minutes, including deciding how to execute each step. Repeated analyses or a laboratory exercise could be done in about 15 minutes on a comparable computer. It is also worth noting that our 1000 values had a sample mean of 4.94 and variance of 6.44. In contrast, a chi-square distribution with five degrees of freedom has a mean of 5 and a variance of 10. Thus, the  $H$  statistics for our randomization data probably did not follow a chi-square distribution, so determining probability levels through randomization was preferable for this problem.

This example illustrates the general applicability of randomization methods to data that do not lend themselves to traditional analyses. Furthermore, such analyses can be conducted in the absence of specialized software. Randomization methods are not covered in most current introductory statistical texts, yet ecological studies, especially class projects, frequently result in small data sets sampled from unknown distributions. Thus working through similar problems seems appropriate for courses in ecological data analysis. Once students have worked through examples, the mysteries of jackknifing, bootstrapping, and permutation methods are diminished.

### Acknowledgments

I am grateful to Helen Alexander and Rob Swihart for comments on the text, to Matthew Brady for the example problem, and to students at the University of Kansas for their interest in learning modern approaches to statistics.

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## QUADRAT SAMPLING: A COMPUTER-GENERATED LABORATORY EXERCISE

Perhaps no methodology in ecology has produced such a large gap in the classroom between promise and realization as the analysis of spatial distributions by quadrat sampling. Not only is this sampling technique fundamental to many field studies, but as a class exercise it has much to offer—it certainly provides an excellent opportunity to get students into the field and close to the organisms! Moreover, it is easy to lay out the quadrats (providing they are not too large), and usually in any habitat a few species are numerous and easily identified even by inexperienced students. But the data collection itself can go very slowly, and all too frequently a long afternoon of quadrat sampling yields results that are equivocal or simply too sparse to reveal the underlying patterns; with inconclusive data, students will not discover the utility of the statistical analyses that the ecologist uses as stock in trade. To alleviate the problem of insufficient data, I have written a computer program that generates different spatial patterns for two species.

The computer simulation [program Spatial] allows for an arbitrarily large number of quadrats and a [relatively] high density of individuals per quadrat. Consequently, it generally produces meaningful comparisons between two or more (usually three, in my course) different “habitats.” Perhaps most importantly, this exercise enables students to see the analytical consequences of using different quadrat sizes when sampling the same area; this is often quite difficult to do when relying solely on data collected in the field.

In my population biology course, I use Spatial prior to class to generate the worksheets for the students, who usually work in pairs or threesomes. These worksheets simulate different “habitats” in which individuals of two species are placed on a 100 x 100 grid; see Figs. 1–3. I run the program repeatedly for each habitat with the same parameter values but with a different random seed, so each group is given a replicate of each of three environments. The designations such as Forest, etc., are arbitrary and, for convenience in the student exercise, usually the circles and diamonds are assumed to represent the same species in all three habitats (obviously an unrealistic assumption).

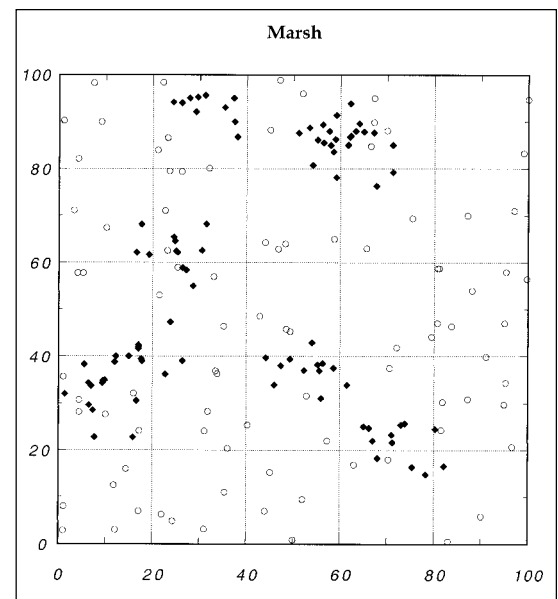
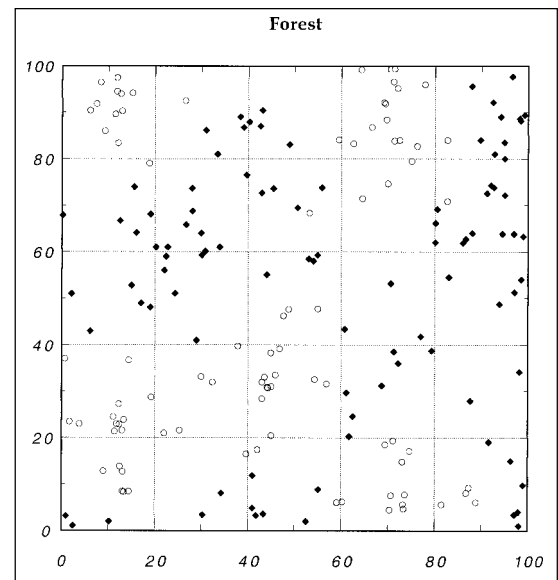
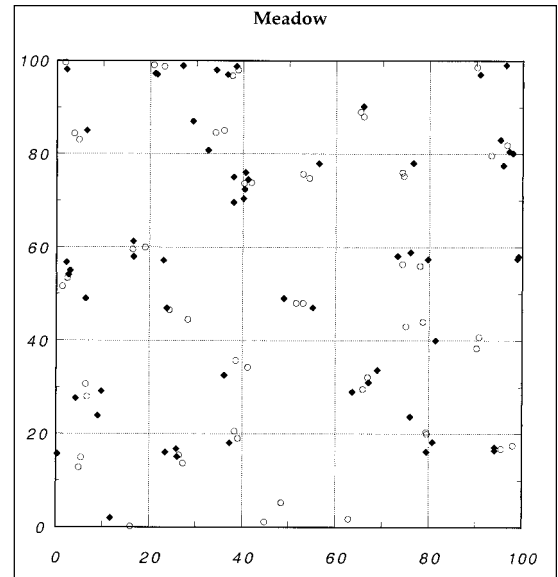
The students begin by enumerating the two “species” on a quadrat-by-quadrat basis, each quadrat being 20 units on a side. Thus their primary data consists of a 2 x 25 table for each habitat. Their write-up proceeds in three stages:

### 1) Single-species analysis

For each species in each environment, students determine the mean number of individuals per quadrat and the associated variance. These are used to compare the densities between the habitats on a pairwise basis, and then they derive the aggregation (or dispersion) indices based on the respective mean/variance ratios.

### 2) Two-species analysis

Students carry out both graphical [plotting  $n_{1i}$  vs.  $n_{2i}$  for each habitat, where  $n_{1i}$  and  $n_{2i}$  are the number of each species in the  $i$ th quadrat,  $i = 1$  to 25] and chi-square analysis of species association. Several different approaches to quantifying two-species association are discussed.



### 3) Effect of quadrat size

Each group reanalyzes *one* of their three habitats by using the 10-unit tick marks to subdivide each quadrat into four 10 x 10 quadrats. Fifteen of the original 25 large quadrats are randomly selected and then two small quadrats (e.g., upper-left and lower-right) in each of the 15 are tallied, so that the primary data for this part of the exercise is a 30 x 2 table. The students then repeat the steps taken in parts 1 and 2.

Spatial is written in Think Pascal for the PowerMac; the only nonstandard coding is the pseudo-random number generator (*function random*) which uses an integer variable of type *computational*, but most machines and languages will have their own intrinsic pseudo-random number function. However generated, the pseudo-random variables must be uniformly distributed over the interval [0,1].

The innovative feature of this program is the use of Cluster Points, which determine the interactions both within and between species, but which are not themselves drawn on the grid. The symbols corresponding to individuals of each species are placed at random in proximity to their respective Cluster Points—either closely (low R1 or R2 values) or more widely dispersed (R1 or R2 in the range of 8 to 20). There are eight parameters supplied by the user (four for each species):

CP1, CP2: specifies the number of Cluster Points for each species.

PointsOne, PointsTwo: specifies the number of individuals in each cluster.

R1, R2: determines the region around each Cluster Point within which individuals are restricted.

D1, D2: determines the separation of Cluster Points from each other.

Species One Cluster Points are generated first—they can be random with respect to each other (D1 = 0) or overdispersed by placing each Cluster Point a minimum distance (D1 > 0) from all other Cluster Points. Species Two Cluster Points are placed with respect to Species One's Cluster Points. For D2 = 0 they are indepen-

dent; a positive D2 places the points at least this distance from any of Species One's Cluster Points, whereas a negative D2 places the points within D2 units of a Species One Cluster Point.

The simplest distribution is attained by using a D1 value of 0 and assigning just one individual (PointsOne = 1) to each Cluster Point (the value of R1 is immaterial in this case); the resulting distribution of species One is Poisson with mean and variance = CP1/25. The parameter values for the illustrated habitats (in order of CP1, PointsOne, R1, D1/CP2, PointsTwo, R2, D2) are: Meadow: 30, 2, 3, 14 / 60, 1, 1, -5; Forest: 5, 20, 16, 32 / 100, 1, 2, 18; Marsh: 90, 1, 1, 0 / 8, 12, 10, 0.

The program Spatial does not draw the grids, which are standard X, Y scatter plots that all graphing programs provide. My worksheets are drawn using the commercial application, *Kaleidograph* (the data from the file, "RunName," are transferred to the graphing program using the `IMPORT` command). The grids as printed are 15 cm on a side, and therefore fit on letter-size paper (this is a satisfactory size when using a laser printer). Although all the cluster points lie within the grid, a few of the individual points may lie outside it, and these are eliminated by the Spatial program prior to export to the graphing program; as a consequence, the total number of individuals for a species may be a little less than CP1 x PointsOne or CP2 x PointsTwo.

As presented, the program generates a constant number of points per cluster; this does not diminish the program's pedagogical effectiveness, but some teachers might wish to have a more realistic version in which the number of individuals in each cluster is a random variable with an expectation equal to PointsOne or PointsTwo. It would be straightforward to add a procedure that chooses the values for PointsOne and PointsTwo for each Cluster Point from an explicit stochastic distribution.

I find it rewarding to put on the board for each habitat the analyses of

several different laboratory groups. The variability among the replicate worksheets is usually striking, particularly in the values of the dispersion indices based on the mean/variance ratio. The students also discover that the human eye/brain complex is sometimes superior to numerical analysis in discerning the essentials of the spatial patterns placed before them. Overall, however, the principal objective of the exercise is achieved—the student analyses reveal the significant differences between the habitats and the different spatial relationships between the two species. (And the students are introduced to the Poisson distribution, a fundamental concept in many areas of biology, which does not seem to be presented in any other courses, including the required cognate math courses.)

The code (as a text listing) for Spatial is available at the web site <<http://www.union.edu/PUBLIC/BIODEPT/Quadrat.txt>>. I also include a listing for the program used by the students in selecting the 10 x 10 quadrats.

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## EXPERIMENTAL AQUATIC ECOLOGY IN STORM-WATER DETENTION PONDS

### Introduction

Ecologists are especially fond of having our students study natural ecosystems for many important reasons, not least of which is to understand "how things ought to work if people don't mess them up." However, human impacts are becoming ubiquitous, and it makes sense to study altered, even constructed, ecosystems as well, especially considering how easy it is to find them. For example, recognition that stormwater runoff is a significant source of water

pollutants has led to regulations implementing various stormwater management practices in the last decade, including the widespread use of stormwater detention ponds (Schueler et al. 1992). Wet detention ponds, which retain a pool of standing water between storm events, can be designed for flood management, sedimentation control, or other water quality improvements based on catchment basin characteristics, storm event characteristics and frequency, and the desired level of management (Wanielista and Yousef 1993). The recent proliferation of stormwater detention ponds offers new opportunities for aquatic ecology exercises. These small aquatic ecosystems, although artificial, are rapidly colonized by a diverse biota and readily lend themselves to various studies of basic and applied aquatic science problems.

Stormwater detention ponds are now quite common in urban and suburban areas, particularly in locations where development activity is rapid. New Hanover County, a coastal county in North Carolina that includes the campus of the University of North Carolina at Wilmington (UNCW), the city of Wilmington, and adjoining unincorporated suburbs, now has well over 250 permitted stormwater detention ponds, more than twice as many as in 1992. Many of these lie within a short distance of the UNCW campus, facilitating their use for aquatic science instruction.

Here, I describe some representative laboratory exercises using local stormwater detention ponds that I have conducted during the last 4 years in a senior undergraduate/graduate limnology course. The exercises presented here are part of a larger set. For those interested in more detail and a description of the complete set of exercises, the "Pond Project" is presented on a Web page: <http://www.uncwil.edu/people/cahoon/>

### **Student exercises using stormwater detention ponds**

I first assign students to find five stormwater detention ponds in their neighborhoods, measure their

approximate dimensions, and draw a simple map showing the layout of each pond with its inlets and outlet. The aims of this exercise include making them aware of how many of these ponds there are, demonstrating their basic characteristics, and allowing the students to observe the biological features of these artificial aquatic ecosystems, which typically include various aquatic plants, amphibians, small fishes, and other wildlife that are almost always established by natural colonization processes. The students are then asked to plot their ponds on a master map of the area so they can see patterns of distribution. One of the take-home messages is that newly developed areas have lots of ponds, but older neighborhoods do not, meaning that stormwater may not be as effectively managed in those areas, and that aquatic habitats are differently distributed. The class can also use the master map (included in the "Ponds Project" Web page) to locate ponds for subsequent exercises.

Another exercise focuses on management of sedimentation, which is recognized as a major cause of water quality problems and aquatic habitat degradation in much of the country. Sediment control efficiency is one of the principal design criteria for stormwater detention ponds (Wanielista and Yousef 1993). Measurement of total suspended solids (TSS) is one way to evaluate pond performance. I assign groups of students to sample water in the inlets, outlets, and midsections of old (> 1 year) and new (<1 year) detention ponds, and then measure TSS by gravimetry (APHA 1995). We compare TSS values between "old" and "new" ponds (expecting newly constructed ones to have higher levels from recent soil disturbance), and between inlets and outlets (expecting that TSS levels should decline in the outlet water). We have also measured light attenuation using quantum sensors to test the hypothesis that TSS levels drive light attenuation properties. We have usually found that stormwater detention ponds perform as intended, with low TSS values in

most effluents and ponds (except the very newest). We have also found no significant correlation between TSS levels and light attenuation, which forces the students to consider alternate explanations, such as varying levels of coloring substances, and the differences between the parameters: suspended solids and turbidity.

Nutrient management is another important function of stormwater detention ponds (Schueler et al. 1992) that lends itself to investigation by student exercises. Stormwater detention ponds are typically designed to have relatively high turnover times, i.e., short water residence times, so that nutrient loading rates are usually high. We have evaluated the trophic state of detention ponds using Carlson's Trophic State Index (Carlson 1977), which derives from measurements of Secchi depth, total phosphorus, and chlorophyll *a*. Owing to the shallow depths of detention ponds, use of a Secchi disk is often impractical, so we calculate Secchi depth from measurements of the light extinction coefficient obtained through simultaneous measures of light intensity at two different depths, using a pair of quantum sensors. There are various methods for measuring total phosphorus and chlorophyll *a*; we use the methods of Valderrama (1981) and Welschmeyer (1994), respectively. Most local stormwater detention ponds have trophic states in the mesotrophic to moderately eutrophic range (50–70 on Carlson's 0–100 scale). Somewhat surprisingly, even relatively young (< 2 years) ponds have fairly high trophic state scores (owing to relatively high light extinction coefficients), whereas the oldest ponds (> 8 years) have higher total phosphorus and chlorophyll *a* component scores. However, we rarely find ponds with highly eutrophic scores (>85), perhaps because most of the plant growth in eutrophic ponds occurs as periphyton and macrophytes, which are not measured as chlorophyll *a*.

We have evaluated nutrient limitation patterns in stormwater detention ponds using nutrient deletion bioas-

says, in which all but one of the macronutrients, nitrogen (N), phosphorus (P), or silicon (Si), are added to pond water in experimental vessels, and phytoplankton growth response is measured as changes in chlorophyll *a*. One such experiment compared the response of phytoplankton biomass to additions of various combinations of macronutrients in “new” and “old” detention ponds (Fig. 1). Phytoplankton in the “new” pond were apparently not limited by any nutrient, suggesting light limitation in this highly turbid pond, whereas the “old” pond’s phytoplankton appeared strongly nitrogen limited, suggesting the accumulation of excess phosphorus over time. The results of these experiments must be interpreted carefully, as they are subject to the limitations of all such bottle experiments (“Level II” experiments in Hecky and Kilham’s (1988) scheme), and reflect only the responses of phytoplankton from ecosystems usually dominated by periphyton and macrophytes.

Stormwater detention ponds also lend themselves to whole-pond experimentation (“Level IV” experiments in Hecky and Kilham’s (1988) scheme) owing to their small size and similar morphometry. We conducted one such whole-pond experiment

investigating nitrogen and phosphorus limitation, with permission from the North Carolina Division of Water Quality. We added nitrate to one pond, phosphate to another, and a combination of the two to a third, and then monitored responses over a week-long period. Phytoplankton biomass increased noticeably in the pond receiving N + P additions, slightly and only initially in the pond receiving N addition, and slightly in the pond receiving P addition. Comparisons of nutrient deletion experiments conducted before and after the N and P additions were made showed that N + P addition appeared to relieve nutrient limitation altogether. That is, none of the nutrient deletion experiments produced results different from controls, whereas addition to ponds of N or P alone yielded less clear results. We suspect that the abundant periphyton and macrophytes in these ponds masked the nutrient addition effects to some degree, but their biomass and responses to nutrient additions are far harder to measure.

The exercises described here exemplify some of the many possibilities for using stormwater detention ponds for instructional purposes, but several caveats apply to using stormwater detention ponds for experimental and observational exer-

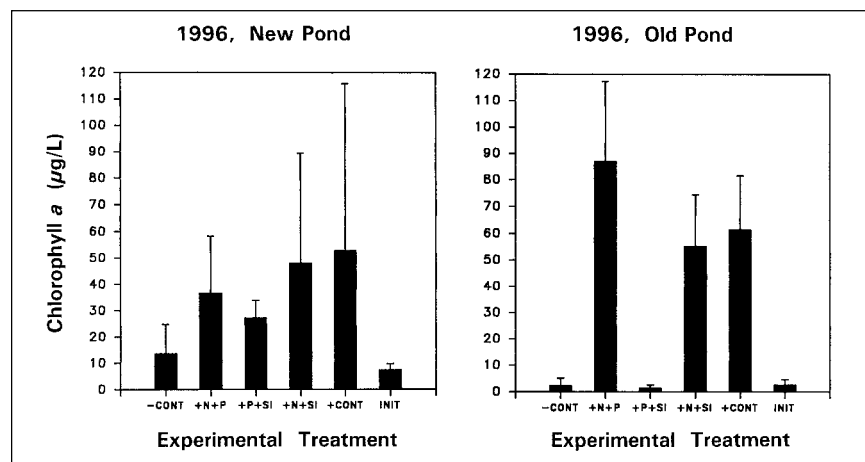
cises. We have found that fecal coliform bacteria levels were high enough in some ponds to suggest a pathogen problem (Cahoon 1996), so direct personal contact with the water should be managed carefully. Some ponds are on private property and are fenced, so access is difficult and might involve trespass; we avoid such problems by sampling other ponds. Whole-pond manipulations are relatively easy to do, but permission should be sought and manipulations should not create water quality problems downstream. In some cases, management agencies may actually be pleased to facilitate studies that may help them. The use of stormwater detention ponds represents an experiment in water quality management that our society has conducted for approximately the last decade and must now evaluate. Ecologists can use them to teach our students important ideas in aquatic ecology and learn some valuable new things at the same time.

### Acknowledgments

The work presented here was supported by grants from the National Science Foundation’s Division of Undergraduate Education (DUE 9551941), the Northeast New Hanover Conservancy, and New Hanover County. This is Contribution Number 210 of the UNCW Center for Marine Science Research.

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**Fig. 1.** Results of nutrient addition experiments conducted in new and old stormwater detention ponds in 1996 in New Hanover County, North Carolina, measured as biomass (chlorophyll *a*) response 1 week after nutrient additions. Control treatments are no nutrients added (–cont) and all nutrients added (+cont).

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# *What Are Your Dreams?*



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