

Institute of Wildlife and Environmental Toxicology

TITLE: The Effects of Golf Course Activities on Wildlife

INVESTIGATORS:

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CLIMATIC REGION: Warm Arid

USGA REGION: Western

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Executive Summary

In the spring of 1991, the United States Golf Association funded a proposal entitled "Environmentally Sensitive Techniques in Golf Course Management: A Model Study of the Ocean Course, Kiawah Island, South Carolina" submitted by The Institute of Wildlife and Environmental Toxicology (TIWET) at Clemson University. The basic objective of the project was to understand the "Golf Course ecosystem." This includes an understanding of how birds, fish, and plants respond to golf course chemical inputs as well as pesticide and nutrient behavior in water, soil, and sediments. This information will be integrated into an ecological risk assessment which can be applied, initially, to the Ocean Course and ultimately, to a wider distribution of courses. In addition to the research that provides a strong scientific foundation upon which to build an ecological risk assessment, there has also been a great deal of regional, national, and international interest in the project to date. Regional television and radio stations as well as state newspapers have picked up the progress of the project. More recently the project has been covered several times by CNN News. The golf course research group consists of seven graduate students and five faculty members from the Department of Environmental Toxicology and TIWET at Clemson University. An update on the research will be provided at the annual meeting of the United States Golf Association Green Committee to be held at Kiawah Island in November 1992.

INTRODUCTION and OBJECTIVES

The United States Golf Association (USGA) is committed to promoting environmentally safe management techniques on golf courses. The USGA is pursuing a variety of research projects on golf courses that address pesticide fate and other issues such as potential disturbance to wildlife but the work here at the Ocean Course in South Carolina represents a true interdisciplinary project involving integration of chemical transport and fate, water quality, plant and wildlife exposure to chemicals, and the integration of these data into an ecological risk assessment.

1992 OBJECTIVES

1. To establish a model study at Kiawah Island Course, South Carolina, Ocean Course to promote environmental issues associated with Golf Course maintenance.
2. Evaluate wildlife utilization of the course during the four seasons of the year and through the Golf Course development operations process.
3. Assess both surface and ground water resources for pesticide residues and other contaminants which might effect environmental quality of the site.
4. Work with Kiawah Island Golf Course Operations to develop an environmentally sensitive management plan that will encourage safe wildlife utilization of the area, minimize chemical use, and support environmental design features which recycle water and minimize the use of pesticides.

In order to establish a model study representing an interdisciplinary research effort to evaluate the "Golf Course Ecosystem" the Kiawah Island Ocean Course was selected as an excellent model for the following reasons:

1. A diverse wildlife habitat.
2. Significant thought and design features were built into the course by the architect and builders.
3. The architect and owners have a sincere interest in cooperating in verifying the environmental features of the Course.
4. The Course exists in a very sensitive ecological zone between wetlands and ocean frontage in the proximity of TIWET allowing easy access to the site.

The Ocean course has offered challenging opportunities to characterize pesticide fate as well as evaluate the response of plants and animals to pesticides and other management practices on the Golf Course. In addition, the integration of our research findings with golf course management is critical if we are to have an impact on the industry. Indeed with the press coverage from the local, regional, national, and international levels the transfer of what we are learning into public awareness has been and will continue to be extremely important. We have also involved the Golf Course Superintendents Association of America. Recently the President, Mr. Bill Roberts, had an opportunity to make a statement on CNN News on the importance of the environment related to Golf Course Management. This model study of the Ocean Course at Kiawah Island represents an opportunity to not only develop scientific information but catalyze the transfer of new knowledge to environmental approaches of golf course management.

Update as to research progress and the present report will include the following chapters:

1. Assessment of Potential Exposure and Impacts of Organophosphate Pesticides to Avian Species on the Kiawah Island, South Carolina Ocean Course.
2. Biochemical Assessment of Wildlife Exposure to Pesticides.
3. Estimating Anti-Cholinesterase Sensitivity in Avian Species.
4. Determining Pesticide Movement in Representative Soil Matrices from the Kiawah Island Ocean Course.
5. Golf Course Chemical Management Practices and Their Effects on the Susceptibility of Sheepshead Minnows (*Cyprinodon variegatus*) and Mosquitofish (*Gambusia affinis*) to Disease and Nitrogen Excretion, a Comparison of *in situ* and Laboratory Data.
6. Hydrographic Characteristics and Planktonic Productivity: Baseline Estimates of Chlorophyll a and Organic Biomass to Ocean Course Lagoons.
7. Determination of Uptake and Peroxidase Response as Indicators of Sublethal Pesticide Exposure to Aquatic Macrophytes.

Chapter 1

Assessment of Potential Exposure and Impacts of Organophosphate Pesticides to Avian Species on the Kiawah Island, South Carolina Ocean Course

Thomas Rainwater and Ronald J. Kendall

INTRODUCTION

The use of organophosphate (OP) pesticides on golf courses to control turfgrass pests has resulted in numerous incidents of exposure and mortality to avian species. Stone and Gradoni (1985) report 16 exposure incidents from 1970 to 1985 involving 8 states and resulting in over 900 mortalities. One incident resulted in the death of 700 brant (Branta bernicla) from exposure to the OP diazinon (O,O - diethyl O - [2-isopropyl - 4 - methyl - 6 - pyrimidinyl] phosphorothioate) on a Long Island golf course. In 1986, 85 American wigeon (Anas americana) died after grazing on one diazinon-treated fairway in Bellingham, Washington (Kendall et al., 1992).

Organophosphate pesticides act by inhibiting the enzyme cholinesterase (ChE). Cholinesterase inhibition produces an accumulation of acetylcholine at nerve synapses which disrupts the normal transmission of nerve impulses. Symptoms of severe OP poisoning include respiratory difficulty leading to respiratory arrest, paralysis, convulsions, and death (Stone and Gradoni, 1985). Waterfowl seem to be at the greatest risk to OP exposure on golf courses because many of them consume the very turfgrass to which pesticides are commonly and directly applied. However, other avian species can also be exposed; directly, by making contact with the treated turf, or indirectly, by consuming contaminated food items.

The Ocean Course on Kiawah Island, South Carolina is situated in a sensitive ecosystem and is surrounded by saltwater marsh, brackish marsh, maritime forest, beach, and dune habitats. With these different habitat types comes a wide variety of wildlife, including a diverse and

abundant avian population. Numerous species of seabirds, wading birds, raptors, and passerines are attracted to the different habitats on the course and are commonly observed year round. Like other golf courses on Kiawah Island, the Ocean Course employs some use of OP pesticides to control turfgrass pests.

The terrestrial component of the Kiawah Island Ocean Course project is focused on determining if avian species are being exposed to OP pesticides on the course, and if so, determining the levels of exposure and the resulting effects. The overall objective is to determine the feasibility of a golf course monitoring program to assess potential exposure and impacts of OP pesticides to avian species under normal management practices. The following information is an overview of the activities conducted and data collected during the 1992 field season.

1992 OBJECTIVES

1. To determine what avian species are present on the ocean course
2. To determine which species on the course are readily usable for sampling purposes
3. To collect biological samples to measure pesticide exposure

METHODS and MATERIALS

In mid February 1992, twenty-seven bluebird nest boxes were placed on the Ocean Course for two purposes: 1) to help determine if eastern bluebirds (Sialia sialis) were present on the course, and 2) to encourage bluebirds to nest in the boxes. If bluebirds were present on the course, and if they actively used the nest boxes, the nesting population could be closely monitored for pesticide exposure. Generally, bluebird nest boxes are placed in open areas away from overhanging vegetation. However, in order to minimize interference with golfers, nest boxes were placed close to trees and wooded areas. Several small groves of live oaks (Quercus virginiana) are located on the front nine while very few trees are present

on the back nine. Hence, the majority of the nest boxes, 21, was placed on the front nine and the remaining 6 were placed on the back nine.

Avian surveys were conducted to help determine what species are present on the course. The surveys were conducted in the early morning (05:30 - 08:00) and in the late evening (18:30 - 21:00). The observer would begin at a tee box and walk slowly to its corresponding green, recording data along the way. At other times, the direction of the survey would be reversed, and the observer would walk from green to tee box. When necessary, the observer used binoculars to identify far-away species and was also allowed to deviate from the normal survey route to identify a bird that fled from the survey area. The recycling lagoon was considered part of the front nine during the surveys, and the driving range was considered part of the back nine. Birds observed were placed into three categories: songbirds (passerines and non-passerine land birds), water/wading birds (seabirds and wading birds), and raptors. Waterfowl would have been included in the water/wading birds category, but none were observed during the surveys. Data collected during avian surveys included species observed, numbers within each species observed, location, habitat, and activity.

Walk-in traps were placed on the Ocean Course to determine catchability of different species. A total of 10 traps, 5 quail-funnel traps and 5 passerine-funnel traps, were used. Trapping began June 10, and traps were moved frequently to sample different areas on the course. Sites on which traps would be placed were cleared and leveled, and then 2 weeks prior to trapping, the sites would be baited with wild birdseed to attract avian species. Each bird captured was identified and released. Mist nets (12 and 12.6 meter) were placed on the course at several different locations to catch birds in flight.

Three types of biological samples were collected to measure potential exposure of avian species to OP pesticides: 1) Blood samples, to measure ChE activity, 2) Footwash samples, to serve as indicators of dermal exposure, and 3) Avian carcasses, to analyze brain ChE and body residues. The collection of biological samples from trapped and mist-netted birds began August 4. Blood samples were collected from juvenile bluebirds in early June. Blood samples were taken from the jugular vein

using a 1cc syringe equipped with a 27-gauge needle. Samples were then transferred to microcentrifuge tubes and stored on ice. Once out of the field, samples were centrifuged at 2000 rpm for 10 minutes using a Damon IEC centrifuge. The supernatant, plasma, was then pipetted into a new microcentrifuge tube which was labeled and stored in a freezer at approximately -25 C. Footwash samples were collected by holding a bird's feet over a stainless steel funnel that led into a Nalgene bottle and rinsing the feet with 100% denatured ethanol. Samples passed through the funnel into the bottles which were then sealed with teflon lids. Once the lids were secure, the bottles were labeled, placed on ice, and ultimately refrigerated. Each avian carcass collected on the course was placed in a ziploc bag, labeled, and frozen at approximately -25 C.

PRELIMINARY RESULTS and PERSPECTIVES

Bluebird nesting activity began in late April and lasted through early August. This first year, four nest boxes were used and yielded 5 nests, 21 eggs, 17 young, and 13 fledgings (Table 1). Failure of eggs to hatch can most likely be attributed to infertility or inexperience of the female as a breeder, in which case the eggs may not have been properly incubated. Mortality of juveniles prior to fledging is most likely due to predation. Three nests were built in boxes on the back nine of the course, and two nests were built in boxes on the front nine. The two nests on the front nine were built in the same box, and all boxes used for nesting were situated among high or overhead vegetation. Although nesting activity was low, it was encouraging to learn that bluebirds were in fact present on the course. In addition, it is very possible that nesting activity will significantly increase next spring after bluebirds have had a year to become more accustomed to the nest boxes.

Approximately 782 birds were observed during avian surveys: 600 water/wading birds, 180 songbirds, and 2 raptors. The large difference between the number of water/wading birds observed and the number of songbirds observed is due to several flocks of brown pelicans (Pelicanus occidentalis) and laughing gulls (Larus atricilla) that flew overhead or were seen on the driving range and were included in the survey. The majority of the songbirds observed during the survey were located on the front nine, while the most of the water/wading birds and raptors were

Table 1. Bluebird nest box activity (Spring - Summer 1992)

Box #	# Eggs	# Hatched	# Fledged
3	4	3	3
24	4	4	4
26	5	5	1
23	4	4	4
3	4	1	1
Total	21	17	13

observed on the back nine (Table 2). Songbird predominance on the front nine of the course is most likely attributed to the presence of trees. While very few trees are present on the back nine, several live oaks are located on the front nine and provide vital cover for songbird species. Most songbirds observed during surveys were either perched in or flying to and from live oak trees.

The predominance of water/wading birds on the back nine can be attributed to the large brackish creek connecting Willet and Ibis ponds. The majority of water/wading birds observed during surveys was located in this creek, and birds were actively probing and feeding on fish and other aquatic organisms. Water/wading birds were also observed on the two ponds located along #16 fairway and #17 green.

Although only two raptors were observed during the survey, both were located on the back nine. Raptors feed primarily on rodents, which are abundant in the dune habitat of the back nine. With practically no tree cover to impair their vision, the raptors were undoubtedly attracted to this large and somewhat vulnerable rodent population.

In addition to location, avian surveys also showed bird activity in relation to time of day. Songbirds and raptors were both equally active in the early morning and late evening, while water/wading birds were slightly more active in the early morning (Table 3). By knowing when and where species are located on the course and when and where OP pesticides are applied, we can better predict which species are at the greatest risk of chemical exposure.

In addition to the avian surveys, birds observed at random were also noted. Twenty species were recorded during random observation, but this included only those species that were not observed during surveys or species that were observed together in large numbers. Several species were observed on the actual golf course turf. Some species, especially laughing gulls, congregated in large numbers and loafed on fairways. Several other species actually probed and fed from the golf course turf. By coming in contact with the very turf to which OP pesticides are applied, these species greatly increase their potential for chemical exposure. Following pesticide application, species walking on treated

Table 2. Occurrence of species on the front 9 and back 9 of the Ocean Course

Category	Total Birds	% Birds Front 9	% Birds Back 9
Songbirds	180	73	27
Water/wading birds	600	28	72
Raptors	2	-	100
Total	782		

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Table 3. Morning and evening occurrence of species on the Ocean Course

Category	Total Birds	% observed in AM	% observed in PM
Songbirds	180	51	49
Water/wading birds	600	64	36
Raptors	2	50	50
Total	782		

areas can be exposed dermally. Species probing for food items in the turf are at a great risk of oral exposure and subsequent ingestion of the chemical applied (Stone and Gradoni, 1985).

As was mentioned in the introduction, waterfowl seem to be at the greatest risk of OP pesticide exposure on golf courses. No waterfowl were observed on the Ocean Course during the summer 1992 field season, but data concerning waterfowl presence on the course were obtained through personal communication. Mr. Robert W. Cowgill of St. John's Island, SC generously provided a copy of a survey he conducted on the Ocean Course during the winter of 1991-1992. The survey was primarily concerned with migratory waterfowl on Ocean Course ponds but also included other avian species observed near the ponds. Four species of waterfowl and 10 species of water/wading birds were observed. Table 4 provides a categorical breakdown of all species observed during avian surveys and random observation as well as those species observed by Mr. Cowgill. Also included are those species that were trapped and not seen elsewhere. Overall, 60 different avian species were observed on the Ocean Course from November 1991-August 1992 including 36 species of water/wading birds, 18 species of songbirds, 4 species of waterfowl, and 2 species of raptors.

Seventy-five birds representing 9 species were trapped on the course from June 10 through August 7 (Table 5). Traps were moved to different locations across the course to examine trapping success in other areas. However, most trapping was conducted on the front nine of the course because it provided more cover and prevented traps from interfering with golfers' play. As a result, most birds were captured on the front nine. Five of the nine species were trapped more frequently in the evening than in the morning, but the three most frequently trapped species were captured more often in the morning. These three species, common grackles (Quiscalus quiscula), red-winged blackbirds (Agelaius phoeniceus), and boat-tailed grackles (Quiscalus major) will most likely be designated as target species. Only one bird, a least bittern (Ixobrychus exilis), was captured in a mist net. Mist-netting failure can most likely be attributed to a lack of background vegetation which helps camouflage the net from a bird's view. Most birds caught in walk-in traps are seed-eaters, so we relied on mist nets to catch insect-eating species. Because

Table 4. Categorical breakdown of all avian species observed on the Ocean Course

CATEGORY	# SPECIES
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SONGBIRDS	18
Top 5:	
1.	Red-winged Blackbird
2.	Tree Swallow
3.	Eastern Kingbird
4.	Northern Mockingbird
5.	Mourning Dove
WATER/WADING BIRDS	36
Top 5:	
1.	Laughing Gull
2.	Brown Pelican
3.	Snowy Egret
4.	Forster's Tern
5.	Black Skimmer
WATERFOWL (Cowgill, pers. comm.)	4
Top 4:	
1.	Bufflehead
2.	Hooded Merganser
3.	Lesser Scaup
4.	Northern Shoveler
RAPTORS	2
Top 2:	
1.	Red-tailed Hawk
2.	Osprey
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Total # Different Species -	60

Table 5. Avian species trapped on ocean course (Summer 1992)

Species	#	%AM	%PM
Common grackle	37	62	38
Red-winged blackbird	12	58	42
Boat-tailed grackle	7	57	43
Mourning dove	6	33	67
Painted bunting	5	20	80
Northern cardinal	3	33	67
Brown-headed cowbird	2	100	-
Brown thrasher	2	-	100
Northern mockingbird	1	-	100
Total	75		

of the lack of success in mist-netting, alternative trapping methods will be used in the future.

Biological samples were collected to measure potential exposure of avian species to OP pesticides. Three blood samples were collected from juvenile bluebirds. Seventeen blood samples were collected from 7 species of trapped and mist-netted birds. Twelve footwash samples were collected from 5 different species, and 4 avian carcasses were collected. These biological samples will be analyzed and will serve as baseline data to which future samples can be compared.

FUTURE GOALS

To assess potential exposure of avian species to OP pesticides, goals have been set for winter 1992-93, spring 1993, and the summer 1993 field season. The information obtained during the 1992 field season will serve as the foundation for the remainder of the study.

GOAL #1 -- CONTINUE AVIAN SURVEYS - Avian surveys are currently being conducted in the fall of 1992 and will continue throughout winter 1992-93 and spring 1993. Once completed, a full year of survey data will have been accumulated and will be used to compare seasonal use patterns of avian species on the course. This information will be useful in predicting at what time(s) of the year pesticide applications are more likely or least likely to affect birds on the course.

GOAL #2 -- ENCOURAGE MORE BLUEBIRD NESTING ACTIVITY - Additional bluebird nest boxes will be placed on the course to encourage more nesting activity. If a large nesting population can be established, it can be closely monitored to assess potential exposure and possible reproductive impacts of OP pesticides to an insect-eating species.

GOAL #3 -- CONTINUE TRAPPING AVIAN SPECIES - Walk-in funnel traps will continue to be used to capture avian species, but other trapping techniques will be employed in attempts to increase the number and diversity of species captured.

GOAL# 4 -- COLLECT MORE BIOLOGICAL SAMPLES - More blood and footwash samples will be collected during the summer 1993 field season and compared to samples collected in 1992. In addition, all avian carcasses collected in 1993 will be analyzed for brain ChE activity and body residues and compared to those collected in 1992.

Accomplishing these goals will enable us to achieve the overall goal of the terrestrial component of the Ocean Course project -- to determine the feasibility of a golf course monitoring program to assess potential exposure and impacts of organophosphate pesticides to resident and migratory avian species.

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Chapter 2

Biochemical Assessment of Wildlife Exposure to Pesticides

Vincent A. Leopold and Michael J. Hooper

INTRODUCTION

Many pesticides commonly used on golf courses are organophosphorus (OP) compounds. Their mechanism of action is the inhibition of cholinesterases (ChEs), vital for the proper function of cholinergic neurons. In such neurons, acetylcholine transmits nerve impulses by diffusing across the synapse, activating a receptor and causing depolarization of the post-synaptic cell. The enzyme acetylcholinesterase (AChE) catalyzes the hydrolysis of acetylcholine in the synaptic gap allowing the repolarization of the post-synaptic membrane. Thus ChE serves to regulate transmission of nerve impulses by controlling the amount of neurotransmitter, acetylcholine, present in the synapse. OP pesticides characteristically have a high affinity for ChEs. Butyrylcholinesterase (BChE) may protect the nervous system against OP pesticides. However, when the plasma concentration of inhibitor is sufficient AChE is inhibited, acetylcholine accumulates in the synapse, and the post-synaptic cell remains depolarized causing paralysis of that nerve and its affected tissue, whether nervous, muscular or glandular. When enough nerves controlling vital functions are inhibited by an anti-ChE, the animal becomes incapacitated and may die.

Though many turf pests are effectively controlled with OP insecticides, concurrent poisoning of non-target species can result. Avian species are particularly susceptible to non-target poisoning as they feed on turf or on insects containing chemical residues. The level of free, uninhibited ChE is depressed in animals exposed to OP insecticides. Thus, the magnitude of OP exposure can be determined by measuring the amount of ChE inhibition.

PURPOSE AND OBJECTIVES

The purpose of this project is to determine whether or not avian species are exposed to OP insecticides on Kiawah Island's Ocean Course. This determination entails measuring relative amounts of the different types of plasma ChE and measuring the amount of ChE bound by OP compounds.

1. Determine levels of acetylcholinesterase (AChE), butyryl - cholinesterase (BChE) and total ChE in plasma samples collected from the Ocean Course.
2. Determine levels of OP inhibition of ChE in plasma samples from the Ocean Course.

METHODS

Blood samples were taken from several avian species, the cellular fraction discarded, and the plasma frozen until analysis according to SOP 302-01-04. Levels of individual plasma AChE, BChE, total ChE and OP - inhibited ChE were measured in a single assay using the methods of Ellman et al. (1961) and Martin et al. (1981) modified for use on a 96-well plate reader according to SOP 202-07-03. Samples were incubated with iso - OMPA (tetraisopropyl pyrophosphoramidate), a specific BChE inhibitor, to determine AChE activity, whereas BChE activity is simply the difference between total ChE and AChE. Incubation of plasma samples with 2-PAM (pyridine-2-aldoxime methochloride) reactivates any OP-inhibited enzyme. Thus, the amount of inhibited ChE is the difference between ChE activity with and without 2-PAM treatment.

RESULTS AND RESEARCH PERSPECTIVES

Seventeen plasma samples, representing eight avian species have been collected and are currently frozen. Plasma from four individual red - winged blackbirds (Aegelaius phoeniceus) have been analyzed and show no evidence of OP exposure. Analysis of the remaining samples is expected to be completed by November 7, 1992. The results are not expected to show inhibition by OP compounds because no pesticides were used on the

course just prior to sampling. However, the results are expected to be valid baseline data describing plasma cholinesterases in the 17 individual birds.

FUTURE GOALS

More avian plasma samples will be collected. It is particularly important to collect samples pre- and post-application of any OP pesticides. This capability depends on frequent communication with the golf course superintendent prior to the use of such pesticides.

Strategies for applying the above methods to laboratory-raised sheepshead minnows (Cyprinodon variegatus) dosed with acephate and clorpyrifos will be developed. Then, field-sampled sheepshead minnows will be assayed and any ChE inhibition quantified. Thus, the scope of ChE inhibition as a measure of OP exposure will be expanded to include both avian and fish species.

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Chapter 3

ESTIMATING ANTI-CHOLINESTERASE SENSITIVITY IN AVIAN SPECIES

Vincent A. Leopold and Michael J. Hooper

INTRODUCTION

Because of increased public awareness and concern about chemicals in the environment, scientific research is seeking methods by which deleterious effects of such chemicals can be predicted. Currently, for registration of all outdoor-use pesticides, the U.S. Environmental Protection Agency (EPA) regulations require test data using bobwhite quail (*Colinus virginianus*) and mallard ducks (*Anas platyrhynchos*) (Federal Insecticide, Fungicide, and Rodenticide Act, section 162.82). These species were selected because they can be easily bred and maintained in controlled laboratory conditions and because they are two important game bird species. The ideal test species would be that which exhibits measurable toxic effects at the lowest dosage of a particular chemical.

The toxicity of anti-cholinesterase pesticides to wildlife can vary considerably between orders, species, age groups and sexes. This variability can make it difficult to select the most sensitive (ideal) test animal. However, Schafer and coworkers (1972, 1973, 1979 and 1983) have demonstrated patterns of susceptibility to organophosphate (OP) and carbamate pesticides among several avian species. These patterns indicate that passerine species (except the adult European starling) tend to be the most sensitive avian group. Therefore, a passerine species is a valid choice for a test species in the evaluation of a carbamate or OP pesticide.

How does one choose which passerine species' survival to quantify and evaluate in actual field application experiments? One method is to make LD50 or LC50 determinations (respectively, dosage and concentration which result in 50% mortality) in potential species with the

chemical of interest. Considering the large number of pesticides that need testing, the enormous number of bird kills needed for LD50 or LC50 determinations makes this method unsatisfactory. A non-lethal method that could predict sensitive species would be a desirable way to select a test species for large field evaluations of pesticide effects.

Introduction of an anti-ChE pesticide into non-target species (birds, for example) is predominantly by ingestion. The pesticide's mode of toxicity is high affinity binding to ChE, decreasing the availability of this enzyme for its normal function of hydrolyzing acetylcholine and allowing repolarization of postsynaptic cells. The result of toxic exposure is permanent depolarization of postsynaptic cells, i.e. paralysis.

The toxicity of a carbamate or OP is determined by a number of factors. These include, in order of their occurrence after the pesticide's absorption from the gut, the following:

- 1) The ability of the plasma to degrade or sequester the active ChE-inhibiting form of the chemical via inhibition of plasma esterases, degradation by plasma OP hydrolases and non-specific hydrolysis by plasma proteins;
- 2) The rates at which the liver activates (OP's primarily) and degrades (both OP's and carbamates) the pesticide and subsequently excretes it through the bile;
- 3) The innate sensitivity of brain acetylcholinesterase (AChE) to the pesticide;
- 4) The rate at which the brain activates (OP's primarily) and degrades the pesticide; and
- 5) The rate at which the kidney performs the similar functions as the liver and excretes parent compound and the metabolites into the urine.

OBJECTIVES

The ability of avian plasma ChE to sequester and degrade a particular anti-ChE pesticide is an important part of the animal's defense against that chemical. We will characterize this ability in different species to provide useful information on potentially predicting pesticide

susceptibility. Investigation of the protective function of avian plasma esterases is proceeding in the following four phases:

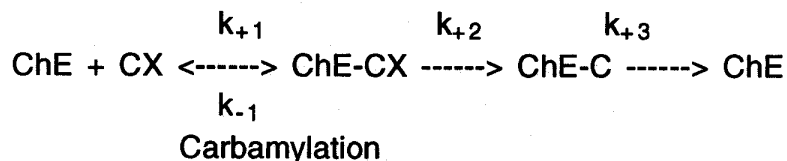
- 1) Quantify acetylcholinesterase (AChE), butyryl-cholinesterase (BChE), total ChE and carboxylesterase activities in the plasma of individual birds. This will determine the mean and variance of enzyme activities in the samples.
- 2) Determine the kinetics of the pesticide's inhibition of AChE, BChE and carboxylesterase in a pooled plasma sample from each species. These kinetics will be used to rank the species by their sensitivity to the pesticide.
- 3) Determine the pesticide buffering capacity of esterases in a pooled plasma sample from each species. This determination will be made by simulating the introduction of the pesticide into the blood from the gut following oral exposure or from the liver following activation and will examine the inhibition time course and total study pesticide buffering capacity of the plasma esterases.
- 4) Rank the predicted sensitivities to the pesticide of all tested species using their respective characterization, kinetic and buffering data.

METHODS

Method practice has proceeded on phase one using the method of Ellman et al. (1961) modified for use on a 96-well plate reader. AChE activity is quantified using the BChE inhibitor, iso-OMPA (tetraisopropyl pyrophosphoramidate). BChE activity is the difference between total ChE and AChE. Carboxylesterase activity will be measured with the assay described by Ashour et al. (1987) modified for the 96-well plate reader. Absorbance activities are reported as micromoles substrate hydrolyzed per ml of plasma.

Method development and practice with iso-OMPA has proceeded on phase two, kinetics of plasma esterase inhibition, using methods described in Aldridge and Reiner (1972), Kurh and Dorough (1976) and Wang and Murphy (1982). A bimolecular reaction constant, k_i , will be determined using the kinetics of the following reaction between ChE and an inhibiting pesticide (carbamate in this example):

Enzyme - Carbamate
De-carbamylation
Complex Formation
Enzyme Regeneration



A logarithmic progression of inhibitor concentrations is incubated with the plasma enzymes for time periods of 0 to approximately four minutes. A slope is determined for the time verses log percent remaining activity curve for each inhibitor concentration. Slope values are then plotted against the log of their inhibitor concentration and the slope of this new line is determined. Constant values are determined from the slope ($1/k_{+2}$) and Y-intercept (K_a/k_{+2}) of this line.

The reaction constant, k_i , is used to describe the overall potential of the pesticide to inhibit the enzyme. It is calculated from the following equation:

$$k_i = \frac{k_{+2}}{K_a}, \quad \text{where } K_a = k_{-1}/k_{+1}$$

Generally, the greater the affinity of a pesticide for an enzyme, the greater the k_i . Therefore, k_i values can be used to rank the potential for inhibition, i.e. the sensitivity of different species to a pesticide.

In phase three, the buffering capacity tests will attempt to mimic the role that plasma esterases play in the sequestering of an anti-ChE pesticide as it would be absorbed into the plasma from the gut, lung or skin. The inhibitor will be slowly infused into a pooled plasma sample from which aliquots will be removed over time and tested to determine

remaining esterase activity. These measurements will describe the ability of plasma esterases to protect the system by binding potentially dangerous anti-ChE pesticides.

The characterization of ChE's and carboxylesterases, inhibition and recovery kinetics, and plasma buffering capacity characteristics will provide the information needed to predict relative sensitivities of any studied species to any studied pesticides. Thus, we hope that the use of this non-lethal method can be used to determine what species should be used in large field studies of pesticide effects.

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Chapter 4:

Determining Pesticide Movement in Representative Soil Matrices From the Kiawah Island Ocean Course

Frank C. Bailey, J. L. Cowles, Carol P. Weisskopf, and S. J. Klaine

INTRODUCTION

To determine the environmental fate of pesticides and nutrients applied to a golf course, the flow dynamics and drainage of the system must be understood. Soil columns have been used traditionally to model the movement of chemicals in specific soil types over time (Grover, 1977; Utermann, 1990). Soil columns will be constructed in the laboratory that model the soil matrices found under greens, tee boxes, and fairways of the Ocean Course on Kiawah Island.

It is important to know the residence time of analytes in the drainage system to assess the ultimate fate of a chemical. If the toxicants are held in the system for long periods of time ($\gg 2$ half lives), they may be degraded into nontoxic components before emerging from the drainage system. In this case they pose no threat to wildlife, as they are not bioavailable. However, if residence time in the subsurface drainage is short (< 1 half life) the resulting exposure to wildlife could be significant.

HYPOTHESIS AND OBJECTIVE

Hypothesis: Compounds applied to soil columns migrate through the columns before degrading.

Objective: To determine the movement of pesticides through representative soils from the Ocean Course using batch adsorption studies (McCall et al; 1981) and soil columns.

METHODS

Batch adsorption studies will be carried out in 50ml Teflon centrifuge tubes. A range of soil to water ratios and aqueous concentrations of each chemical will be utilized in these experiments. Initially, a 5:1 (V:W) of water:sugar sand or green's mix (sugar sand mixed with peat) will be used with 1ppm concentrations of the pesticides Chlorpyrifos and Acephate to determine adsorptions (K_d) values for these chemicals in each matrix. By varying the soil:water and chemical concentration, Freundlich isotherms will be developed for each chemical in both types of soil.

Soil columns of ID 10cm and length 100cm will be constructed. Columns will be made of glass and all connections will be Teflon. All exposed glass will be silanized and solvent washed prior to use.

Columns will be constructed of sugar sand and greens mix separately, and these components in layers and proportions representative of greens and fairways. Pesticides and fertilizers will be applied to the soil columns at rates that are comparable to field conditions. Eluents will be collected and analyzed for said compounds.

In order to determine the percolation rate for each matrix, blue dextran or a nonbinding radiolabeled compound (tridiated water) will be applied to the columns (Logan et al; ES&T in press). This experiment will be repeated using the pesticides that are applied to the Ocean Course on a regular basis. The data collected in this manner will enable us to move into the field with a better understanding of the system and therefore validate the laboratory findings in a more expedient manner.

RESULTS AND DATA TO BE OBTAINED

- K_d 's for each pesticide in each matrix
- Freundlich isotherms for each pesticide in each matrix
- percolation rate
- organic carbon content
- pore volume of each matrix
- pH, hardness and other physical characteristics of each matrix

- flow rates of pesticides and nutrients through columns under dry soil and pre-saturated conditions
- validate lab results with field lysimeter studies on the Ocean Course
- obtain physical/chemical characteristics of the pesticides of interest, such as K_{ow} and solubility

PRELIMINARY RESULTS

As expected, preliminary soil distribution experiments indicate that a large proportion of Chlorpyrifos is found in the soil compartment, most likely bound to soil organic matter. Cation exchange capacity may also come into play in this process, and will be calculated for each soil. When batch adsorption studies are complete, we will have a better grasp of the retention times in soil for each chemical, and we will then move on to soil column work.

Methods for the extraction of each chemical from water, soil, and plants have been explored, and are being fine tuned. Acephate extraction continues to be a problem, with recoveries around 60%. This is not unexpected given the compound's short half-life and high polarity. Chlorpyrifos extractions are going well, with recoveries approaching 90%.

FUTURE GOALS

By November, 1992 - complete batch adsorption studies

December 1992 - June 1993 - complete soil column work

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Chapter 5

Golf Course Chemical Management Practices and Their Effects on the Susceptibility of Sheepshead Minnows (*Cyprinodon variegatus*) and Mosquitofish (*Gambusia affinis*) to Disease and Nitrogen Excretion, a comparison of *in situ* and Laboratory Data

Barry L. Forsythe II and S. J. Klaine

INTRODUCTION

The proposed study of golf course chemical-use management is intended to address the effects of the current practices invoked on the Ocean Course (Kiawah Is., SC) on the susceptibility of sheepshead minnows (*Cyprinodon variegatus*) and mosquitofish (*Gambusia affinis*) to disease. Present management practices call for the use of various organophosphate insecticides such as acephate (Orthene®), chlorpyrifos (Dursban®) and glyphosate (Roundup®). Along with these and other pesticides, a significant volume of fertilizers are also applied to the course.

Sheepshead minnows, the most euryhaline fish species known, and mosquitofish, also euryhaline but a livebearer, are common fish species found on the Ocean Course. Though euryhaline, mosquitofish populations choose bodies of water with low salinity. Both species are hardy and easily reared in a laboratory setting. There is a vast data base in the literature as to the effects of various xenobiotics and environmental factors on both fish species. An interesting note on the selection of mosquitofish; populations in areas of chronic exposure to pesticides have shown the ability to develop resistance (Vinson *et al.*, 1963, Culley and Ferguson, 1969).

Organophosphates (OP's) and their toxic action are well studied. Most notable are the studies of OP's inhibition of AChE. However, there are other effects described in the literature. Acephate has been shown to increase ventilation and buccal amplitude at levels slightly above the 48h LC50 (Duangsawasdi and Klaver Klamp, 1979). OP's have been shown to

cause skeletal deformities in fish (McCann and Jasper, 1972). Parathion - contaminated food was avoided by mullet (Kleerekoper, 1974). It has been reported that organic pesticides cause pregnant, female *Gambusia* to abort its embryos (Boyd, 1963). Abnormalities in the circulatory system were seen in medaka embryos exposed to parathion and malathion (Solomon and Weis, 1979). The runoff of OP's may have an indirect effect on the fish, in that invertebrates utilized as a food source may be severely impacted causing a nutritional deficiency.

The following table exhibits the acute toxicity (96h LC50) of some of the xenobiotics that might be found on the Kiawah site to common North American freshwater fish (Murty, 1986).

<u>Xenobiotic</u>	<u>LC50 Range</u>
Acephate	100->1,000 mg/l
Chlorpyrifos	2.4-280 µg/l
Glyphosate	"Not Toxic"
MSMA	"Low Toxicity"
PCP	0.22 mg/l

There are five environmental conditions that are important to consider when looking at pesticide toxicity. Increases in temperature cause an increase in metabolic rate and ventilation. This increased ventilation obviously increases the chance of uptake of water soluble toxicants. Reports are conflicting as to whether this causes increased toxicity or not because of the concurrent increase in metabolism of the xenobiotics. The hydrolysis of OP's is controlled by pH. Hardness has been shown to have little effect on OP toxicity (Pickering *et al.*, 1962). A few studies have shown the role salinity plays in pesticide toxicity. *Gambusia* exhibited decreased accumulation of organic pesticides with increased salinity (Murphy, 1970). Very little has been done to characterize the part dissolved oxygen (DO) plays in this convoluted scheme (Pickering, 1968).

The use of susceptibility to pathogens as an endpoint provides a very sensitive measurement of sublethal exposure that may dictate the need for alternative management practices in these systems. The overt toxicity of the chemicals applied may not be sufficient to protect fish from pathogen infection which leads to some altered behavior.

Additionally, unpublished data have revealed that pesticides may influence the excretion of nitrogen waste products, such as ammonia, by fish (Personal Communication, Dr. Mike Hooper, Clemson University). This appears to be promising tool as a biomarker for pesticide contamination.

OBJECTIVES

1. To characterize the water quality of the selected sites on a diurnal basis so as to produce a baseline of possible variables that might produce an effect or mask the effect being tested.
2. To determine the exposure levels of fish to pesticides (OP's).
3. To determine the toxicity of the expected exposure to lab strain (LS) and wild strain (WS) fish (sheepshead minnows and mosquitofish) in a laboratory setting.
4. To determine the effect of sublethal exposures on the susceptibility of the two species to pathogens in the lab.
5. To determine the effect of sublethal exposures on the susceptibility of the two species to pathogens *in situ*.
6. To determine if non-pesticidal agents (i.e. fertilizers) are in fact more of a contributor to susceptibility to pathogens.
7. To determine the effects of chemical management on nitrogen excretion by fish, in the laboratory and field.

METHODS

Water quality data are collected on a bi-weekly basis. Thus far, laboratory analyses indicate the absence of pesticide residues. This is probably due to the fact that pesticide application has been minimal to date.

The third objective stated above will be achieved by utilizing EPA approved acute toxicity tests. A laboratory strain and wild strain will be

maintained in a laboratory setting and exposed to selected pesticides for 96 hours with lethality as the endpoint.

Following these series of tests, a sublethal exposure will be forced upon the fish in the laboratory concurrent with a pathogenic challenge. Contraction of infection will be the endpoint. This information will lead to *in situ* field studies .

Caged fish will be maintained on the course throughout pesticide application events and examined periodically for infection. The type of infection will be characteristic of various environmental conditions. Therefore, the influence of fertilizers on dissolved oxygen and subsequent infection outbreaks can be monitored also.

Since the method of conducting nitrogen excretion assays is unpublished, there have been arrangements to learn the technique.

PRELIMINARY RESULTS AND PERSPECTIVES

In August of 1992, I was charged with the duties of collecting current golf course management strategies used on the coastal courses of South Carolina. The South Carolina Coastal Council wants this information in an attempt to provide superintendents of the courses with various available options to use in managing their course(s) on the coast. A copy of this survey is attached.

A survey of fish species and water quality data collection have been the most significant results to date. The sheepshead minnow and mosquitofish are the only species on the course, except a recent introduction of grass carp. Dissolved oxygen in the lagoons appears to be a widely variable water quality parameter, with concern to the fish.

Currently the process of setting up a culture system for the fish is nearing completion, at which time I will begin conducting acute toxicity tests with the compounds of interest and making comparisons of my data with the LC50's found in the literature.

FUTURE GOALS

Following these initial tests I plan to begin the proposed immunological work. The beginning of 1993 should see the start of these tests. By middle to late spring, I plan to be conducting the tests *in situ*.

Estimations of population densities for the fish on the Ocean Course will be conducted seasonally. Currently I am pursuing the possible use of other ecological measures of chemical perturbation.

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Chapter 6

HYDROGRAPHIC CHARACTERISTICS AND PLANKTONIC PRODUCTIVITY: BASELINE ESTIMATES OF CHLOROPHYLL A AND ORGANIC BIOMASS IN TWO OCEAN COURSE LAGOONS

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CAROL P. WEISSKOPF

INTRODUCTION

Intense fertilizer application to golf turfgrass often generates high nutrient levels in golf course lagoons, as runoff enters the aquatic system. Productivity of algae and other planktonic organisms increases greatly in response to nutrient availability and excessive reproduction may result in adverse effects (Mason 1981). Algae have the capability to add tremendous amounts of organic matter to lentic systems (Hayes and Greene 1984). Hypertrophic systems may become anoxic as dense algal mats prevent oxygen from dissolving into the water's surface. Reductions in dissolved oxygen can provoke "fish kills" over large areas. Extensive phytoplankton growth may generate offensive odors and tastes as anaerobic decomposition occurs.

Seasonal application of chemicals (fertilizers and pesticides) to the golf course, and subsequent runoff, may generate a multiplicity of effects within the aquatic community. Management of nuisance algal populations often involves algicides and biological controls. Because phytoplankton form the foundation of aquatic food chains, alteration of the algal community may have repercussions on higher trophic levels.

Construction of the irrigation water recapture system on the Ocean Course, Kiawah Island, provides researchers the opportunity to track golf course runoff to contained lagoon systems and to examine the effects of turf management practices on the aquatic organisms. Two Ocean Course lagoons were chosen for analysis in the 1992 field season. The lagoon on the back nine receives recaptured water from the 13th, 14th, 15th and 16th fairways, greens and tee boxes. This water may contain chemicals

and nutrients from the soil or surface runoff from a quantifiable area. A "recycling" lagoon is the second study site. This lagoon contains all the water recaptured from the back nine holes of the golf course. Cumulative effects of diverse management techniques may be detectable at this point.

The concentration of the photosynthetic pigment chlorophyll *a* is commonly used as a measure of primary productivity (Marker, *et al.* 1980). Biomass or standing crop is a quantitative estimate of the mass of live organisms within a given area or volume and is most accurately generated by measuring ash free dry weight. Chlorophyll *a* comprises about 1-2% of the dry weight of planktonic algae. These two measurements will be used to assess phytoplankton productivity in the Ocean Course lagoons.

1992 OBJECTIVES

- 1 Estimate primary productivity in the Ocean Course lagoons using measurements of chlorophyll *a* and ash free dry weight.
- 2 Trace seasonal changes in lagoon primary production.
- 3 Correlate levels of primary production and golf course management techniques.
- 4 Describe, quantitatively and qualitatively, the Ocean Course Lagoon ecosystem.

MATERIALS AND METHODS

WATER QUALITY

Each lagoon is divided into three transects, and each site has three transects along it, giving nine sampling sites in each lagoon. At each site on each sampling day water quality measurements are taken at depths of 0.3 m, 1.0 m, 1.5 m, every 0.5 m until the bottom is reached. All samples are collected from a 16 foot aluminum canoe. Five water quality parameters are measured at each depth at each site, including dissolved oxygen (DO), pH, temperature, conductivity, and redox potential. Measurements are taken using a Hydrolab Surveyor 3 Water Quality Logging

System (Hydrolab Corp., Austin, TX) by lowering the probe of the unit to the sample depth, waiting for the readings to stabilize, then storing the information in the Hydrolab. The location and support data are simultaneously recorded by hand on waterproof data sheets. The water samples for chemical and plankton analyses are collected at each site along with the water quality data.

Once water quality data are collected and the Hydrolab is returned to the laboratory, the information is transferred from the Hydrolab to computer and into a spreadsheet program, which allows the data to be put into presentable form both numerically and graphically.

PHYTOPLANKTON DYNAMICS

Each lagoon was divided into equal thirds by three transect lines. Three sampling stations were selected along each transect. Water samples were collected every two weeks at each of the 18 sampling stations using a liter capacity Kemmerer bottle. Samples were collected 50 cm below the water's surface and transferred to 1 quart Cubitainers®. The containers were kept in the dark at 4°C for transport to the laboratory.

In the laboratory, samples were filtered using millipore apparatus, a vacuum pump and glass fiber filters (Gelman AE; 1 µm porosity). Chlorophyll *a* was extracted by placing the filter in a vial and treating with 15 ml of 90% aqueous acetone (10% saturated magnesium carbonate solution). The samples were then steeped for 24 hours at 4°C in the dark.

The concentration of chlorophyll *a* was determined spectrophotometrically on a Beckman DU-70 spectrophotometer. The optical density at 664 and 750 nm was measured and recorded for each sample. Following this reading each sample was acidified using 0.1 N HCl and absorbance measured at 665 and 750 nm to correct for absorbance by the pigment pheophytin.

Corrected values for chlorophyll *a* (OD 664 - OD 750_{before} = 664_b and OD 665 - 750_{after} = 665_a) per cubic meter were determined using the following:

$$\text{Chlorophyll } a, \text{ mg/m}^3 = \frac{26.7(664_b - 665_a) \times V_1}{V_2 \times L}$$

where:

V_1 = volume of extract, L,

V_2 = volume of sample, m³,

L = light path length or width of cuvette, cm, and 664_b, 665_a = optical densities of 90% acetone extract before and after acidification, respectively.

The value 26.7 is the absorbance correction, $A \times K$. Where A is the absorbance coefficient of chlorophyll *a* at 664 nm = 11.0 and K is the ratio correcting for acidification. (APHA, 1989.)

Extracts and filters were then transferred to porcelain crucibles and placed under a hood until the acetone had evaporated. The crucibles and contents were dried at 105°C for 24 hours and dry weight was measured and recorded. The samples were ashed in a muffle furnace at 500°C for one hour. Ash free dry weights were calculated and recorded.

The composition of the phytoplankton community was determined by examining water samples from each transect during each sampling period using an inverted microscope at 300X.

RESULTS

WATER QUALITY

In general, the Recycling lagoon and the back lagoon behaved similarly with respect to water quality parameters (Figure 1). The recycling lagoon will be used here as an example for discussion. The lagoon exhibited thermal and dissolved oxygen (DO) stratification, as might be expected from a eutrophic system (Figures 2 - 6). The DO values varied greatly at some sites, from near 20% saturation at the bottom to over 120% in the surface water (Figure 2). On some dates (as 08/06 and

FIGURE 1

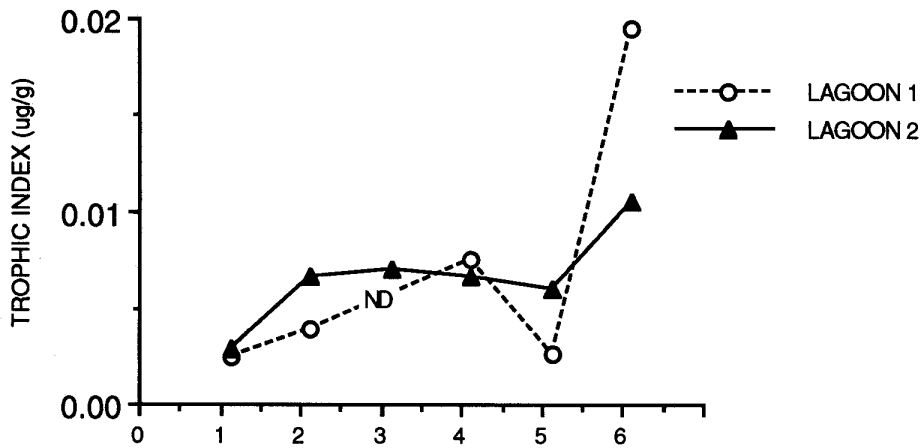
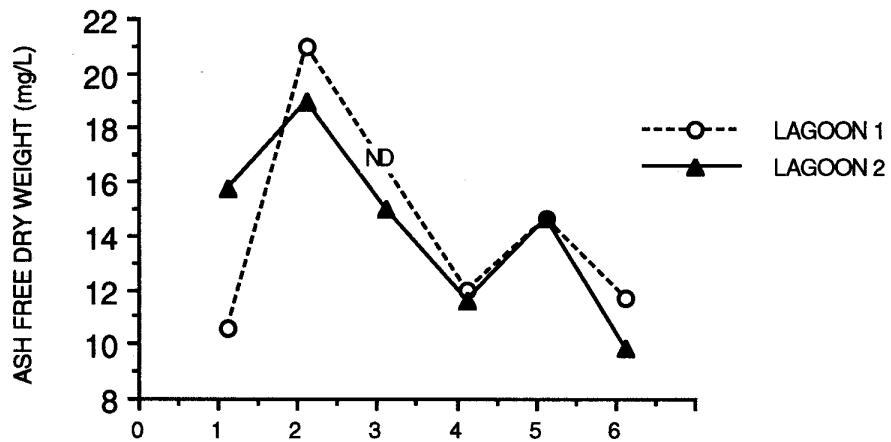
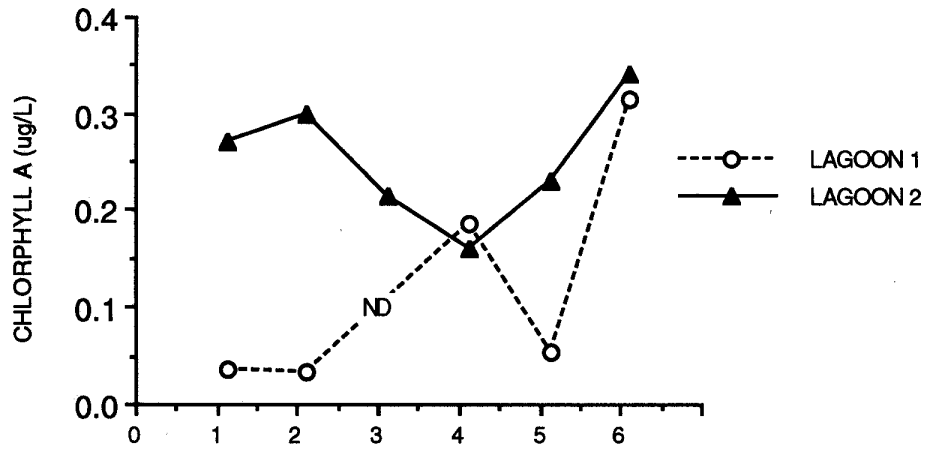
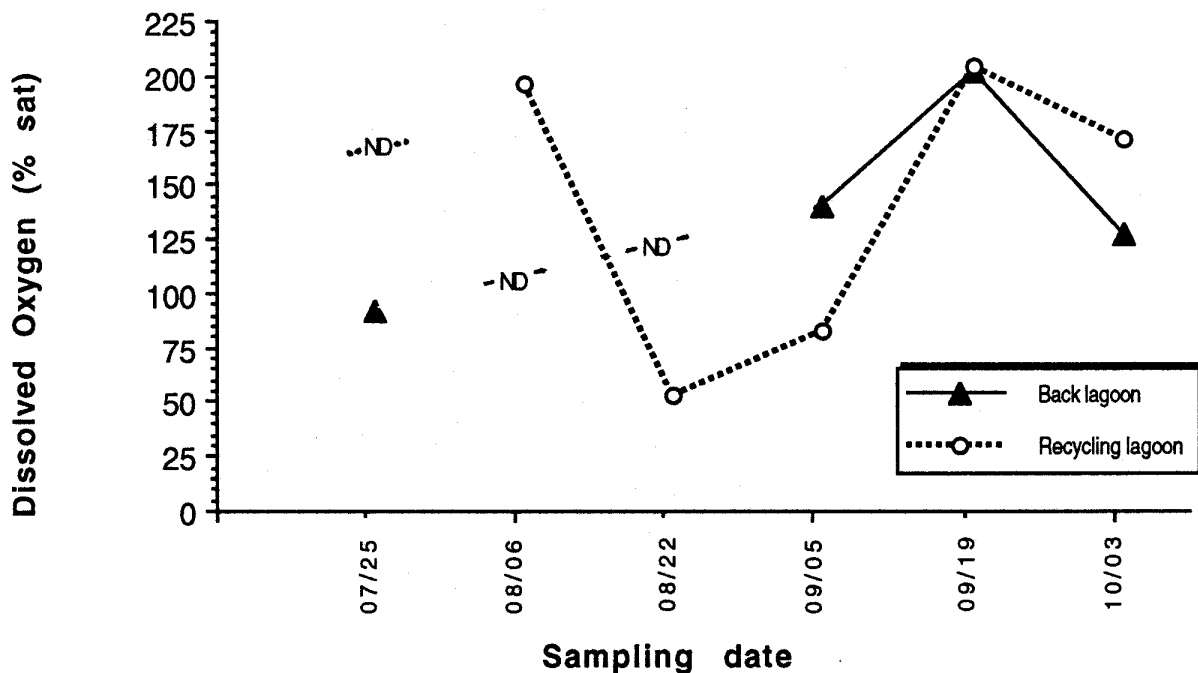


Figure 2

Average Dissolved Oxygen (% sat) levels at 0.3 m for both lagoons



Average pH values at 0.3 m for both lagoons

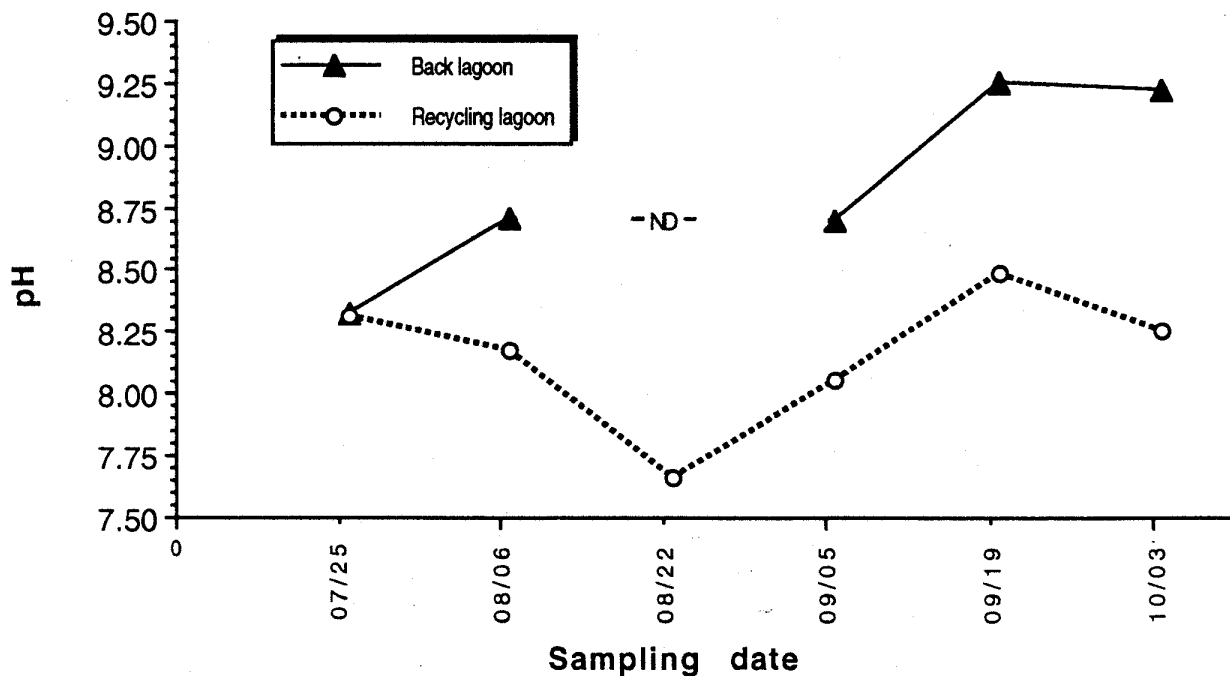


Figure 3

Dissolved Oxygen profile (% sat) for
the Recycling Lagoon on 10/03/92

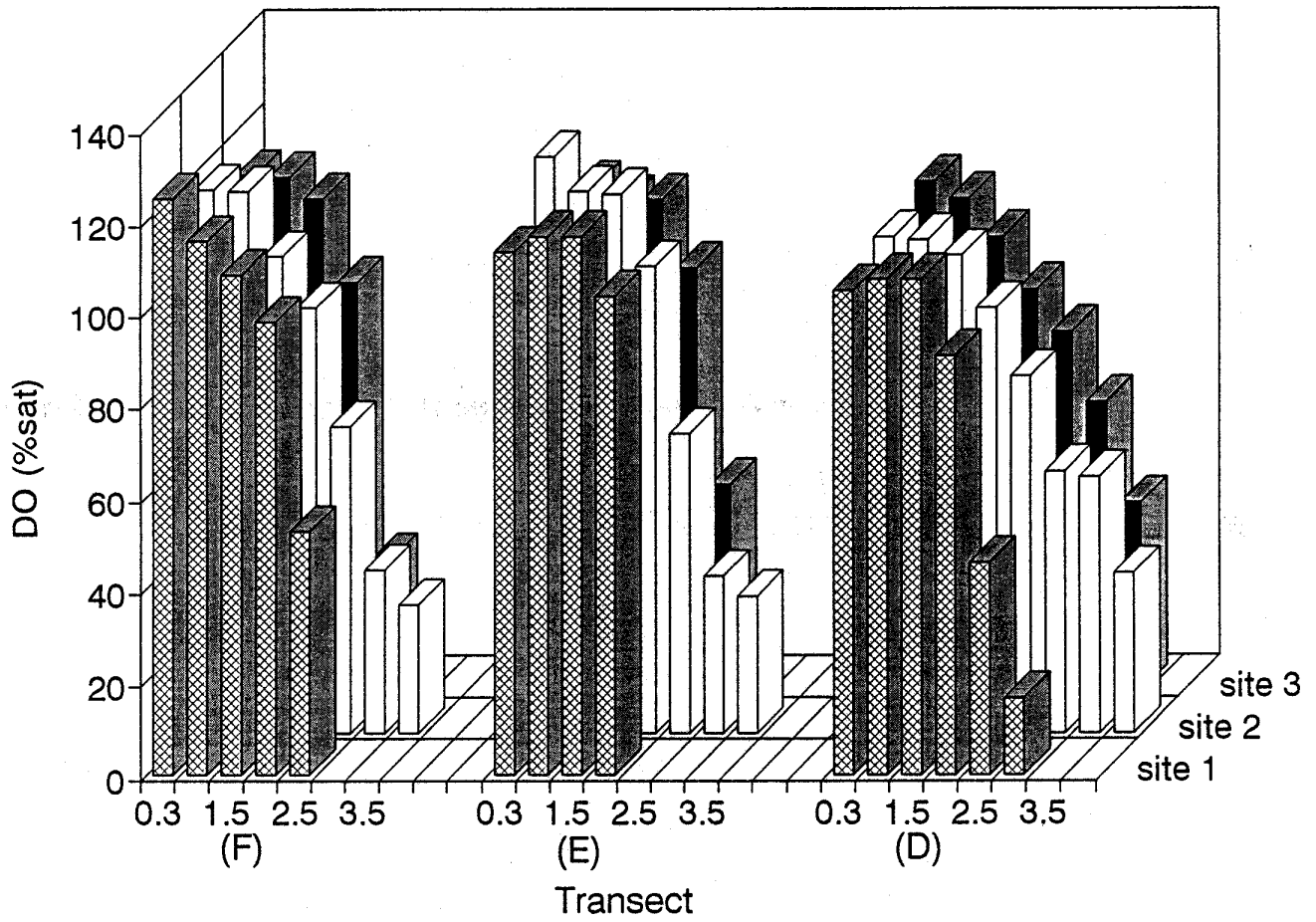


Figure 4

Temperature profile for the Recycling Lagoon on 10/03/92

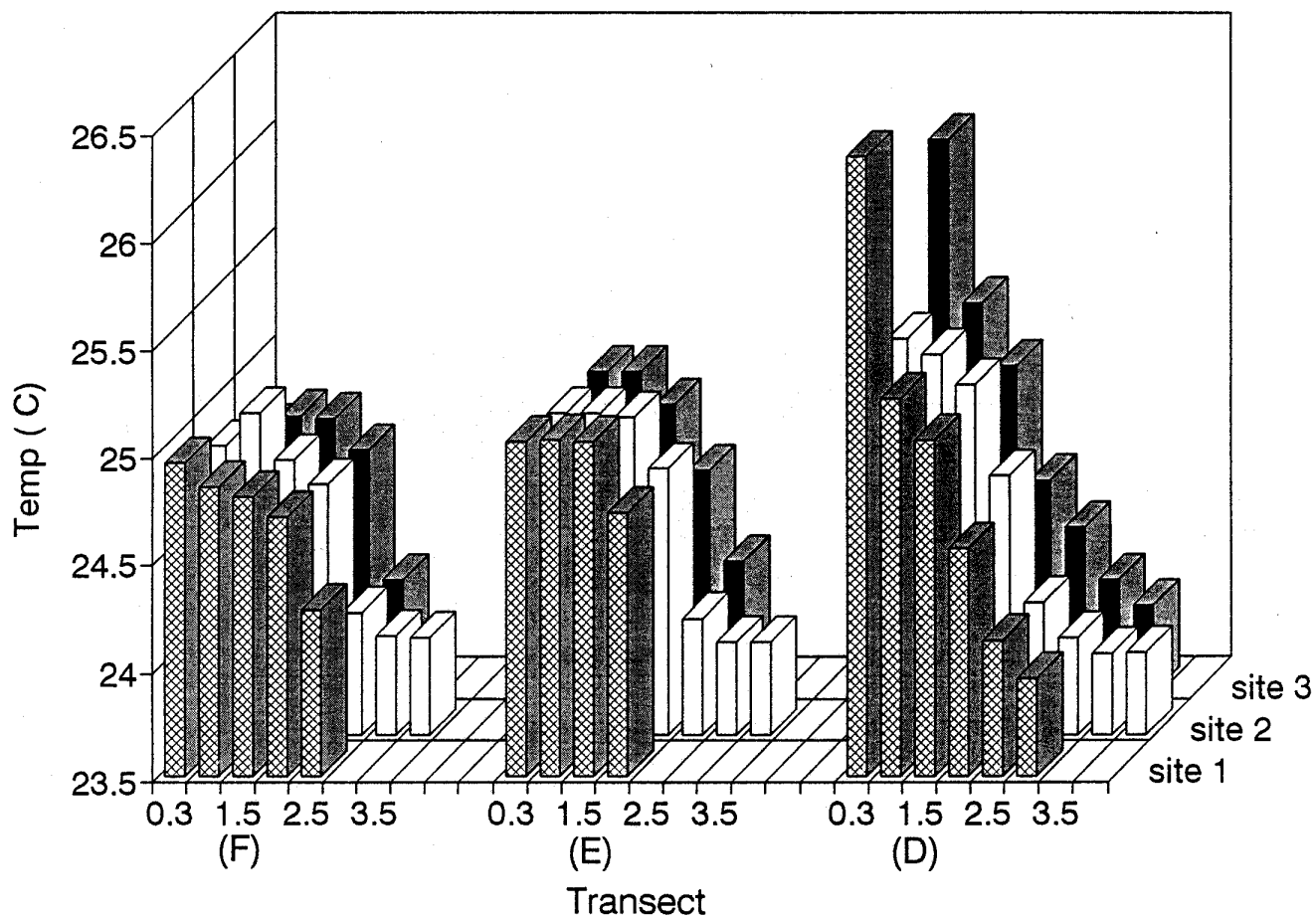


Figure 5

pH Over Time at 0.3 m depth for
transect E, Recycling Lagoon

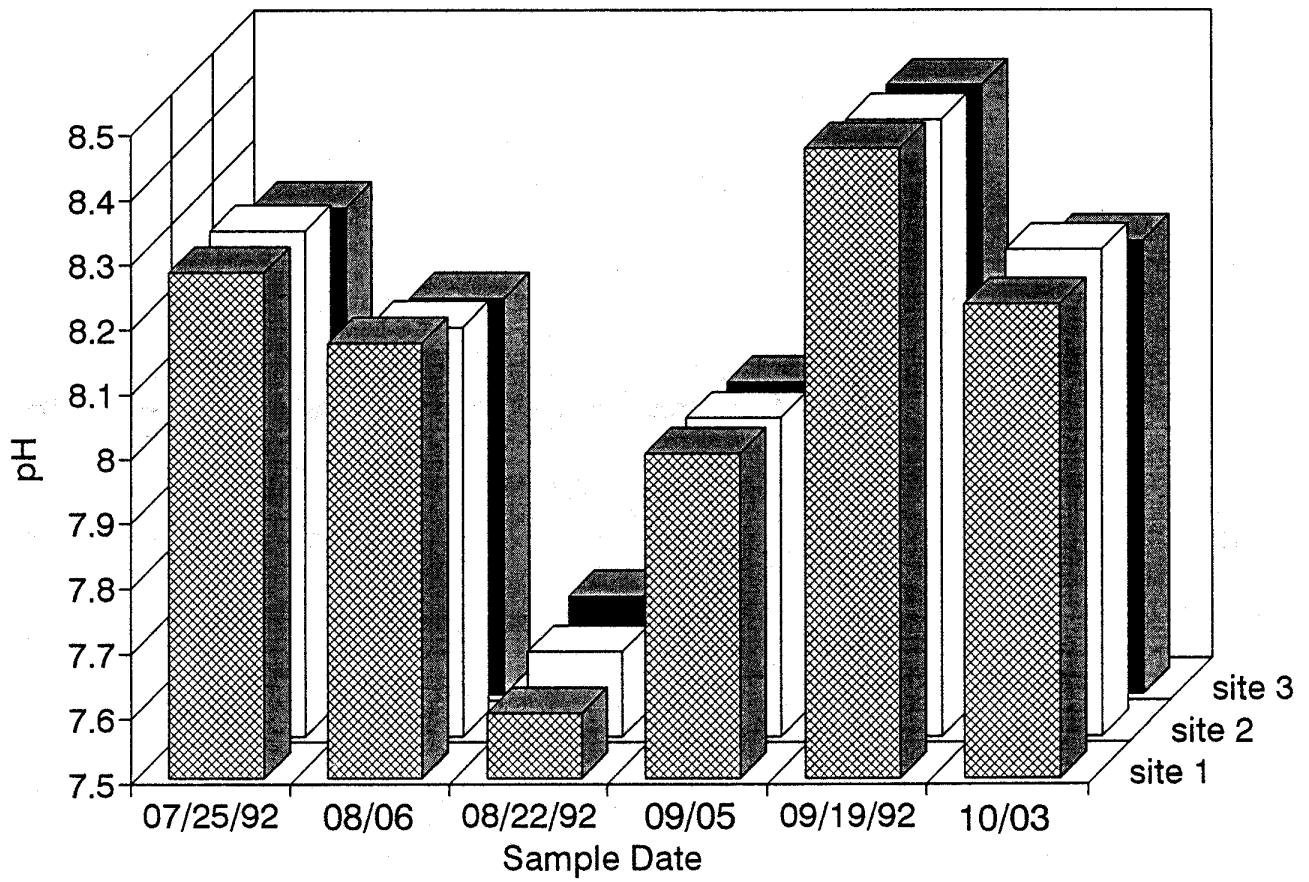
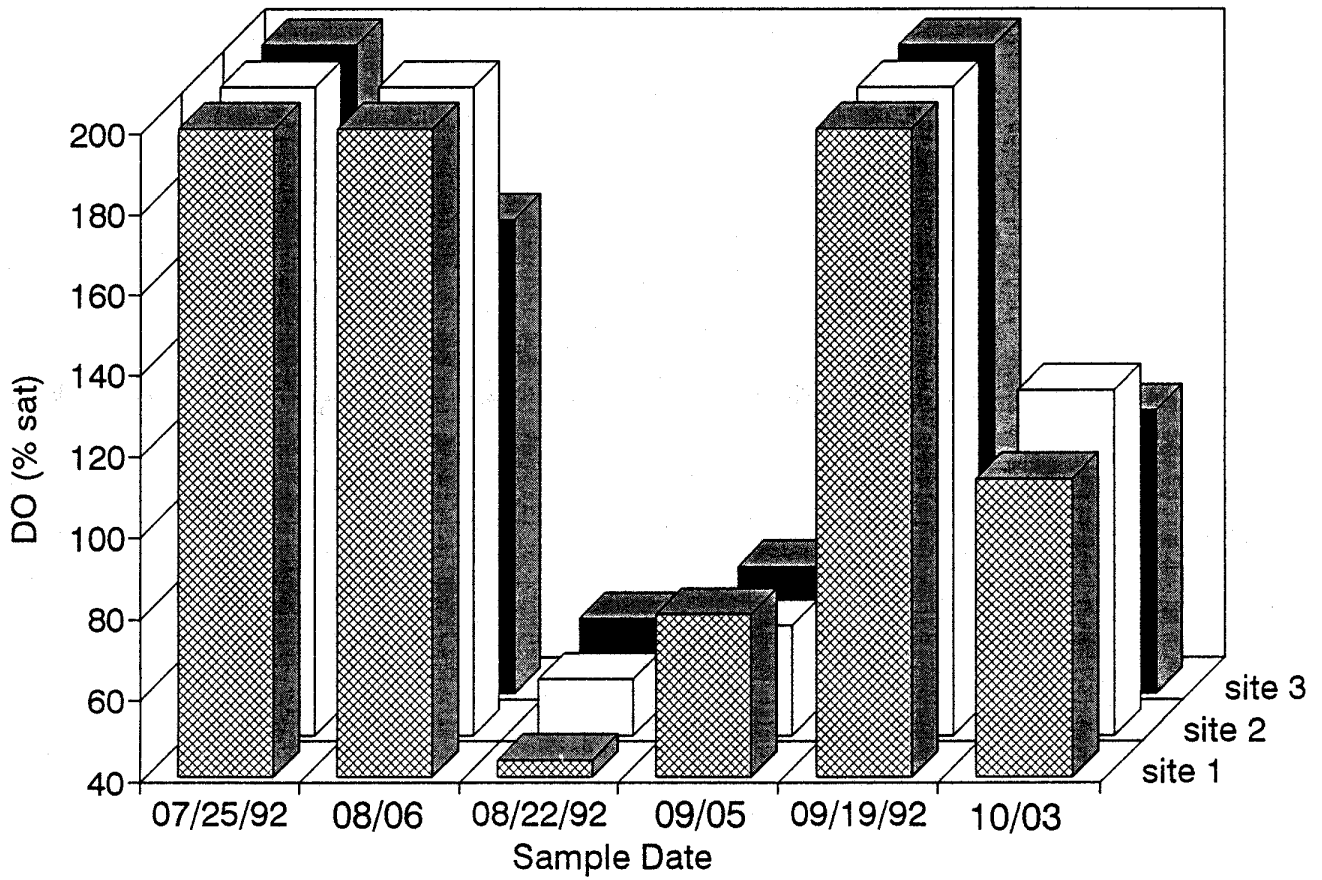


Figure 6

Dissolved Oxygen (% sat) over time at 0.3 m for transect E, Recycling Lagoon



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09/19) the DO in the surface water was near 200% saturation. These DO phenomena are indicative of an extremely productive system. Fertilizers in the runoff from the course are a possible factor contributing to this productivity.

Over the summer and fall the Recycling lagoon experienced notable variation in DO and pH over time. Values for a representative transect (E) at 0.3 m depth were plotted to display the levels (Figures 4 & 5). Changes in weather patterns or changes in the runoff from the front nine course could be the cause for these changes, but these parameters have not yet been correlated with the water quality data. The DO and pH followed generally similar trends in the lagoon. There was not much variation along the transect (across the width of the pond) on a given date, indicating uniform conditions horizontally across the pond. Most of the variation occurred vertically (from top to bottom) in the lagoons, although some data (not included) do show some vertical homogeneity of conditions right near the pond inflows, due to the mixing that occurs there. Stratification then occurs quickly as one moves away from these turbulent areas.

PHYTOPLANKTON DYNAMICS

Concentrations of chlorophyll *a* and ash free dry weights have been estimated for each of the 18 sampling stations over six sampling periods (Table 1). With the exception of the 08/22/92 expedition, when extremely high water levels prohibited sampling on the back nine site. Measures of chlorophyll *a* per ash free dry weight or trophic index, estimate the level of autotrophic biomass relative to the total biomass (Clark *et al.*, 1979). The trophic index does not measure a specific community function, but rather characterizes the photosynthetic efficiency of the community. Measures of chlorophyll *a*, ash free dry weight and trophic index did not vary greatly within each lagoon, during each sampling period (Table 2). Data from the central sampling station along each transect were chosen as representative of the sampling period for temporal analysis (Figure 1).

The dominant plankton species observed during each sampling period was a cyanobacterial genera, *Anacystis*. This blue-green algae is common to hypereutrophic systems. Only rarely were other phytoplankton taxa encountered. This is not surprising since established populations of

TABLE 1. Sampling periods for the 1992 field season on the Ocean Course, Kiawah Island.

SAMPLING PERIOD	SAMPLING DATE
1	07/25/92
2	08/06/92
3	08/22/92
4	09/05/92
5	09/19/92
6	10/03/92

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TABLE 2. Chlorophyll a, ash free dry weight (AFDW) and trophic index ^a for sample stations along three transects in the "back nine lagoon" (1) and the "recycling lagoon" (2) on the Ocean Course, Kiawah Island, 09/05/92. See text for details.

LAGOON	TRANSECT	SAMPLING STATION		
		1*	2	3
1	1	0.164	0.166	0.221
		11.9	16.0	15.6
		0.004	0.003	0.004
	2	0.080	0.127	0.118
		9.8	10.0	11.3
		0.004	0.004	0.004
	3	0.075	0.232	0.064
		9.5	8.7	9.7
		0.003	0.013	0.003
2	1	0.320	0.199	0.280
		11.3	14.4	12.3
		0.007	0.006	0.007
	2	0.128	0.130	0.115
		8.7	10.7	8.7
		0.006	0.007	0.006
	3	0.115	0.118	0.106
		11.3	8.6	8.5
		0.005	0.006	0.005

* Tabled values are $\mu\text{g/L}$ for chlorophyll a, milligrams/L for AFDW and $\mu\text{g/g}$ for trophic index.

^a Trophic Index = chlorophyll a/AFDW

cyanobacteria may actually alter their surroundings, physically, chemically and biologically, in ways which favor the persistence of the species (Vincent 1989).

FUTURE RESEARCH OBJECTIVES

- 1 Continue to track levels of autotrophic biomass in the Ocean Course lagoons, using estimates of chlorophyll a and ash free dry weight.
- 2 Examine secondary productivity in the lagoons, by describing quantitatively and qualitatively, the invertebrate and fish populations.
- 3 Attempt to relate any alteration of the lagoon community's structure and function with golf course management practices.

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Chapter 7

Determination of Uptake and Peroxidase Response as Indicators of Sublethal Pesticide Exposure to Aquatic Macrophytes

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INTRODUCTION

The predominant aquatic macrophytes in the lagoons on the Kiawah Island Ocean Course are Cattails (*Typha sp.*) and wigeon grass (*Ruppia maritima*). *R. maritima* is a rooted submerged plant and Cattails are rooted emergent plants. Most work will be carried out with *R. maritima*, as it is a known waterfowl food (Fosset, 1940) and may be involved with food chain effects of pesticides. Also, *R. maritima* is in culture in our lab, which will more easily accommodate comparison of laboratory and field studies. In other words, controlled studies in the laboratory can be validated in the field.

Submerged rooted aquatic plants were chosen over floating plants and other species for this study. These were chosen because they have contact not only with the water column, but also intimate root contact with the sediment pore water. This root contact could be important, as root to shoot translocation of nutrients by *R. maritima* (Thursby and Harlin, 1984) and pesticides by Hydrilla (Hinman and Klaine, 1992) has been demonstrated. If the plants are capable of "sampling" the sediment compartment with their roots, and transporting chemicals to their shoots, they may be able to reintroduce sedimented compounds back into the aquatic food chain.

In many instances, acute toxic responses of organisms to low levels of pesticides are not seen. However, there may be underlying sublethal impacts, which can potentially cause long term shifts in ecosystem structure and/or function. These effects would not be noticed by conventional chemical analysis or acute bioassays. In order to determine sublethal effects of pesticide exposure to aquatic plants, the method of Byl (1992) will be used to determine the peroxidase response.

The use of peroxidase in plants as a biomarker of toxicant exposure is a new and very important field of study. It may be possible, using this technique, to identify areas in an aquatic system that are being impacted by chemicals before the problem becomes unmanageable. This technique was tested in Milltown Reservoir, in Montana, which has highly metals - contaminated sediments, and was found to correlate well with conventional chemical analysis in identifying "hot spots" in the sediments (Klaine, 1992).

HYPOTHESES AND OBJECTIVES

General Objective: To determine the extent of exposure of aquatic macrophytes to pesticides, using accumulation and peroxidase as endpoints.

The following hypotheses and related specific objectives have been proposed for this study:

Hypothesis 1: Pesticides are mobile and/or bioavailable in the irrigation water system. These compounds migrate to the irrigation lagoons and end up in the water column and sediment pore water and are taken up by aquatic plants.

- Objectives:**
1. Sample plants in conjunction with pesticide application to the Ocean Course.
 2. Extract and analyze the plants for appropriate pesticides.

Hypothesis 2: Pesticides/nutrients are impacting the aquatic macrophytes in a sublethal manner, causing a measurable peroxidase response.

- Objectives:**
1. Determine the peroxidase activity of plants in the lagoons before and after pesticide application.
 2. In situ study with *R. maritima*.

METHODS

Aquatic macrophytes (*Ruppia* and *Typha*) will be collected in conjunction with application of pesticides on the course. Soil column studies (Cowles and Bailey, this report) will aid in determining when chemicals should theoretically arrive in the lagoons.

- Whole plants with roots will be harvested from the impact area, placed into ziploc bags, and stored on ice until placement in a lab freezer
- Appropriate digestions/extractions will be carried out on plant tissues for pesticide analysis.

For in situ studies, laboratory raised plants will be placed in the lagoons, at a reference site with no pesticide application, and under controlled laboratory conditions just prior to application of pesticide to the course. For peroxidase response determination, plants will be harvested from each condition at time 0 to find background levels under each condition, and at 5 days post application.

PERSPECTIVES

This work will aid in determining the persistence of the applied pesticides in this system and will give some indication of the possibility of food chain uptake. The peroxidase work is exciting as it will give us a more sensitive measure of ecosystem response than a simple bioassay type approach. Long-term chronic or sublethal community responses may be more easily discovered using the peroxidase method with plants than by more traditional strict chemical analysis of the water.

FUTURE GOALS

By the end of January, 1993, chemical analysis of the plants from 1992 will be complete. The peroxidase studies, and continuing plant chemical analysis will begin in spring of 1993, and will conclude by October of 1993. A final report of accomplishments of these investigations will be complete by December 1993.

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Chapter 8:

Summary and Research Recommendations

In addition to the research accomplished this past year and consistent with our proposal of February 1991 submitted to the United States Golf Association, we also proposed to work with the Management Staff at the Ocean Course at Kiawah Island to evaluate various aspects of water quality, pesticide use, wildlife habitat utilization and other associated ecological variables. Consistent with these goals we are collaborating with the Department of Horticulture at Clemson University to conduct a survey entitled "South Carolina Golf Course Pesticide Use and Practices Survey" developed by the Clemson University Cooperative Extension Service. Data acquired from this survey will facilitate the transfer of results derived from the Ocean Course at Kiawah Island to a wider distribution of courses.

The integration of plant, fish and wildlife exposure to pesticides and other management practices will give a very good perspective on environmentally sensitive issues in golf course management. In addition these data are being developed and integrated in a way which will lead to an ecological risk assessment. This ecological risk assessment will assist us in applying the information learned towards golf course management procedures. In addition, it will give a basis for interpretation by the USGA Green Committee as to the extrapolation of these data to a wider distribution of courses.

The objectives for 1993 will be as follows:

1. Continue research program at the Ocean Course at Kiawah Island to address the response of fish, plants and other wildlife in both terrestrial and aquatic environments to pesticide use and other environmental management practices on the Golf Course.
2. To integrate these data into an ecological risk assessment to define sensitive variables associated with environmental issues that might be impacted from Golf Course Management practices.

3. To develop the 1993 annual report with a stronger data base to be related in the form of an ecological risk assessment associated with environmental issues directly related to management practices at the Ocean Course at Kiawah Island which might be applied then to a wider distribution of courses.