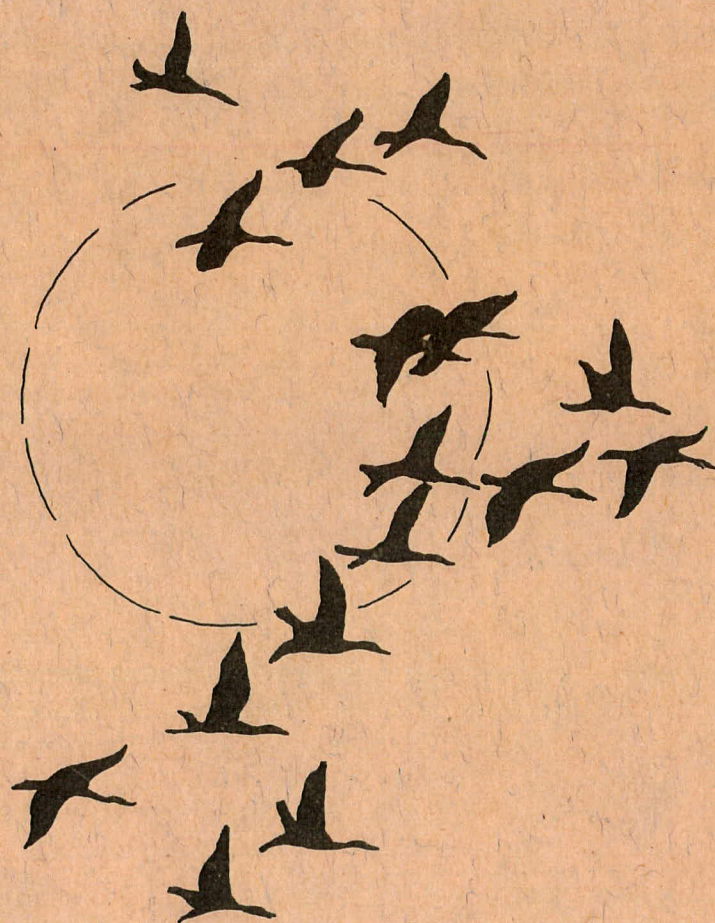


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MASTER

ANNUAL REPORT
of the
Savannah River
Ecology Laboratory

UNIVERSITY OF GEORGIA



MAY 1977

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ANNUAL REPORT OF ECOLOGICAL RESEARCH
AT THE SAVANNAH RIVER ECOLOGY LABORATORY

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BETWEEN THE UNIVERSITY OF GEORGIA
AND THE U. S. ENERGY RESEARCH AND
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May, 1977

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INTRODUCTION

The Savannah River Ecology Laboratory (SREL) is a regional research facility in ecology operated by the University of Georgia for the U. S. Energy Research and Development Administration (ERDA). Research is organized around two major programs: (1) Thermal and Aquatic Stress and (2) Mineral Cycling. These programs are strengthened by a previously established foundation of basic ecological knowledge. Research in basic ecology continues to be a major component of all SREL environmental programs. The resident research staff presently consists of 13 senior scientists and associated technical staff. Laboratory objectives are to carry out appropriate applied and basic research and to disseminate the findings through publication in the open scientific literature or through other forms of communication to the public and scientific community.

Emphasis in all programs has been placed upon field-oriented research relating to regional and local problems having broad ecological significance. For example, extensive research has been conducted in the Par Pond reservoir system and the Savannah River swamp, both of which have received thermal effluent, heavy metals, and low levels of radioisotopes. Furthermore, the availability of low levels of plutonium and uranium in both terrestrial and aquatic environments on the Savannah River Plant (SRP) has provided an unusual opportunity for field research in this area. The SREL studies seek to document the effects, to determine the extent of local environmental problems, and to establish predictable relationships which have general applicability. In order to accomplish this objective it has been imperative that studies be carried out in the natural, environmentally unaffected areas on the SRP as a vital part of the overall program. As indicated above, studies in basic ecology designed to understand natural biological systems are the only means of providing the sure-footing upon which applied research can proceed. This combination of thermally and radioisotopically altered environments in the immediate vicinity of natural areas which are protected and preserved for research purposes has resulted in one of the outstanding field research facilities in the United States.

As indicated, both written and oral communication are considered to be an essential part of our activities as scientists. Besides publishing in peer-reviewed scientific journals, we give many talks as invited speakers at universities, colleges, and high schools and to local church and civic groups. A variety of visiting groups tour the SREL facilities and we annually hold an open house for university personnel, new students, and interested local citizens. An established research participation program has involved students who, after completion of their SREL research, return to their home institutions and promote the SREL program and ecological awareness through discussion of their experiences. We have sponsored four annual symposia (Thermal Ecology and Mineral Cycling) in which representatives from government, industry, and academia have participated. The combination of these activities and efforts has led to a high level of communication with both the professional community and the lay public.

The resident research staff has professional associations at national and international levels as well as within the southeastern region. Several serve on committees or as officers of scientific societies. Numerous scientific papers and grant proposals are reviewed as part of each staff member's professional responsibility. SREL staff members hold adjunct academic positions in universities in several states and serve on the committees of graduate students from these universities.

Professional interaction with academic colleagues is further enhanced through the SREL Education Program. This program provides stipend support for 40-50 undergraduate and graduate students per year involved in research participation with individuals of the SREL professional staff. The Education Program also provides stipends for a limited number of faculty members to conduct short-term research at the laboratory site. Visiting scientists, resident graduate students, and undergraduate research participants allow the SREL staff to conduct selected facets of their research in collaboration with individuals from other colleges and universities. The staff time and facilities devoted to these activities are highly beneficial in providing a stimulating professional environment with new ideas and perspectives.

As always, the SREL's progress during a particular year should be judged in terms of the quality and quantity of the research conducted and disseminated. The effectiveness with which these research findings are presented to the public and scientific community must also be weighed in this evaluation, as should our response to the local environmental problems on the SRP, regional problems of the coastal plain, and matters of national concern. The professional advancements made by staff and students must also be held as an important measure of our success as a scientific laboratory as this is indicative of our credibility and respect by colleagues in the scientific community.

The objective of this report is to indicate the direction and progress of research in the major programs by selecting highlights of the research conducted during this current fiscal year. The two major areas covered in the report are studies in mineral cycling and in thermal and aquatic stress. These, along with the SREL Education Program and the Transuranic Elements Program, correspond to our current funding units. The latter is included in the Mineral Cycling Program since the environmental behavior of transuranics is related to ecosystem mineral cycling processes.

I. THERMAL AND AQUATIC STRESS PROGRAM

D. H. Nelson	E. H. Liu ²
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OBJECTIVES OF THE THERMAL AND AQUATIC STRESS PROGRAM: IDENTIFICATION AND SCOPE

Since the development of the Savannah River Ecology Laboratory's (SREL) Thermal Ecology Program, we have sought to identify and pursue those specific areas wherein our scientific expertise could best contribute to a greater understanding of elevated temperature as an environmental regulator and stressor on aquatic ecosystems. As with all SREL programs, these efforts have been preceded by and coordinated with basic ecological studies designed to understand the structure and function of natural, undisturbed environments. The thermal and aquatic stress studies have been designed to provide information relevant to applied environmental problems of energy production as well as to basic and theoretical considerations which further the conceptual advancement of ecology as a science.

The availability of diverse natural, heated and formerly-heated aquatic stream, reservoir and swamp habitats makes the Savannah River Plant particularly well-suited to field studies on the ecology of stress to aquatic systems from thermal and chemical sources. In recent years, SREL ecologists have assembled information on an array of lentic and lotic habitats ranging from ambient temperatures to those lethal (50-60°C) for most higher organisms. Research both in the field and laboratory has identified a number of responses and processes that clarify current concepts about stress ecology at the individual, population and community levels. Long-term field studies of largemouth bass, fish parasites, emergent aquatic macrophytes and aquatic invertebrates have helped develop principles that are operative at all levels of biological organization.

¹ERDA contractor, No. E(38-1)-900, Department of Biology, Wake Forest University

²Department of Biology, University of South Carolina

³ERDA contractor, No. AT(11-1)-2502, included because of close collaboration on ongoing research, State University College at Buffalo (N. Y.)

*Graduate student

The SREL facilities have permitted experiments focusing on basic biological processes such as growth, development, reproduction, thermal tolerance, thermal preference, behavioral patterns, isozyme activity, host-parasite interactions, and genetics in several plant and animal species. Temperature control is maintained in the laboratory by the use of environmental chambers and aquaria in conjunction with water baths. Temperature gradients are also maintained in order to assess animal thermoregulatory capacities.

SREL research on temperature effects can be categorized into examination of structure, function and synergistic interactions as follows: (1) Thermal studies of ecosystem structure attempt to quantify the descriptive and physical aspects of (a) trophic levels (producers, consumers, decomposers); (b) ecosystem organization (organism, population, community); and (c) transfer components (e.g., biomass, energy, nutrients and information). (2) Thermal studies on the function of ecological systems are generally concerned with physiology, behavior, or population dynamics, and deal with (a) biological processes such as metabolism, respiration, productivity; (b) behavioral processes such as thermal preferences, aggression; (c) ecosystem perturbation assessment (vulnerability, elasticity, resilience); and (d) population processes (density, growth, mortality). (3) Thermal studies of synergisms and interactions relate organism, population and community responses to a complex of multidimensional environmental factors. This category includes (a) predator-prey and competitive interactions; (b) synergisms of differential toxicities (heavy metal, chemical, etc.); and (c) abiotic factors such as climatic and water quality parameters.

The scope of certain projects within the thermal and aquatic stress program has expanded in recent years to include synergistic effects of thermal alteration and other environmental contaminants such as heavy metals (e.g., cadmium) and interactions among multiple populations (host-parasite relations). The anticipated completion of the Castor Creek experimental facility will result in further studies dealing with synergisms and multiple stresses.

One goal of the Thermal and Aquatic Stress Program is not only to quantify responses of ecosystems to various levels of perturbation, but also to attain a predictive capacity for specific thermal levels in representative aquatic habitats in the Southeast. Principles resulting from studies conducted on the SRP can be tested for general applicability elsewhere and incorporated into models to accurately predict ecological changes concomitant with aquatic stress.

Research in the SREL Thermal and Aquatic Stress Program continues to be conducted in cooperation and coordination with similar research projects performed by other regional laboratories such as DuPont's Savannah River Laboratory, Oak Ridge National Laboratory, and the Baruch Institute of the University of South Carolina. Cooperative research efforts are also in progress with Wake Forest University, the University of South Carolina, the University of Michigan, State University College at Buffalo (N. Y.), and the Pymatuning Laboratory of Ecology, University of Pittsburgh.

BEAVER STREAM SERVES AS AN OUTDOOR LABORATORY FOR THE STUDY OF AQUATIC STRESS (D. H. Nelson, R. R. Sharitz and R. W. McFarlane)

Castor Creek is a 1.5 km first-order tributary of a thermal stream on the SRP. The creek has been greatly modified by the presence of 22 active beaver dams along its length; these create a series of alternating pool and riffle habitats with rich floral and faunal communities. The creek flows parallel to, and is ultimately confluent with, a thermal effluent canal from a nuclear production reactor (Figure 1). The close proximity of heated effluent has allowed the construction of an experimental field facility to add heat to the Castor Creek ecosystem. Some of the thermal effluent (50-70°C) will flow to the creek through a gravity-fed conduit and pass through a heat exchanger in the creek before being discharged further downstream. This design will create three experimental zones: an upstream ambient-temperature "control" area; a thermally-elevated zone downstream from the heat exchanger; and the final "polluted" section, which will directly receive the actual thermal effluent.

Few studies have allowed the thermal manipulation of a natural stream system within a field setting. By regulating the flow rate of effluent water through the heat exchanger, predetermined temperatures can be maintained along the creek. The currently-constructed installation at Castor Creek is designed to accomodate a potential maximum temperature of 10°C above ambient in the experimental section of the stream. Initial studies, however, will focus on lower levels of thermal input (< 5°C). The facility will allow a precise characterization and quantification of ecological responses that are exhibited by a stream ecosystem subjected to minor levels of thermal perturbation. Thus, Castor Creek provides a unique opportunity to experimentally test, under field conditions, principles that have been derived through field and laboratory studies.

Initial prediversion baseline studies (primarily descriptive) will continue to identify major plant and animal components of the aquatic subsystems. Cooperative efforts of several researchers to date have emphasized the aquatic macrophytes, primary productivity, benthos and fishes. Further studies on these topics will include aspects of community dynamics (including species composition), trophic relations, interspecific interactions and succession. One major objective of the project will be to relate species diversity, productivity, community metabolism, and nutrient cycling to specified levels of thermal loading. Research efforts will test general principles that relate overall ecosystem structure and function to thermal input. A generalized hypothesis may be stated as follows: "In nutrient-deficient, softwater stream systems of the southeastern U. S., each of the above mentioned parameters will increase with thermal enrichment to an optimum level, as the artificial heat provides an energy subsidy. Past the optimum levels, however, these parameters will decline as the thermal perturbation assumes the function of a stress."

Subtopics to be pursued include behavioral alteration in benthic organisms, elemental uptake in aquatic plants, comparative decomposition rates on stream substrates, and synergistic effects of other stresses

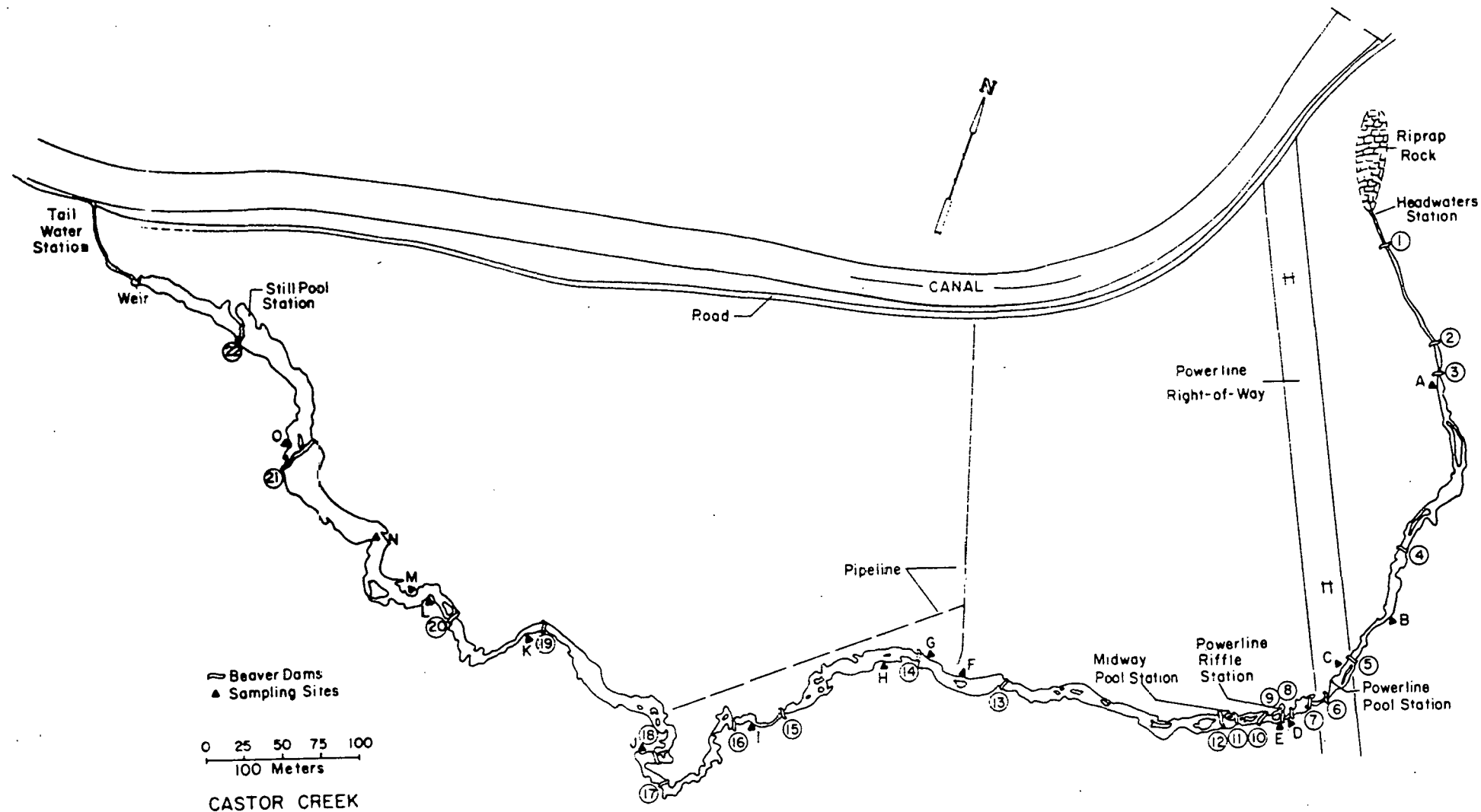


FIGURE 1. SITE OF THE CASTOR CREEK EXPERIMENTAL FIELD FACILITY. THE DASHED LINE REPRESENTS THE LOCATION OF THE PIPE CONDUIT THAT WILL TRANSPORT HEATED EFFLUENT TO THE STREAM. NUMBERS REPRESENT LOCATIONS OF BEAVER DAMS AND LETTERS IDENTIFY VEGETATION SAMPLING SITES.

(such as increased levels of heavy metals) in concert with temperature. Studies at the population and community levels will be integrated within an ecosystem context. Thus, each study conducted at Castor Creek will contribute to the overall conceptual framework of ecosystem responses to temperature stress.

Initial baseline studies have focused on abiotic factors, aquatic macrophytes, aquatic invertebrates and fishes.

1. Abiotic - For over a year, maximum-minimum thermometers have been maintained at selected locations on the creek and checked twice weekly. Local rainfall, % relative humidity, and air temperature have also been recorded at a field weather station at the creek site. In 1976, seasonal temperatures at a representative pool ranged from 11-23°C (winter), 10-28°C (spring), 17-27°C (summer) and 13-24°C (fall). Initial measurements estimate the stream flow at approximately 3.0 - 3.5 cfs. Some basic water quality parameters (temperature, dissolved oxygen, conductivity, oxidation-reduction potential, and pH) are monitored (by use of a Hydrolab surveyor) weekly at four sites along the creek and at the thermal canal. Last year's data show daytime values for D.O. to vary from 6.1 to 10.2 ppm; values for pH range from 4.0 to 5.6 over most of the creek. Conductivity values vary from 0.1 to 0.5 $\mu\text{mho/cm}$, with up-stream values consistently intermediate to higher values of the thermal canal and the downstream creek readings. Data suggest that there is some subterranean seepage of heat and dissolved components into the headwaters of the creek from the nearby thermal canal.

2. Aquatic macrophytes - Seasonal sampling reveals a minimum of 10 (winter) and 17 (spring) species of aquatic macrophytes to be characteristic of riffle vegetation in 15 locations along the creek. Frequencies of occurrence per species ranged from 3% to 87% of the samples. Living biomass estimates at different sites ranged from 12.9 to 151.0 g/m^2 dry wt. (\bar{x} = 79.9) in the winter, and from 27.4 to 250.1 g/m^2 (\bar{x} = 96.1) in the spring.

3. Invertebrates - Preliminary data from an intensive, summer sampling of the invertebrate fauna along Castor Creek has confirmed the presence of 70 species of aquatic invertebrates, representing a total of nine different orders. The more common Odonates exhibited the greatest diversity (17 species), whereas the Plecopterans showed the least (1 species). Initial analysis reveals an apparent lack of large-particle detritivores within the system.

4. Fishes - Although Castor Creek is a first-order stream, the beaver dams have sufficiently modified the ecosystem so as to exclude typical first-order stream fishes. The ten species which do inhabit the pools and riffles are typically found only in higher order streams and accordingly Castor Creek exhibits much higher species diversity than would be expected in a normal first-order stream (Shannon H' = 1.73, Pielou evenness J' = 0.787).

Baseline studies shall continue in the areas of research already initiated. Additional recent efforts have been directed toward aspects of aufwuchs productivity and vegetative decomposition. Since the creek consists of several distinct pool and riffle subsystems, coordinated studies are designed to effectively integrate these components.

PERIPHYTON PRODUCTIVITY IN CASTOR CREEK IS RELATED TO SHIFTS IN SPECIES COMPOSITION (R. R. Sharitz)

As major primary producers, algae are of particular interest in thermally affected stream systems. Algae are likely to be good indicators of environmental quality because of their rapid turnover and high growth rates. In addition, the use of algal assays is especially suitable for monitoring the effects of contaminants in aquatic systems since they integrate all stresses which may deleteriously affect growth and reproduction. Periphytic algal communities may be excellent indicators of environmental change since they are in a fixed position unlike the plankton which are more mobile and may be transported into or out of an area.

Castor Creek, a natural stream designed to experimentally receive heat and reactor effluent at a later time, was sampled in the winter of 1976-1977 to provide baseline information on the community structure and standing crop of the phycoperiphyton. This creek currently contains a series of pools and riffles due to extensive beaver activity. A series of glass slides were placed in eight ponds to provide artificial substrates for periphyton attachment. After a four-week incubation period in the stream, standing crop (based on ash weight and pigment concentration) was measured and relative abundance of the major taxonomic groups was determined. The cells of filamentous and coenobial forms were counted as individuals.

Phycoperiphytic standing crop was highest at the headwaters of Castor Creek and decreased downstream (Figure 2). Physical and chemical characteristics of the headwaters pond, especially increases in water temperature, conductivity, hardness and alkalinity, suggest that underground seepage from the nearby reactor effluent canal may influence algal productivity in this area. A second peak in standing crop was apparent approximately one-third of the distance along the stream. This increase may be associated with the shift in community structure from a dominance by filamentous green algae to a diatom community.

The abundance of filamentous green algae in the upper portion of Castor Creek may likely be influenced by the seepage into the stream in the headwaters region. It is not surprising that diatoms, which are characteristic of southeastern streams with sandy bottoms and low nutrient levels, are the major component of the periphytic flora throughout most of the length of Castor Creek. The unexpected increase in blue-green algae downstream is due to the bloom of a chroococcacean form in sluggish pools where the flow rate was greatly reduced. Periphytic standing crop and community structure in the Castor Creek ponds vary and are strongly influenced by the changes in chemical and physical parameters along the length of the stream.

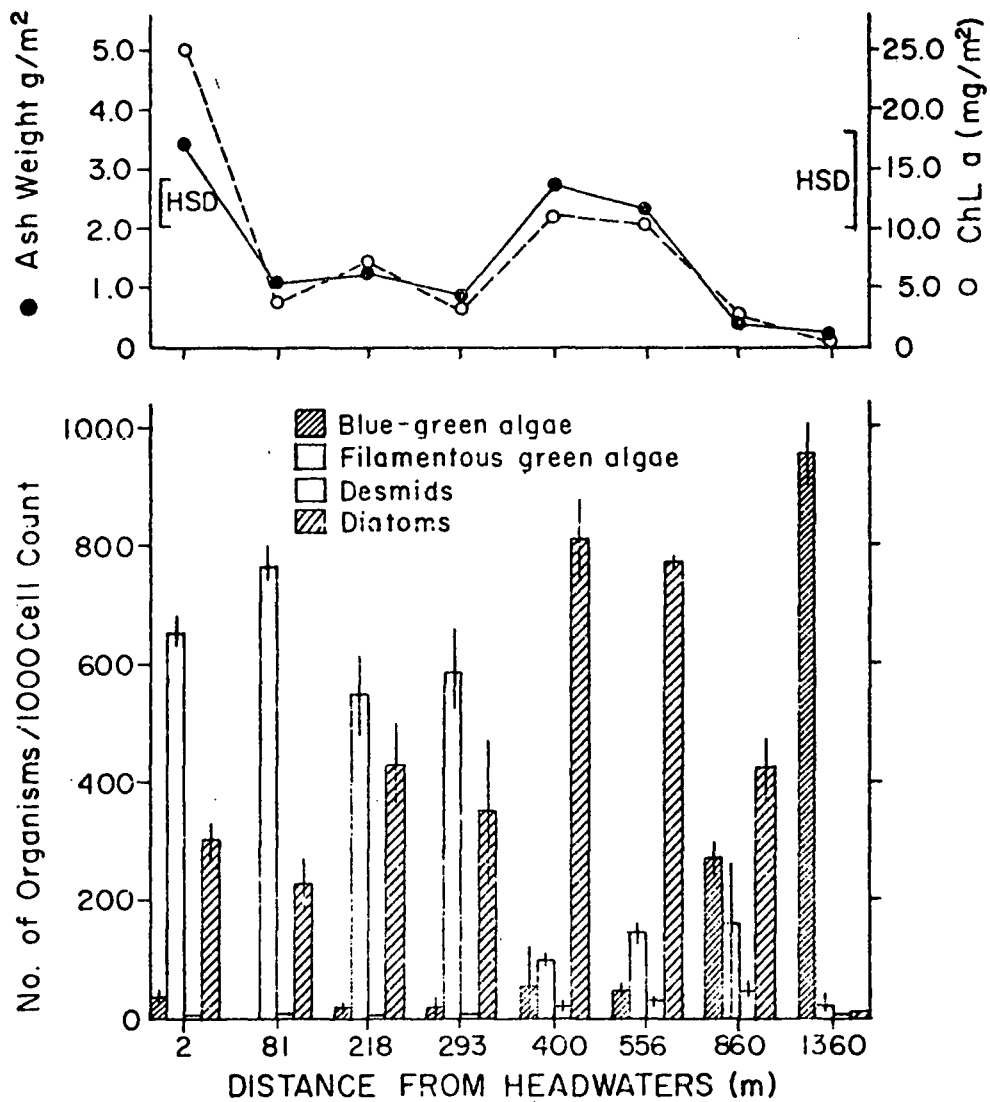


FIGURE 2. STANDING CROP (ASH WEIGHT IN g/m^2 AND CHLOROPHYLL-a CONCENTRATION IN mg/m^2) AND COMMUNITY STRUCTURE OF THE PHYCOPERIPHYTON IN CASTOR CREEK. VERTICAL HSD BARS REPRESENT THE DIFFERENCE BETWEEN MEANS WHICH IS NEEDED FOR A STATISTICAL DIFFERENCE ($P \leq 0.05$) CALCULATED BY TUKEY'S W-TEST. $N = 3$ FOR STANDING CROP; $N = 2$ FOR CELL COUNTS.

THERMAL EFFLUENTS CAUSED STOMATAL CLOSURE PRIOR TO MORPHOLOGICAL CHANGES IN NYSSA AQUATICA (K. W. McLeod)

On the Savannah River Plant, large areas of the swamp and bottomland forests have been modified by the influence of the production facilities. The impact has been variable, from very slight to total destruction of the forest. Causative factors include increased water level, temperature and siltation. Temperature is known to strongly affect metabolism. The

metabolic imbalances created by the adverse response to temperature can eventually lead to mortality. A key to plant metabolism is stomatal activity, which regulates gas exchange and ultimately total metabolism. Therefore an experiment examining the stomatal response of water tupelo (Nyssa aquatica) to elevated temperatures was conducted.

A growth chamber experiment with a 3 x 4 factorial design was used. The four temperatures were 22°C (ambient), 35°C, 42°C and 50°C. Within each temperature treatment, three levels of inundation were used (22%, 56% and 100% of soil volume inundated). The stomatal response, as measured by changes in diffusive resistance, was measured twice daily, once each in the morning and afternoon, for one week or until death was imminent. Nine leaves per treatment were measured (3 on each of 3 individuals).

Stomatal closure occurred at 42°C and 50°C but not at 22°C or 35°C. The response was seen most rapidly at 50°C, as soon as 17 hours after the treatment began. Closure was observed for all three inundation levels with 50°C effluent water. The greater the inundation level, the quicker the response occurred. This indicates a significant interaction between depth of inundation and temperature treatment. At 42°C, only the greatest inundation depth produced a significant closure. This response was not observed until comparatively longer into the treatment. Leaves of seedlings and young and senescent one-year old saplings all responded similarly to the treatments. The water potential of the leaves at the conclusion of the experiment was also determined. It was observed that the turgor of the leaves decreased with a temperature increase.

At the higher temperature treatments with the accompanying stomatal closure and low leaf water potential, gas exchange becomes seriously retarded. These circumstances place the plants under stressed conditions, with probable high respiration rates and low photosynthetic rates. Continued imbalance of this nature would certainly lead to death. In fact a necrosis of the leaves was observed following the temperature modification of stomatal activity. The cause of the changes in stomatal activity are thought to be due to changes in hormone production in the root system.

WATER TUPELO SAPLINGS SURVIVE WHEN INTRODUCED INTO THERMAL STREAMS (K. W. McLeod)

Sharitz, Irwin and Christy (1974) have reported large scale modification of the swamp and bottomland forests of the Savannah River Plant. They attribute these modifications to introduction of thermal effluents into these systems. Revegetation by woody plants on islands in the thermal and post-thermal streams is slow. In an attempt to determine the response to thermal inputs and the revegetation potential of water tupelo (Nyssa aquatic), seeds and one-year old saplings were introduced into the streams. Pots supported by styrofoam floats containing seeds and one year old saplings were introduced into the streams. The buoyancy of the float was varied using different amounts of styrofoam to create three levels

of inundation. These floats were placed at four stream locations representing a temperature gradient. Thus a 3 x 4 factorial design was employed. Occurrence of germination, survival, and growth characters (height, basal diameter, and number of leaves) were recorded. In late August, the plants were harvested for biomass determinations.

Germination was excluded at the warmest location (water temperature $\approx 50^{\circ}\text{C}$). As the temperature of the stream locations decreased the percent germination increased. The survivorship of the seedlings was also dependent on the water temperature with 0.3% surviving at 45°C , 2.0% surviving at 38°C and 4.6% surviving at 30°C . Significant interaction between the depth of inundation and water temperature were observed.

The survival of saplings was also affected by temperature and inundation. One plant (3.3% of the population) survived the warmest location. With decreasing temperature, the survival increased from 46% at 45°C to 100% at the remaining two locations (30 and 38°C). Analysis of the growth parameters is not complete but it appears that the most growth occurred in the 38°C treatment.

We must conclude from these results that germination and establishment is the most critical step in revegetation. It does appear that establishment of water tupelo is possible in areas experiencing up to 40°C thermal effluents. Above this temperature, successful establishment is highly unlikely. Since successful establishment was observed in areas where the temperature was below 40°C , the slowness of revegetation by woody plants is felt not to be due to abiotic factors and that possible lack of propagules or biotic interference may be responsible.

ELEVATED TEMPERATURES ENHANCE THE GROWTH OF LUDWIGIA LEPTOCARPA (R. R. Sharitz and E. J. Christy)

Ludwigia leptocarpa, an annual or short-lived herbaceous perennial in the family Onagraceae, is one of the dominant plants which grows in water and submerged sediments along streams receiving heated effluents on the Savannah River Plant. Previous studies of naturally-occurring field populations of this species have indicated an enhancement in density and standing crop per area at temperatures ranging from 32 - 44°C (heated stream conditions) compared with plants growing at 22 - 25°C (normal water temperatures). In addition, plants growing at normal temperatures in the field produced fewer flowers and seeds than did plants in the thermal habitats.

Ludwigia leptocarpa is characteristic of disturbed localities, and it is not surprising that it should be found at higher densities and greater standing crops in the marshes along the effluent streams where the original flora has been eliminated and the canopy of the floodplain forest destroyed. It is not possible to determine from the field studies whether this apparent enhancement in growth is directly related to the increase in water and sediment temperatures or is due instead to reduction in competition with other plant species intolerant of the heat. Growth

responses were therefore examined under controlled environmental conditions in which temperature was the major variable. Seeds of plants from field populations at normal (22-26°C), intermediate (30-38°C) and hot (40-44°C) water temperatures were germinated and seedlings grown in controlled environmental chambers at constant temperatures of 22°, 32° and 42°C. Homogenized floodplain soils and a 16-8 hour photoperiod were used for each temperature treatment.

After 78 days of growth, shoot length, shoot weight and root weight of plants from each seed location were greatest at 32°C (Figure 3). The only exception was root weight in plants grown from ambient temperature seeds which showed a decline relative to that at 22°C. Plants were generally tallest and most robust at 32°C and smallest at 42°C. Significant differences in growth response between populations was seen at 32° and 42°C but not at 22°C. Seedlings grown from populations occurring at elevated temperatures in the field exhibited significantly greater height and weight responses at 32°C than did seedlings from ambient-temperature populations. No plants from populations grown under ambient temperatures survived in the 42° conditions.

Growth responses of Ludwigia leptocarpa to elevated temperature can therefore be summarized as follows: (1) at normal environmental temperatures, plants grown from seeds collected at each temperature location showed similar growth responses; (2) at temperatures elevated approximately 10°C, growth of plants from all three populations increased; however, plants from thermally altered habitats showed greater enhancement than plants from normal temperature habitats; (3) at temperatures elevated approximately 20°C, plants from the normal habitat did not survive and plants from the thermal habitats exhibited a severe growth reduction as a result of thermal stress. Thus it appears that the response of Ludwigia leptocarpa is dependent upon both the present temperature and habitat temperature to which the parent population was adapted.

THE ORGANELLE LOCALIZATION OF HEAT SENSITIVE AND HEAT TOLERANT ISOZYMES OF MALATE DEHYDROGENASE IN CATTAILS (E. H. Liu, J. C. Jones and R. R. Sharitz)

Two species of cattails, Typha latifolia and Typha domingensis dominate the shoreline of Par Pond. T. domingensis does not appear to be tolerant of the thermal stresses which are imposed at the hot arm of Par Pond, and has not colonized this area. We have investigated the thermal sensitivities of one isozyme system, malate dehydrogenase (MDH), in these two species of cattails.

The MDH system in cattails consists of six anodically migrating isozymes. In the two species, these six isozymes have identical electrophoretic mobilities. However, the thermal stabilities of these isozymes differ markedly between the two species. The malate dehydrogenase activity of T. domingensis is sensitive to heating at 50°C and denaturation of activity at this temperature follows first order

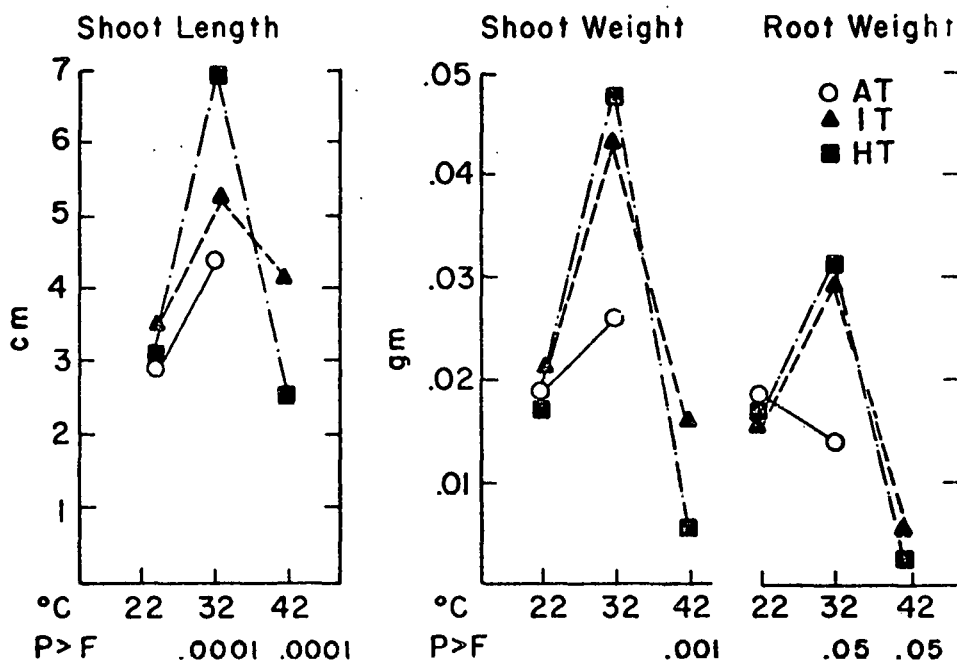


FIGURE 3. SHOOT LENGTH, SHOOT WEIGHT AND ROOT WEIGHT OF LUDWIGIA LEPTOCARPA GROWN AT 22°, 32°, and 42°C MEASURED 78 DAYS AFTER GERMINATION. HT = HABITAT TEMPERATURE OF 40-44°C, IT = HABITAT TEMPERATURE OF 30-38°C AND AT = HABITAT TEMPERATURE OF 22-26°C. FOR THE 22°C AND 32°C CHAMBERS, N = 20; FOR THE 42°C CHAMBER, N = 0 FOR THE AT SEEDLINGS, N = 38 FOR THE IT SEEDLINGS, AND N = 32 FOR THE HT SEEDLINGS.

decay kinetics. The MDH activity of T. latifolia, however, consists of two populations, one of which is heat sensitive and the other which is heat tolerant. T. latifolia thus has a biphasic denaturation curve when heated at 50°C. Fifty percent of the enzyme is denatured according to first order decay processes, while the other 50% is completely stable to this temperature.

These differences in the thermal stabilities of MDH isozymes between the two species can be explained by determining the response to heat stress by individual isozymes. In the case of T. domingensis, all six isozymes are heat sensitive. In T. latifolia, isozymes 1, 2, and 3 are heat sensitive, while isozymes 4, 5, and 6 are heat resistant.

It has been suggested by McNaughton that ecotypic variants of T. latifolia may differ in enzymatic thermal properties. We have investigated the thermal sensitivities of individual MDH isozymes of T. latifolia specimens which were collected as rhizomes from montane, coastal plain, and central valley habitats in California, and then grown under constant greenhouse conditions. In all cases, isozymes 1, 2, and 3 were heat sensitive, while isozymes 4, 5, and 6 were heat tolerant. Preliminary results suggest that these cattails might differ in the relative proportions of stable vs. labile forms of the enzyme.

The subcellular localization of MDH isozymes from Par Pond cattails was determined by sucrose gradient centrifugation. In the 30-60% sucrose gradients employed, mitochondria band at a density of 1.21. MDH activity in this gradient is shown to have its peak of activity precisely at this density (Figure 4). Cytochrome oxidase, which is an internal marker for mitochondria also has its peak of activity at 1.21. Chloroplasts have a density of 1.17, as can be seen by the peak of chlorophyll readings. No MDH activity is associated with this organelle.

Starch gel electrophoresis of the organelle fractions revealed that isozymes 4, 5, and 6 were localized in the mitochondrial fraction. Differential centrifugation techniques were used to demonstrate that isozymes 1, 2, and 3 are cytoplasmic forms of MDH. We were also able to determine by differential centrifugation that 50% of the MDH in cattails is organelle localized.

The fact that the mitochondrion is exclusively responsible for the differences in thermal sensitivities of MDH isozymes between T. domingensis and T. latifolia suggests that this organelle might be important in determining the upper thermal limits of viability in plant species. We are now studying the thermal sensitivities of other organelle localized forms of enzymes in cattails.

A HISTOCHEMICAL STAINING PROCEDURE FOR THE DETERMINATION OF THERMAL TOLERANCE IN PLANTS (E. H. Liu, W. T. Austelle and R.R. Sharitz)

Tetrazolium salts have proved to be useful histochemical stains for determining the presence of metabolic activity in plant tissues. These compounds have been used to examine the salt and thermal tolerance of various woody and herbaceous plants and to determine seed viability. Tetrazolium salts are also employed in conjunction with other substrates to detect the presence of dehydrogenase isozymes in electrophoretic zymograms of tissue extracts.

Although the removal and transfer of electrons occurring in aerobic respiration is catalyzed by various dehydrogenase enzymes, the electrons themselves are transferred to coenzymes such as NADH. Dehydrogenases can also transfer electrons to certain organic compounds not found in living cells, such as tetrazolium salts. Tetrazolium, in the reduced state, forms a water-insoluble formazan.

Nitro Blue Tetrazolium (NBT) was used to monitor the production of reducing equivalents such as NADH in leaf meristem sections of Typha latifolia and Typha domingensis collected from thermally stressed and unstressed portions of Par Pond. Positive reactions, which are indicative of active plant metabolism, result in the precipitation of blue formazan on the cut surfaces of the plant tissue. Thus, the visual inspection of blue color on plant tissues after incubation in NBT constitutes a qualitative test of tissue viability. We have quantified this procedure by extracting the blue formazan product with pyridine and determining its absorbance at 523 nm.

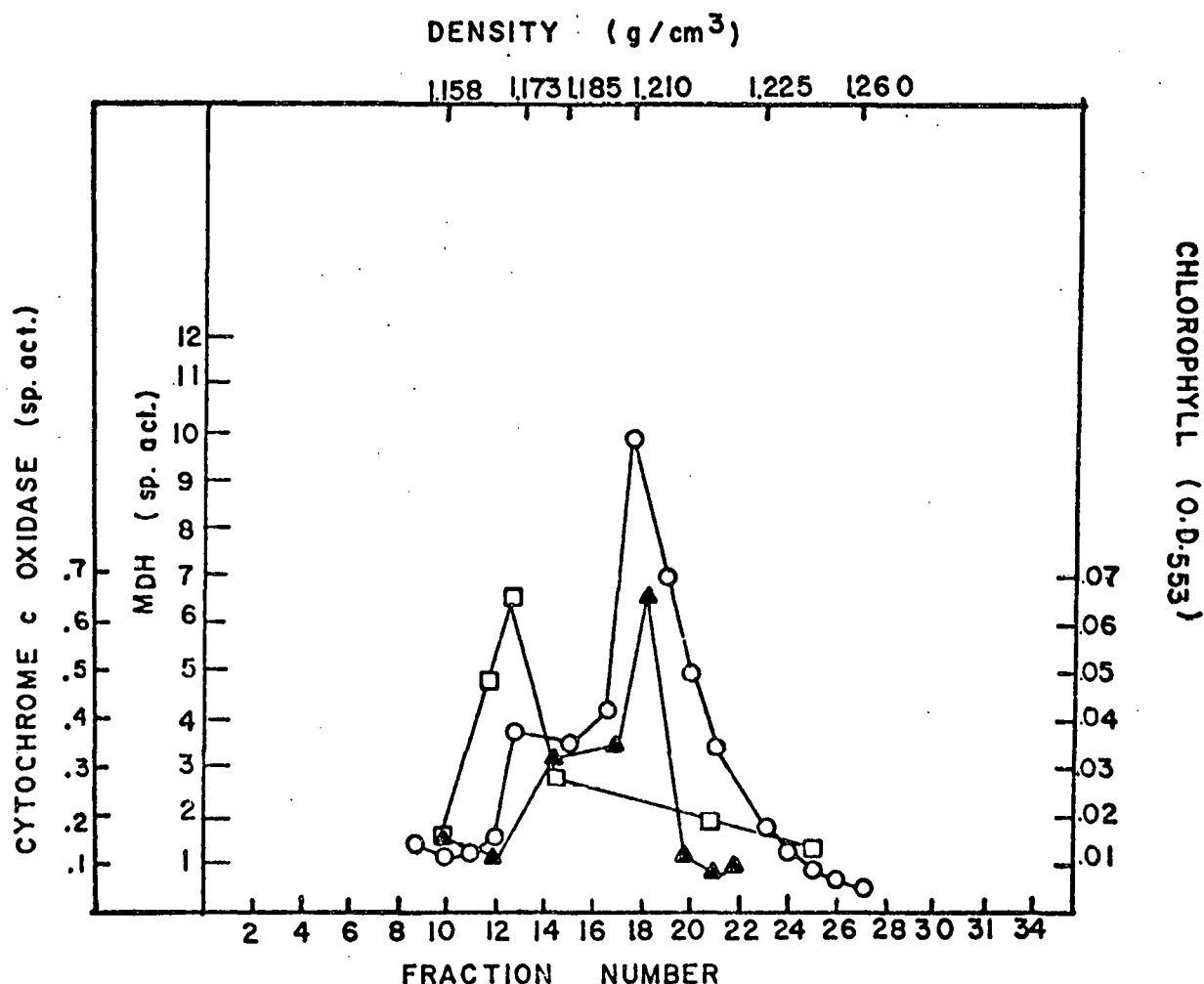


FIGURE 4. SUBCELLULAR FRACTIONATION OF CATTAIL ORGANELLES BY SUCROSE DENSITY GRADIENT CENTRIFUGATION. THE MITOCHONDRIAL FRACTION HAS A MEAN DENSITY OF 1.21. THE CHLOROPLAST FRACTION HAS A MEAN DENSITY OF 1.17. ○—○ : MALATE DEHYDROGENASE. ▲—▲ : CYTOCHROME C OXIDASE, A MITO CHONDRIAL MARKER. □—□ : CHLOROPHYLL (553 NM).

Two millimeter thick cross sections of cattail leaf meristems were heat shocked by incubation in a water bath at elevated temperatures. Several cross sections can be cut from a single meristem. This allows a single plant to be tested at a number of different temperatures. After 30 minutes at the elevated temperature, the tissue was then incubated for 8 hrs at room temperature in a dilute solution of NBT. The blue color which appeared on the surface of the meristem section was then extracted in pyridine and the absorbance at 523 nm determined.

Color yield was found to decrease with increasing temperatures. Also, the older outer leaves of the cattail stalk are not as thermally tolerant as are the youngest leaves in the center of the meristem. At 62°C

T. latifolia completely lost its viability and no further color was produced. T. domingensis completely died at 60°C. These temperatures are much higher than any physiologically encountered by the plant, but it should be noted that the heat shocking period of 30 minutes is a relatively short one. Incubation of these sections for longer periods of time at elevated temperatures results in lower maximum temperatures. Thus, length of exposure of plant segments to elevated temperatures appears to be crucial in determining the upper thermal limits of plant viability.

Our preliminary data indicate that T. latifolia is more tolerant of thermal stresses than T. domingensis. This agrees with field observations. Our Nitro Blue Tetrazolium test for tissue viability provides a simple quantitative measure of plant thermal tolerance. Heat shocking for short periods of time is useful in the comparison of the relative thermal tolerances of different plant species. Longer incubation periods are now being used to determine the actual physiological thermal tolerance of these plants.

AGGRESSION IN THE CRAYFISH CAMBARUS LATIMANUS IS INVERSELY RELATED TO TEMPERATURE (J. H. Thorp)

Until recently, water quality bioassay techniques have been limited to observations of the effects of lethal concentrations of a pollutant. Our knowledge of the sublethal effects of thermal and chemical pollution is quite restricted even though sublethal stresses can be equally detrimental to populations over an extended period. The effects of sublethal exposure to elevated temperature on the agonistic (aggressive) behavior of an important aquatic macroinvertebrate, the crayfish Cambarus latimanus, were investigated during FY 1976 and 1977.

During the summer of 1976, sixty male and female crayfish were acclimated for two weeks at 9.5, 14, 22 or 30°C. At the end of this period the crayfish were paired with another crayfish of the same sex and size, and tested for one hour at the acclimation temperature. The frequency and duration of aggression as well as latency to attack were recorded with a video-tape system for the thirty pairs. A total of four acclimation and test periods were run with each crayfish pair subjected in a random sequence to each of the four temperatures. In the winter of 1977 new crayfish pairs were acclimated and tested at 9.5 and 22°C. Results of these two experiments (i.e., summer and winter) were compared in order to test the effects of season (and concomitant reproductive-form changes) and temperature on aggressive behavior of crayfish.

A statistically significant inverse relationship between temperature and aggression was found in both summer and winter experiments. Additionally, crayfish were more aggressive at a given temperature during the reproductive season (winter) than the non-reproductive season (summer). These results suggest that reproductive form and temperature are related in their effects on aggression.

Since Cambarus latimanus is reproductively active during the winter, it is not surprising, that organisms of this species are also more aggressive at lower temperature. Reproductively active crayfish are morphologically adapted for increased aggression through a seasonal increase in chelae size. I hypothesize that the inverse linear relationship between temperature and aggression would be altered or reversed for crayfish species which are reproductively active during seasons other than winter. Such knowledge is requisite for accurately predicting the effects of environment stress on the behavior of aquatic organisms.

ACCLIMATION AT ELEVATED TEMPERATURES ENHANCES THERMAL TOLERANCES OF FRESHWATER SHRIMP (D. H. Nelson)

The freshwater shrimp Palaemonetes paludosus is a decapod crustacean commonly inhabiting streams of the southeastern United States. Extremely abundant in some streams on the SRP, these benthic organisms reportedly contribute significantly to the growth and reproduction of centrarchid fishes such as bass and bluegill. Thus, these small (< 30 mm) organisms represent important components of the detritivore - carnivore food web. Laboratory experiments were designed to test the thermal tolerances of shrimp that were acclimated for two weeks at one of four constant temperature regimes: 20°C, 24°C, 28°C, or 32°C.

Temperature tolerance values were determined for a total of 104 shrimp (26 specimens at each acclimation temperature). Individual shrimp were heated in pond water at a rate of ca. 1°C/minute until the death point had been attained. During the testing procedure, freshwater shrimp manifested four distinct responses to thermal stress:

1. Initial disorientation (ID) - first, temporary loss of balance
2. Complete disorientation (CD) - total loss of balance; animals at this stage were unable to return to normal posture
3. Onset of tail folding (OF) - tail beginning to fold ventrally and caudally, ultimately to lie appressed to the thorax
4. Loss of movement (LM) - complete cessation of appendage or body movement.

Initial disorientation was shown to be positively related to the temperature of acclimation (Figure 5). Of the four responses recorded, values for the I. D. were most variable; variances ranged from 2.2 to 15.2. Among all four acclimation temperatures, values for ID and CD differed significantly ($P < 0.05$) whereas values for OF and LM did not. Two thermal responses (ID and CD) were related to the temperature of acclimation. These initial two criteria are believed to represent behavioral responses on which evolutionary selective pressures might operate. The OF and LM, however, are interpreted as physiological responses that represent limiting mechanisms; these processes may not be related to selective pressures.

THERMAL STRESS PARAMETERS ($\bar{X} \pm SD$) FOR FRESHWATER SHRIMP,
Palaemonetes paludosus

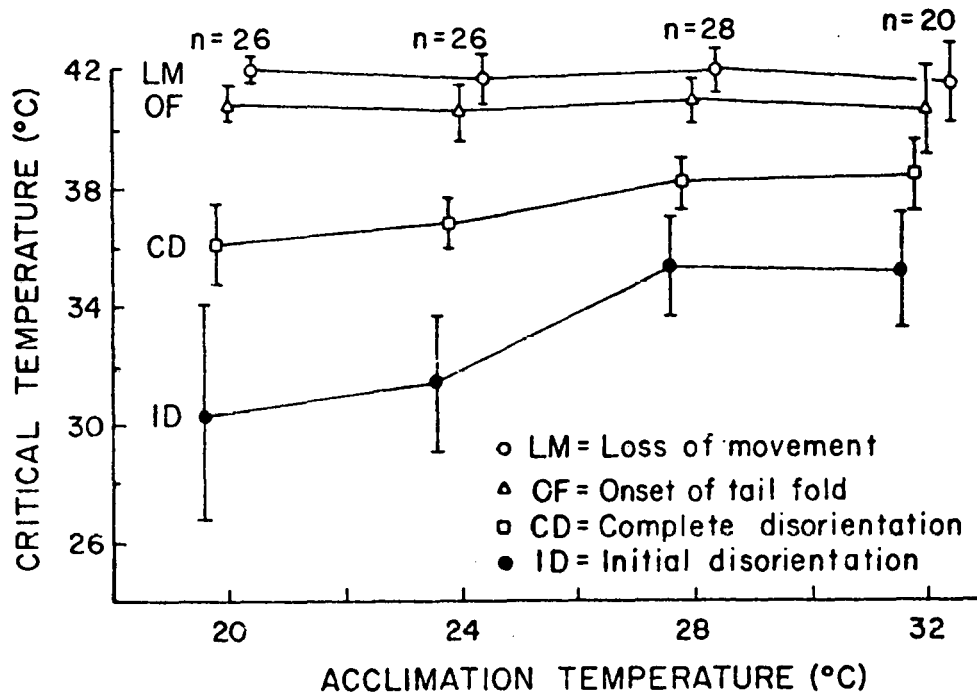


FIGURE 5. THERMAL STRESS RESPONSES OF FRESHWATER SHRIMP ACCLIMATED AT 20, 24, 28, AND 32°C.

Should this interpretation be correct, then behavioral responses become operative roughly from 30 to 39°C, and limiting, physiological responses occur from approximately 40 to 42°C. Additional tests need to be conducted at lower acclimation temperatures to confirm whether this trend is stable.

Values for OF and LM were very highly correlated (0.97) and may be regarded as separate measures of the same parameter. Discriminant function analysis revealed that the value for OF contributed an insignificant amount of information above that already provided by LM. A Duncan's multiple range test also showed that OF and LM values did not differ across acclimation temperature. The earlier occurring responses (ID and CD) represent discrete variables, both of which were distinct from LM. For ID and CD, however, values for 20 and 24°C acclimated shrimp were significantly different from those acclimated to 28 or 32°C. These data suggest that somewhere between 24 and 28°C lies a temperature that is critical to some essential metabolic process, and above which further acclimation does not occur.

Additional experiments are planned which will test whether freshwater shrimp are capable of thermoregulation. The ultimate goal will be to determine whether thermal preferences are manifested at all, and if so, whether they are related to temperature tolerances and acclima-

tion regimes. The ultimate goal of these studies is to integrate the experimental parameters of thermal tolerances, thermal preferences and acclimation temperatures and relate them as an ecologically relevant expression of stress.

CHRONIC, SUBLETHAL EXPOSURE TO CADMIUM CAUSES INCREASED MORTALITY IN CRAYFISH (J. H. Thorp and S. A. Wineriter)

Current knowledge of the effects of chemical stress on aquatic organisms is derived mostly from studies of the effects of acute, lethal concentrations of pollutants. The lack of studies on the effects of chronic sublethal exposure to chemical pollutants severely restricts our understanding of the environmental impact of chemical stresses. During a five-month period in the winter of FY 1977 we subjected male and female crayfish, Cambarus latimanus, to three concentrations of cadmium chloride (0, 5 and 10 ppb) which were sublethal under acute exposure.

One hundred forty-four crayfish were divided by sex and concentration (24 of each sex per concentration), and maintained in individual, flow-through plastic boxes. These boxes received water from the artificial stream system (HWCTR) on the SRP. Crayfish were fed three times per week and any dead crayfish were removed during these periods. Growth and mortality rates were calculated for the entire five-month period. After four months' exposure to 0, 5 or 10 ppb CdCl₂ the social behavior of the crayfish was observed and recorded on a video-tape system. Crayfish were paired for behavioral experiments and tested within and between sexes and cadmium concentrations. At the end of this experiment one-half of the surviving crayfish were immediately sacrificed and analyzed for internal cadmium concentration with an atomic absorption spectrophotometer. The remaining crayfish were exposed to gradually increasing temperatures (1°C/day) until all had died; these crayfish were then analyzed for cadmium concentration.

Since these experiments terminated only recently, we have not had sufficient time to analyze our results; however, several trends are evident. There is a direct relationship between cadmium concentration and mortality. Seventy-five percent of the mortality (approximately 20% mortality at 10 ppb) occurred at the highest concentration and no deaths were recorded for control animals. Growth rates appear equal for all treatments. The lowest growth rates were in the 10 ppb treatment and highest at 5 ppb; however, temperatures (and thus metabolic rate) were highest for the 5 ppb treatment boxes which may have obscured a predicted inverse relationship between growth and cadmium concentration. The effects of cadmium concentration on social behavior and resistance to elevated temperatures have not been analyzed.

In conclusion, we believe that a cadmium concentration of 10 ppb (current water quality standard) causes increased crayfish mortality and would result in a detrimental impact on populations of this important freshwater macroinvertebrate.

STUDIES OF CHEMICAL COMMUNICATION IN AQUATIC ORGANISMS ARE IMPORTANT
FOR AN UNDERSTANDING OF THE EFFECTS OF SUBLETHAL POLLUTION (J. H. Thorp)

If we are to determine safe limits for levels of chemical pollution in the aquatic environment, we must understand the principal mechanisms by which pollutants interact with living organisms. Since the paramount mode of information transfer in many groups of animals is through chemical communication, a knowledge of the effects of sublethal chemical pollution on communication by pheromones is vital. To accurately gauge these effects it is first necessary to obtain a behavioral pattern for an organism which dramatically responds to pheromones. Once this pattern is obtained, the organism can be chemically stressed and its response to pheromones reevaluated.

The ecological importance of pheromones in the agonistic behavior of the crayfish Procambarus acutus acutus (Girard) was investigated with a closed-circuit video-tape system. Four experiments were designed to test the effects of an inflow of water "conditioned" by the presence of crayfish in head tanks, on the behavior of solitary males in observation tanks. The durations of the following behaviors were recorded: meral spread, pincer spread, chela waving, grooming, and searching. Agonism in P. a. acutus is effectively reduced when the receiving animal lacks chemical information indicative of a stressed conspecific, but is not further reduced when those conspecifics are females. The considerable difference in duration of agonism between P. a. acutus (our study) and that reported for other species, suggests that the ecological importance of pheromones is species-specific in crayfish.

Although we have demonstrated chemical communication in P. a. acutus, we do not believe that the response shown by this species was sufficiently strong to justify its use as a target organism for studies of the effects of chemical pollutants on chemical communication. We are investigating, therefore, the potential use of other species of crayfish for this type of stress experiment.

THERMAL WATERS ALTER LIFE HISTORY PATTERNS AND ACTIVITY OF MOSQUITOFISH
(D. H. Smith)

The mosquitofish, Gambusia affinis, is native to many fresh and brackish water systems in the southern United States. It normally inhabits shallow, weed-choked areas of streams, swamps, ponds, and lakes, and is a voracious consumer of many types of small invertebrates, including mosquito larvae (hence its name, the mosquitofish). Because of their mosquito eating habit, Gambusia have been introduced into many areas of world to control pest mosquito populations. Thus they have assumed medical and economic importance.

Mosquitofish are native to most of the natural waterways on the Savannah River Plant, and have become extremely abundant in some areas that receive thermal effluents from nuclear reactors. Because the shallow waters normally inhabited by Gambusia heat rapidly in the summer sun, the fish are already adapted to relatively high water temperatures and to the resulting low oxygen concentrations. This allows them to survive in some heated waters where other species of fish may succumb to thermal stress.

Thermal effluents entering Pond C maintain the winter shallow-water temperatures of the pond in the range of 25 to 35°C during reactor operation. These temperatures are comparable to summer temperatures experienced by Gambusia in natural waters. Although both photoperiod and temperature affect the reproductive cycles of fish, temperature appears to be the determining factor in Pond C Gambusia. While mosquitofish in natural waters breed throughout the summer and cease to reproduce by late September or October, Pond C Gambusia have reversed this trend and their winter population density becomes extremely high (see Figure 6). In December of 1975, for example, fish densities ranged from 40.5 to 141.3 individuals per meter of shoreline (mean of 10 samples = 88.1). This peak density was far in excess of densities observed elsewhere during any season, and occurred when Gambusia populations in natural systems were experiencing severe winter declines. The Pond C sex ratio was approximately 1 : 1 at that time, and 83% of the adult female Gambusia were pregnant. By May, when mosquitofish in natural waters were beginning to breed and increase in numbers, rising water temperatures in Pond C had caused Gambusia populations there to decline so low that 10 hauls with a 4 meter seine yielded no fish. Although small, isolated Gambusia populations survived in cool spring and pool refugia around the pond margin, the population in Pond C proper had declined essentially to zero as a result of lethal water temperatures (greater than 40°C).

Normally, Gambusia populations are dispersed throughout areas of submergent and emergent aquatic vegetation, and thus may extend over areas of deeper water when floating vegetation provides escape cover from predators. In Pond C this is not the case. Not only does Pond C lack most of the higher forms of submergent and emergent vegetation, but a steep thermal gradient normally exists between the hot water in the deeper portions of the pond and the shallow "splash zone" along the pond margins, where water cools more rapidly because of wave action and the effects of the shoreline. Consequently, the mosquitofish there are restricted to a relatively narrow, one to three meter wide zone of water parallel to the shoreline. This compresses an already extremely dense population into an even higher density, and has several significant ecological consequences. The dense congregation of Gambusia close to shore and the general lack of cover in Pond C make the fish extremely vulnerable to avian predators. Kingfishers, mergansers, and several species of herons feed extensively in Pond C during the spring and fall, and resident kingfishers utilize Gambusia as a food supply throughout the winter. Schools of young bluegill (Lepomis macrochirus) utilize the same cool water zone as do the mosquitofish, and these bluegill feed extensively upon the Gambusia as well. During periods of reactor shut-down, water temperatures in Pond C decline to the point where mosquitofish and blue-

gill are able to disperse into deeper water. During such dispersals schools of largemouth bass also leave the larger, cool water refugia of streams entering Pond C. These bass feed on both Gambusia and bluegill. Significant population declines of Gambusia and bluegill are often observed during such bass dispersals.

Another result of the high mosquitofish densities is an increase in parasitism. Trematode parasites with complex snail-fish-bird life cycles are favored in thermal environments such as observed in Pond C because all three hosts for the parasite (freshwater snails of the genus Physa, mosquitofish and bluegill, and predaceous birds) are compressed into a narrow band in extremely high densities. These conditions contribute to a very efficient transfer of the parasites from one host to another. The population dynamics of two species of trematode parasites common to the Gambusia of the Savannah River Plant are described in relation to temperature in another section of this report (Aho and Gibbons).

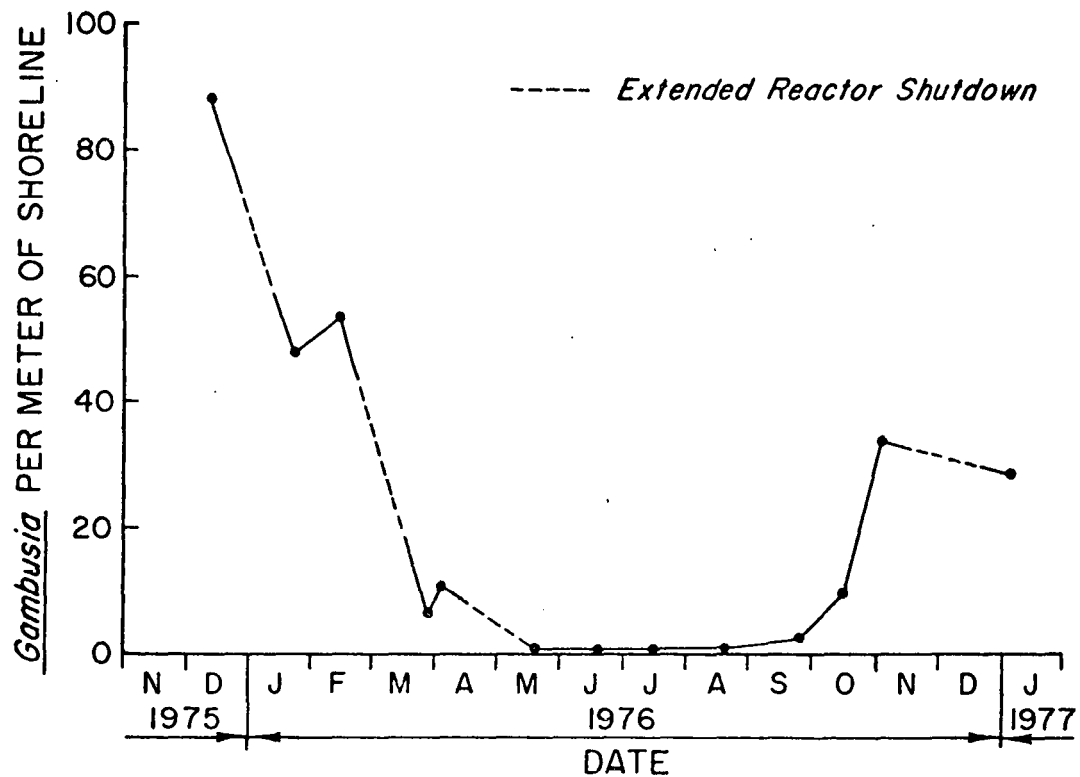


FIGURE 6. POPULATION TRENDS IN POND C GAMBUSIA AFFINIS. BROKEN LINES REPRESENT EXTENDED REACTOR SHUTDOWN.

Both parasite species exhibit seasonal cycling patterns in intensity of infection and incidence of infection. However, distinct differences in incidence of infection and infrapopulation densities of the two species of parasites occurred between the heated, moderately heated, and ambient temperature locations. The mean worm burden of O. ptychocheilus was found to be highest in fish taken from areas directly receiving thermal effluent. Values decreased in mosquitofish taken from areas of reduced water temperature. On the other hand, the mean worm burden of the body cavity metacercaria, D. scheuringi, was found to be highest in fish taken from areas of ambient water temperature. Densities were lower for fish taken from thermally influenced areas. It is interesting to note that no body cavity metacercaria were recovered from mosquitofish sampled from Pond C. Recruitment of new parasites into the host population began in late spring and continued throughout fall, followed by a decline in winter.

There are distinct differences between the heated, moderately heated, and ambient areas of Par Pond in regards to the incidence and intensity of infection of the two species of parasites found in the mosquitofish. However, there is no clear-cut explanation at the present time which accounts for the relationship between thermal loading and parasitism in G. affinis. In other previously examined host-parasite systems, the impact of thermal loading was probably related to a change in the feeding behavior of the definitive host, and hence, changes in the parasite population dynamics of that species. In the case of G. affinis, the feeding behavior of the intermediate host is not directly related to parasitism since the two species of parasites are acquired through cercarial penetration of the body wall. In this particular host-parasite system, differences in incidence of infection and infrapopulation densities for each location over time are probably related to the influence of thermal loading on the larval forms of the parasites, the snails serving as the first intermediate hosts, the distribution of the definitive hosts, or some combination of these possibilities. Upon completion of this study, data will have been generated that will provide answers to our questions concerning just how thermal effluent affects this host-parasite system and others which are linked to aquatic ecosystems.

PARASITES INCREASE VULNERABILITY OF MOSQUITOFISH TO PREDATORS (D. H. Smith)

Mosquitofish, Gambusia affinis, from thermally altered waters of the Par Pond and Pond C reservoir system of the Savannah River Plant suffer extremely high infection rates with two species of trematode parasites. One species, Ornithodiplostomum ptychocheilus, localizes in the eye or on the surface of the brain of the fish, and the other parasite, Diplostomulum scheuringi, is found free in the body cavity of infected Gambusia. These parasites also occur in Gambusia and other small fish in undisturbed waters, but nowhere else do they reach the densities observed in the fish from thermally stressed areas.

In an attempt to assess the impact of these parasites on the fitness of infected Gambusia, a series of experiments were carried out under laboratory conditions. Infected fish collected from three sites on the Savannah River Plant were exposed to predation by largemouth bass, little blue herons, and hooded mergansers in four by eight foot ponds. In each experiment a large sample of fish was collected from the pertinent habitat: Pond C, Par Pond, or the Steel Creek Delta. Half of each sample was sacrificed and the parasite burden of fish was determined as a function of the size of each individual fish. The other half of each sample was exposed to a single species of predator until 50% of the Gambusia had been consumed. The remaining mosquitofish were then examined to determine the number of parasites that each contained.

In every experiment, regardless of the source of the Gambusia utilized or the predator species that was used, fish of a specific size class averaged fewer parasites after predation than they did before being exposed to the predator (Figure 7). This reduction in average parasite burden resulted from predators catching and consuming more of the heavily parasitized mosquitofish than the lightly parasitized ones. This trend was most striking among mosquitofish in the larger size classes. Larger Gambusia normally carry heavier parasite burdens, and the range in number of parasites among the fish of a large size class is greater as well. Consequently, the effects of the parasites may vary from only slight to relatively severe among large fish. Within the smaller size classes of mosquitofish the parasite numbers were generally small, and the contrast between nonparasitized and parasitized individuals was slight. The difference in average number of parasites before and following predation was not as striking among the fish in smaller size classes, indicating that the small numbers of parasites in those fish probably did not have as much effect on their ability to escape predators as did the more severe infections in larger Gambusia.

Similar trends in average parasite burdens among mosquitofish subjected to changing predation patterns in the field tend to support these conclusions. In Pond C, high temperatures resulting from nuclear reactor effluent confine Gambusia to a narrow band of cooler water along the shore. The same high temperatures restrict large predatory fish (largemouth bass and adult bluegill) to refugia in streams or small pools adjacent to Pond C.

THERMAL LOADING DIFFERENTIALLY AFFECTS THE POPULATION DYNAMICS OF TWO
PARASITE SPECIES IN THE MOSQUITOFISH, GAMBUSIA AFFINIS (J. M. Aho,
G. W. Esch and J. W. Gibbons)

Previous studies have shown that thermal effluent released into the Par Pond reservoir system affects host-parasite interrelationships. Input of thermal effluent into Par Pond may act in such a way as to increase the abundance of the acanthocephalan parasite Neoechinorhynchus cylindratus in largemouth bass, or alter the species diversity of enteric parasites in the yellow-bellied slider turtle, Pseudemys (Chrysemys) scripta. Further studies have shown that thermal effluent affects the species composition and infrapopulation densities of parasites utilizing the mosquitofish as an intermediate host.

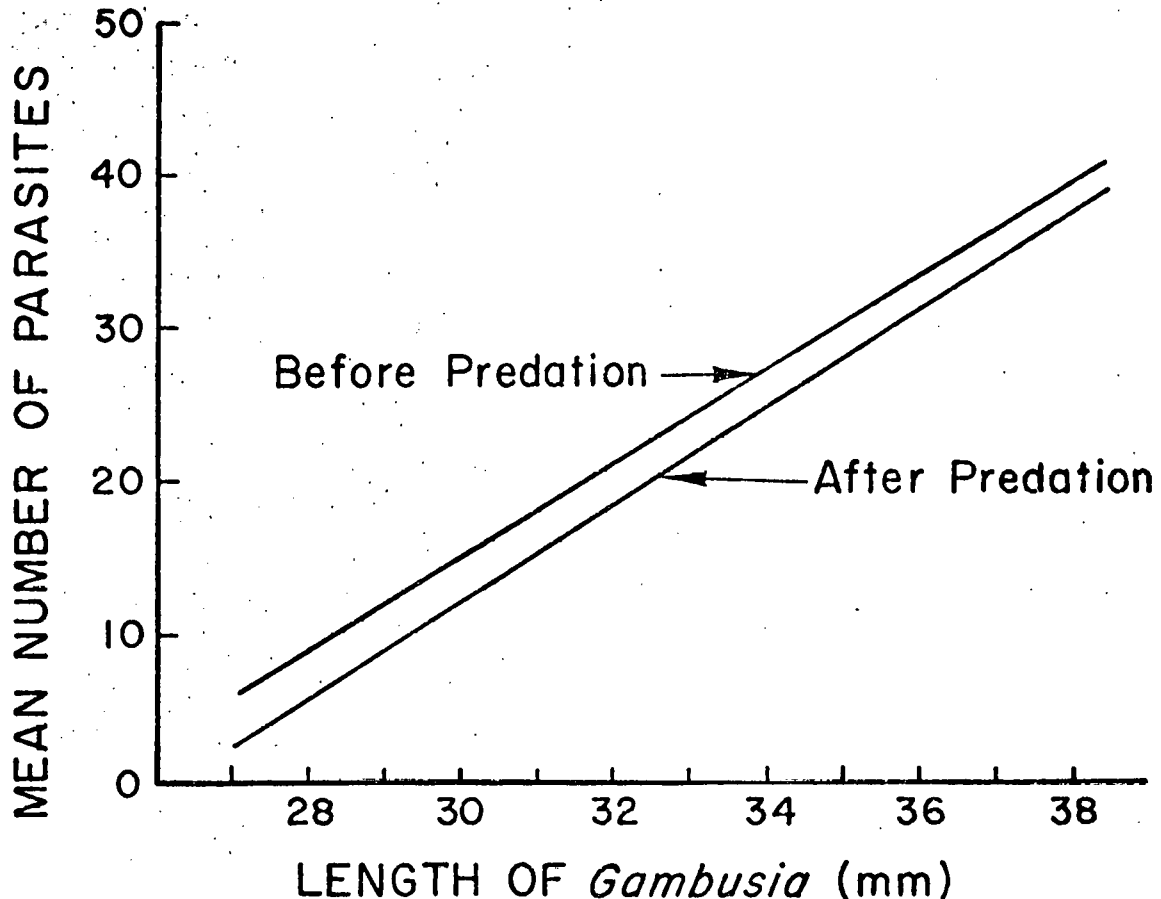


FIGURE 7. COMPARISON OF MEAN PARASITE BURDENS IN GAMBUSIA POPULATIONS BEFORE AND AFTER PREDATION BY LARGEMOUTH BASS.

The mosquitofish has been shown to harbor only two species of larval strigeid trematodes. Both of these parasites are acquired in an active fashion whereby the body of the fish is penetrated by a free-swimming larval stage, called a cercaria, which is released from an infected molluscan host. Snails which serve as the first intermediate hosts for many species of trematodes become infected when they come in contact with the miracidial life cycle stage of the parasite. Miracidia are released from eggs of the parasite as the avian definitive host defecates into the water. The definitive host, generally a species of fish-eating wading bird or diving duck, becomes infected when it consumes the infected mosquitofish.

The two species of larval trematodes have been identified as metacercaria of Diplostomulum scheuringi, which is found free in the body cavity of the mosquitofish, and Ornithodiplostomum ptychocheilus, which is found encysted in the cranial cavity of the mosquitofish. The cercaria of O. ptychocheilus penetrates the body surface of the mosquitofish and appears to migrate through the spinal cord until it reaches the

cranial cavity where it encysts, usually in the humor of the eye, on the backside of the sclera of the eye, or between the brain case and the meninges which cover the brain. On the otherhand, the cercaria of D. scheuringi penetrates the body surface and migrates to the body cavity where it develops as an unencysted metacercaria.

Preliminary studies indicate that thermal loading differentially affects the species composition and infrapopulation densities in the mosquitofish. In an effort to elucidate the effects of thermal effluent on parasite population dynamics, an extensive sampling program has been underway since January 1976 and will extend through May 1977. Mosquitofish (at least ten males and ten females) have been collected twice a month from nine locations on the Par Pond reservoir system representing areas directly heated by thermal effluent, areas of intermediate water temperature, and areas of ambient water temperature. The parameters of interest were the intensity of infection, the incidence of infection, size structure of the parasite population, and the dispersion pattern exhibited by the different species of parasites over time.

Although the mosquitofish are exposed to avian predators while confined to shallow water, they become subject to the larger fish predators only when reactor shutdown causes water temperatures to drop. Gambusia then forage out into deeper waters that are normally too hot for them, at the same time that schools of bass and adult bluegill disseminate from their refugia. The mean number of parasites per Gambusia declines during these periods of more intense predation. The pattern of change in parasite numbers parallels that observed in the laboratory experiments very closely. The rate of disappearance of heavily parasitized fish is greater in the larger size classes than among the smaller size classes.

MOLE SALAMANDERS REPRODUCE AS LARVAE (K. K. Patterson and J. W. Gibbons)

For 18 months, three populations of the mole salamander (Ambystoma talpoideum) were studied to determine the life history pattern of this species on the southeastern coastal plain. In each of the populations several unexpected life history events occurred. Prior to this study, these events were considered atypical of southeastern salamander populations. This research indicates that these phenomena are indeed common rather than unusual, at least in this area of the range.

Portions of the life history of the study species were discovered through interpretation of terrestrial migrations. As part of the study, a drift fence completely encircling one of the study ponds was erected in February, 1975 to catch all individuals moving toward or away from the water (Table 1). The salamanders were classed either as terrestrial adults (no gill remnants) or recently metamorphosed individuals (gill remnants). Terrestrial, adult mole salamanders move to ponds to breed in late fall or winter and return to land in the spring. The larvae hatch in the spring and remain in the water for approximately 14 months. In their second fall as larvae, the aquatic forms in all populations become

TABLE 1. MONTHLY VARIATION IN THE MIGRATIONS OF A. TALPOIDEUM FROM A STUDY POND ON THE SAVANNAH RIVER PLANT IN 1975 AND 1976. THE ADULTS WHICH PRODUCED OFFSPRING IN 1975 LEFT THE BAY IN FEBRUARY, THE LARVAE HATCHED IN SPRING 1975 AND LEFT THE POND IN SPRING, 1976.

	1975		1976	
	IN	OUT	IN	OUT
January			92	54
February	178	1238	24	372
March	32	452	19	230
April	63	814	82	1111
May	27	288	1	14
June	1	22		
July	3	6		
August	0	0		
September	0	1		
October	0	0		
November	306	16		
December	806	29		

sexually mature and reproduce. Larval sexual reproduction is termed paedogenesis. After reproduction and overwintering as larvae, a majority of the animals metamorphose and emerge onto land. There is some evidence, (not yet fully substantiated) that a few individuals may never transform. Retaining larval characteristics throughout life is termed neoteny. Apparently, stress from environmental factors can induce metamorphosis at any time after the animal has achieved a minimum size necessary for transformation. Some larvae, trapped by the drying pond, transformed in the late summer, 6 months after hatching and before reproducing.

One theory, advanced by others, proposed paedogenesis as adaptive in areas where temperatures are extreme, humidity is low and there is a lack of cover or food. Moist, warm terrestrial habitats would, therefore, be conducive to early metamorphosis. Since the climate of the southeastern coastal plain is warm and moist and provides adequate shelter and food for terrestrial salamanders, this hypothesis is not adequate to explain the life history events of the mole salamander on the SRP.

An alternative idea is suggested by the present research. The conditions of the terrestrial habitat may not influence the metamorphic strategies of larvae as strongly as the conditions of the aquatic habitat surrounding them. The potential for larval survival in a body of water may determine whether larvae metamorphose before sexual reproduction or remain in the aquatic habitat through the first reproduction. After attaining a certain minimum size, salamanders may have the option to

metamorphose or not, depending on the particular environmental stimuli the animals encounter. Paedogenesis in southeastern mole salamanders is not a consistently employed strategy, but is probably a consequence when larvae are not subjected to the particular environmental stresses which precipitate metamorphic processes.

It is not understood why the larvae do not remain neotenic, if they do not encounter adverse environmental conditions. Nonetheless, despite high water levels in the spring the majority of overwintering larvae transform, en masse. A possible explanation is that metamorphosis may be a mechanism of gene dispersal. After reproducing as a larva and introducing one's gene complement into one gene pool, it may be evolutionarily advantageous for an individual to leave that population and move overland to another one. Leaving offspring in several ponds would insure exposure to different environmental stimuli and increase the chances of some offspring surviving to reproduce. Another possibility is that insurance of favorable conditions for two consecutive seasons in such habitats is not high; therefore, after successfully breeding as an aquatic larva, one's chances may be best to forego an aquatic existence and become terrestrial, rather than risk a second year in such an uncertain environment.

SAVANNAH RIVER PLANT (SRP) HERPETOLOGICAL FIELD DATA PROVIDE CRITICAL BACKGROUND INFORMATION FOR SOUTH CAROLINA NONGAME AND ENDANGERED SPECIES PROGRAM (J. W. Gibbons and D. H. Nelson)

An objective of the South Carolina Endangered Species Symposium held at The Citadel in November 1976 was to consider the status of species in each taxonomic unit and make appropriate category assignments. As expected, most species were deemed to be in no foreseeable danger and tacitly fell into the implicit category of "no immediate concern." A few species were clearly deserving of "Endangered" or "Threatened" status, whereas a large number were placed by committees into the category of "Special Concern." This latter action was primarily a consequence of a lack of information about the ecology, abundance, or distribution of many of the species found within the state of South Carolina. The need for quantitative assessments of these critical facets of the biology of each species was boldly apparent. The "Special Concern" designation was particularly useful to the committee charged with placement of reptiles and amphibians into categories. As revealed at the meetings, detailed knowledge of South Carolina herpetofauna is limited, resting in the minds of a few individuals who have familiarity with a particular taxonomic group or region of the state.

Because published contributions about the herpetofauna of South Carolina are limited primarily to general works considering the entire or eastern U. S., a formal documentation of relative abundances and habitat associations of selected groups in particular regions within the state would be useful in identifying the gaps in our knowledge. Our objective was to provide this information for the reptiles and amphibians of the SRP, as a region representative of the Atlantic Coastal Plain. The

approach was to indicate the presence and relative abundance of each species, to note those species which might be expected to occur but apparently do not, and to add appropriate information on habitat associations that might be useful in future considerations of status assignments.

The SRP is an approximately 777 sq. km (300 sq. mi) tract of land bordered on the southwest by the Savannah River and encompassing portions of Aiken, Barnwell and Allendale Counties, South Carolina. The northern boundary is 32 - 48 km (20 - 30 mi) south of the Fall Line and the entire site lies within the Atlantic Coastal Plain geologic province. Two physiographic subregions, the Aiken Plateau (82 - 122 m elev.) and the Pleistocene Coastal Terraces (30 - 82 m elev.) are recognized. Although herpetofaunal lists and information have been derived from the entire SRP, concentrated efforts have been made at selected sites. Because of the bias inherent in using particular habitats and collecting methods both must be taken into consideration when establishing relative abundances of species.

Since 1965, 52 species of reptiles and 42 species of amphibians have been reported from the SRP (Table 2). At least 16 of these species might not be expected to occur on the site on the basis of general range maps. On the other hand, the occurrence of 19 species whose ranges might

TABLE 2. TOTAL NUMBER OF AMPHIBIAN AND REPTILE FAMILIES, GENERA AND SPECIES REPORTED FROM THE SRP SINCE 1965.

	Families	Genera	Species
AMPHIBIANS			
Frogs	5	8	26
Salamanders	6	9	16
Total Amphibians	11	17	42
REPTILES			
Turtles	4	8	11
Snakes	3	20	31
Lizards	4	6	9
Crocodilians	1	1	1
Total Reptiles	12	35	52
Total Amphibians and Reptiles	23	52	94

conceivably extend to the SRP has not been documented. Finally, despite the recognized biases associated with collecting method, habitat, and season, an impression of relative abundance of SRP species can be obtained through the comparison of numbers obtained from a variety of sources. Thus, the most common of more than 11,000 salamanders were Ambystoma talpoideum and Notophthalmus viridescens; the most frequently captured of more than 36,000 anurans were Rana utricularia (pipiens) and Bufo terrestris. The most frequently represented species of fewer than 1100 lizards were Sceloporus undulatus and Cnemidophorus sexlineatus, primarily because of their being the focal species in two separate studies. Coluber constrictor emerged as the dominant snake species of more than 3000 specimens. However, a dozen species are represented by more than 100 individuals each. In some instances the large numbers of a species may be attributed to a particular study or sampling technique. Chrysemys scripta is unquestionably the most ubiquitous and abundant turtle on the SRP. This is further confirmed in additional population studies on the SRP which are not yet reported. Nine species (2 amphibians and 7 reptiles) listed in the "Special Concern" category by the Endangered Species Symposium Committee for Reptiles and Amphibians are documented inhabitants of the SRP. Only one species with Endangered status (Alligator mississippiensis) has been reported.

The obvious value of this effort is that it systematically relates what is known about the herpetofauna of an area which is representative of a large segment of the state. Such an account helps to identify habitats and species that require further attention and provides a basis for comparison with other regions. At this time, several species of reptiles and amphibians deserve special attention because uncertainty exists about their status in the SRP herpetofauna. For some, the question is whether a reported species actually does occur on the site. For others, the question is whether they occur but simply have not been captured or reported. This always exists as a possibility, especially when most of our information comes via particular times, habitats, and techniques, so that certain forms are overly represented whereas others may be overlooked. However, the establishment of relative abundances, despite the recognized collecting biases, provides a foundation from which to proceed in determining the status of critical species in this or any given area.

NINE YEAR RESEARCH PROGRAM REVEALS LONG TERM GROWTH PATTERNS AND AGING PHENOMENON OF REPTILES (J. W. Gibbons and J. L. Greene)

Understanding growth and size phenomena of reptiles, specifically turtles, is important for several reasons. For one thing maturity is related to size, not just age, in some species; for another, a positive relationship exists between body size and clutch size in several species of turtles. To understand individual growth patterns within a species, the impact of factors which influence growth must be determined. For example, what effect do varying environmental conditions, population density, age, sex, or size structure have on the growth strategy and related population dynamics of a particular species?

The obvious growth pattern in turtles (as well as in many other organisms) is that juveniles grow rapidly, and adults grow more slowly. In the most useful growth model now available for turtles, juvenile growth is best characterized by a logarithmic relationship whereas adult growth patterns are linear. Several studies have shown variability in growth rate between populations of turtles of the same species, even if populations were in close geographical proximity. The difference between populations has been accounted for by varying environmental conditions. However, the general patterns of growth appear to be similar.

Due to the reported variations, and because growth is related to a number of variables, a thorough, critical examination of growth patterns is necessary to provide an understanding of the phenomena. The objective of this study was to examine several facets of growth in selected populations of aquatic turtles in order to characterize growth patterns and to address a number of questions relating to their evolution and ecology. We have a unique opportunity on the SRP to examine long-term growth rates because we have mark-recapture data from populations over a 9-year period.

Two populations of the yellow-bellied turtle (Chrysemys scripta) from the SRP ($N \approx 1100$) were used in this study. One population was from Ellenton Bay, a Carolina Bay which has varied in size from a few meter area in 1967-68, when the population was first marked, to its current size of about 10 ha. The other population was from Risher Pond, a 1.1 ha farm pond constructed in the 1930's. Approximately 850 turtles have been marked at Ellenton Bay, and over 200 have been marked at Risher Pond.

Capture methods included a 20 cm high drift fence of aluminum flashing with paired 20 l buckets sunk at 10 m intervals alongside the fence to check terrestrial movement of the animals. Aquatic fyke nets and chicken wire swim-in-traps baited with fish or beef heart were also used. Animals were captured, sexed, measured, weighed, and released. They were permanently marked by drilling the outer scutes of the carapace. Change in plastron length was used as the indicator of growth.

Long-term growth rates (turtles recaptured 6-9 yrs. after original capture), short-term growth rates (turtles recaptured 1-3 yrs. after original capture), and changes in population structure were examined.

The long-term growth data (Figure 8) indicated that the larger a turtle was at original capture, the more slowly it grew. This is interpreted as a growth pattern showing not only a gradual slowing trend from immature to adult but also showing a declining growth rate as an adult gets older. This contrasts with the earlier mentioned, generally accepted model which states that growth rates of adult turtles are linear.

It has also been previously reported by others that males of a given size grow more slowly than females. Although true, it should be emphasized that males mature at a much smaller size than females (100 mm plastron length compared to 160-170). Consequently, if adjustments are made for this size discrepancy at maturity, adult males and females grow at the same rate, according to our data (Figure 8).

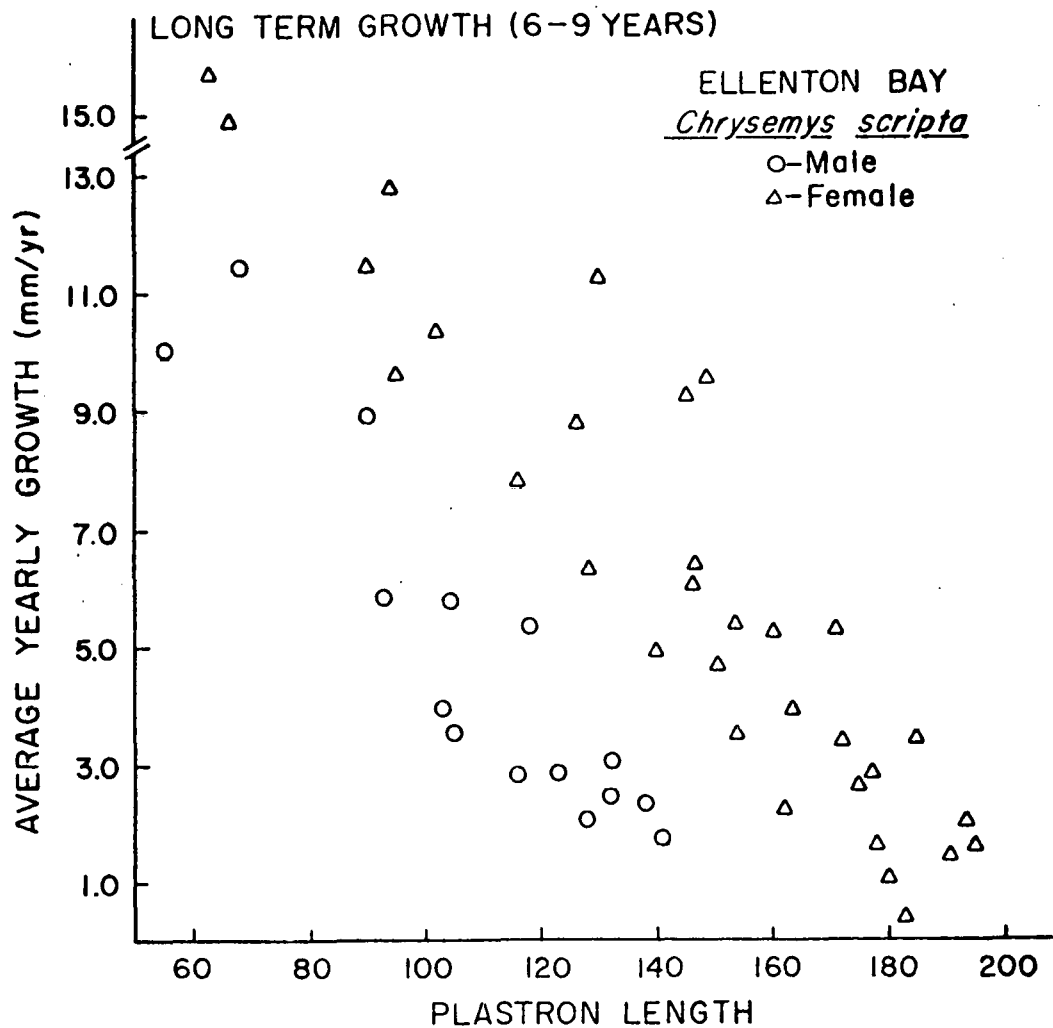


FIGURE 8. MEAN ANNUAL GROWTH RATES FOR TURTLES BETWEEN SUCCESSIVE CAPTURES.

Short-term growth information was found to be much more variable than the long-term data, but does suggest the same trends. Higher variability in growth would be expected over a short time due to extreme, though temporary, environmental conditions. Short-term data would also suffer more from experimental error than long-term data. Therefore, short-term growth rates, though useful, should be viewed cautiously and should not be taken out of context of the entire growth pattern of the individual.

A population size structure comparison for Ellenton Bay between 1967-68 and 1975-76 showed the peak in proportion of mature males being in the 100-119 mm size category in 1967-68 whereas in 1975-76 the peak was in the 120-139 mm size category. No shift was obvious for females.

The most interesting observation gained from the population structure information however, was that no males larger than 149 mm in the 1967 population were captured in 1975 even though individuals up to 229 mm were marked in 1967. No females larger than 199 mm in 1967 were recaptured in 1975 even though animals up to 245 mm had been marked in 1967. This suggests that the largest turtles of both sexes from 1967 have left the population either by emigrating or dying. The former reason seems improbable since there are turtles of the large size classes currently living in Ellenton Bay. Our data, therefore, suggest that not all Chrysemys scripta live as long as 75 or even 40 years which are the maximum and average lifespan reported in the literature.

In conclusion, our studies of growth rates in C. scripta indicate that the larger a turtle is, regardless of sex, the more slowly it grows, and that if we adjust for sex, adult males and females grow at the same rate. Furthermore, adults in our populations do not show linear growth rates; instead, the adult growth pattern best fits an asymptotic model. Finally, the size structure and recapture data imply that individuals in some populations of C. scripta do not live as long as was previously thought.

DISSECTABLE FAT BODIES MAY BE MAJOR ENERGY SOURCE FOR REPRODUCTION IN SNAKES (J. W. Gibbons and R. D. Semlitsch)

The concept of reproductive effort has played a major role in the development of theories explaining life history patterns but, as recently indicated by several authors, the development of these theories has outweighed the accumulation of empirical data. The most common estimate of reproductive effort has been the clutch to body ratio which offers a quick measure of what some investigators believe to be the major portion of energy expended in reproduction. However, as may be seen from the criteria set forth by Williams (1966), the clutch to body ratio is not as estimate of total reproductive effort but merely one component thereof. Even in organisms that do not exhibit parental care, significant proportions of their total energy budgets are probably spent on courtship displays, mating, territoriality, and migration to breeding sites.

Total energy budgets for each species are needed to determine the proportion of energy available to the organism that is partitioned into reproduction. One component of the energy budget is reproductive allocation, which designates the proportion of total body tissues partitioned into reproductive tissues. Although numerous studies have analyzed and discussed factors influencing patterns of reproductive allocation in lizards, few have dealt with these aspects in other reptiles. The purpose of this study was to collect information on several critical variables influencing reproductive allocation in snakes in order to more fully understand their ecology and evolution. The initial effort concentrated on one species of water snake, Natrix taxispilota.

Brown water snakes (N. taxispilota) were collected during the spring and summer from freshwater streams (Upper Three Runs Creek, Steel Creek and Tinker Creek) on the SRP. Specimens were brought to the laboratory, measured (snout-vent length) to the nearest millimeter, and weighed (total body weight) to the nearest g. Dissections were made to determine sex, number of yolked and unyolked follicles or embryos in the present year's litter, mean diameter of three, randomly chosen follicles in the largest size class, and weights of the total litter, fat bodies and stomach contents. The litter destined to be dropped that year was assumed to be represented by the largest set of follicles present. Care was taken to include only that year's litter and to separate mesentery, oviducts and fat bodies from the follicles being measured. Body (minus stomach contents), follicles and fat bodies were freeze-dried separately to determine dry weight. Each component was ground on a Wiley mill into a homogeneous mixture. A Goldfish Fat Extractor was used to extract total lipids from each component (two 1 g aliquot samples of follicles and body; one 1 g aliquot sample of fat bodies). Dried fat bodies were usually small enough to include the total sample in a single extraction. Petroleum ether was used as a solvent and extractions were run for four hours each. The petroleum ether was evaporated from preweighed beakers and the remaining lipids weighed with accuracy to 0.0001 g. Reproductive allocation was calculated as the ratio of litter weight to total body weight plus lipid weight. Both wet and dry weight determinations were made.

A significant correlation ($P < 0.01$) exists between the number of follicles per litter and snout-vent length in N. taxispilota. This is consistent with results obtained for lizards. These data for N. taxispilota support the hypothesis of Williams (1966) that the resources allocated to reproduction increase with age (size of N. taxispilota is assumed to increase with age). However this does not indicate an increase in reproductive effort (a proportional determination) with age, but only that the absolute amount of reproductive tissues increases.

Reproductive allocation calculated with three variables (wet body weight, dry body weight and lipid weight) for 17 females N. taxispilota collected within a short time span (26 days) was not significantly correlated with snout-vent length ($P > 0.05$ for all three variables). The slope of each regression line by ANOVA did not differ significantly from zero ($P > 0.10$ for all three slopes). These data indicate that the absolute amount of body tissues partitioned into reproduction increases with body size in N. taxispilota but that the proportion does not. This does not eliminate the possibility that reproductive effort increases with size or age.

Since all N. taxispilota females greater than 91.0 cm snout-vent length found during the summer months had enlarged follicles, it is assumed that reproduction occurs annually, in contrast to biennial reproduction in some viperid species. This, coupled with the fact that water snakes deposit their young during the late summer or early fall,

lead us to expect that an increasing amount of resources would be partitioned into eggs and developing young during the summer months. Reproductive allocation in *N. taxispilota* was found to vary seasonally, increasing from a value of 0.0059 ± 0.0009 in early March to a value of 0.2133 ± 0.0054 for mid-July and to 0.3344 for a single individual taken in August. Not only did the litter weight increase through the summer months but total lipid content of the litter also increased from $5.91 \pm 0.57\%$ in early March to $26.19 \pm 0.19\%$ by mid-July, just prior to parturition.

This increase in reproductive allocation and lipid content of the clutch was most closely related to the weight of fat bodies in *N. taxispilota*. Percent fatbodies increased in females from $1.38 \pm 0.45\%$ in early March to a maximum value of $3.16 \pm 0.38\%$ in July, 30 days before the peak reproduction allocation value. A decline to $2.18 \pm 0.41\%$ was observed at the same time reproductive allocation increased sharply. Other body components maintained relatively constant levels of lipids throughout the summer (Table 3). These data are consistent with those of other investigators and indicate that fat bodies may be an important source of lipids during follicle development.

TABLE 3. LIPID AND WATER COMPOSITION OF BODY COMPONENTS IN *NATRIX TAXISPILOTA*. VALUES PRESENTED ARE MEANS \pm 1 STANDARD DEVIATION: SAMPLE SIZES ARE INDICATED IN PARENTHESES.

Sex	Body	Percent Total Lipids		Percent Water
		Fatbodies	Follicles	Body
♀	2.24 ± 0.80 (36)	91.22 ± 8.01 (37)	19.78 ± 9.44 (27)	74.8 ± 1.81 (37)
♂	2.27 ± 0.68 (24)	87.33 ± 12.74 (24)	-	75.2 ± 2.13 (24)

TERRESTRIAL DRIFT FENCE PROVIDES EXPLANATION FOR WORLDWIDE BEHAVIORAL PHENOMENON IN TURTLES (J. W. Gibbons and D. H. Nelson)

Turtles of most temperate region species are characteristically presumed to lay their eggs in the spring or summer and emerge from the nest as hatchlings in the fall. However, actual dates of hatchling emergence are well-known for only a few species. The extensive use of terrestrial drift fences and pitfall traps on the SRP has allowed a unique opportunity to gather empirical evidence on the dates that hatchling turtles of several aquatic species actually emerge from their

nests. Our approach has been to obtain field data on this phenomenon and to complement this difficult-to-obtain information with a thorough review of world literature on the subject. The ultimate goal is to provide an evolutionary and ecological explanation for variation in emergence time within and among turtle species.

When available, information on emergence habits of turtles is anecdotal, generally based on no more than one or a few observations for any given species. Collectively, these indicate that delayed emergence (or overwintering in the nest in cool temperate climates), is a phenomenon of worldwide occurrence in turtles and is far more common than traditionally believed. Delayed emergence has been reported from localities as varied as Nova Scotia, South Africa, Japan, Russia, southern Australia and the southeastern and midwestern United States and has been confirmed in more than a dozen genera and six families.

To determine the extent of delayed emergence in turtles from South Carolina, we have carried out studies on populations associated with three habitats on the SRP. Each aquatic site was completely enclosed by a terrestrial drift fence and pitfall traps to monitor terrestrial movements of all sizes and species of turtles. Traps were checked daily for at least two years at each site. Data, location and size were noted for each hatchling captured. Maximum and minimum temperatures and precipitation for the previous 24 hr were recorded.

Data collected during those years when year-round drift fence studies were conducted revealed that hatchlings ($N = 383$) of the five most abundant aquatic species moved toward the water primarily during early spring (Table 4). Most freshwater turtles have incubation periods of at least 60 days and no species in the South Carolina populations begins laying before mid-February. Therefore, hatchlings collected before May are assumed to be holdovers from the previous year's clutches. The only other South Carolina turtle species for which extensive hatchling data are available is the loggerhead sea turtle (*Caretta caretta*). This species rarely, if ever, overwinters, on South Carolina beaches (John Mark Dean and Rhett Talbert, pers. comm.).

Hatchling aquatic turtles emerged only during warm periods, primarily in the spring. Nonetheless, fall temperatures at times when most eggs would have already completed development were higher than spring temperatures during the period of actual emergence. For example, temperatures in fall 1975 (September $\bar{x}_{\max} = 29.1^{\circ}\text{C}$, $\bar{x}_{\min} = 18.1^{\circ}\text{C}$; October $\bar{x}_{\max} = 26.5^{\circ}\text{C}$, $\bar{x}_{\min} = 12.1^{\circ}\text{C}$) were higher than those in spring 1976 (March $\bar{x}_{\max} = 23.2^{\circ}\text{C}$, $\bar{x}_{\min} = 7.6^{\circ}\text{C}$; April $\bar{x}_{\max} = 26.3^{\circ}\text{C}$, $\bar{x}_{\min} = 8.4^{\circ}\text{C}$). No relationship was apparent between rainfall and hatchling emergence ($\chi^2 = 3.40$, $df = 1$, $P > 0.05$).

Our data challenge the ideas advanced by others that hatchling emergence is dependent upon rainfall or that overwintering is a phenomenon of delayed development as a result of cold conditions. Hatchling emergence dates on the SRP were in no way correlated with amount or date of precipitation. Delayed development was also not a suitable explanation,

TABLE 4. SEASONAL EMERGENCE PATTERN OF HATCHLING FRESHWATER TURTLES INTO THREE AQUATIC HABITATS ON THE SRP. LETTERS REPRESENT MONTHS OF THE YEAR.

	F	M	A	M	J,J,A	S,O,N	TOTAL	% F,M,A
<u>Deirochelys</u>								
<u>reticularia</u>	-	113	45	1	4	1	164	96
<u>Kinosternon</u>								
<u>subrubrum</u>	10	74	10	0	1	1	96	98
<u>Chrysemys</u>								
<u>scripta</u>	-	47	24	5	3	3	82	87
<u>Chrysemys</u>								
<u>floridana</u>	-	22	9	-	1	-	32	97
<u>Sternotherus</u>								
<u>odoratus</u>	1	3	-	-	2	3	9	44
	11	259	88	6	11	8	383	93

as adequate temperatures for incubation occur in South Carolina long after the nesting peak. Instead, our findings are best explained by a general hypothesis applicable to all situations.

A cost/benefit assessment is useful in questioning why some turtles delay emergence whereas others of the same or different species apparently do not. The primary benefit to the individual emerging immediately upon completion of embryonic development is the potential to initiate feeding and growth toward maturity. The primary benefit of delayed emergence from the nest is the proven sanctuary offered during a period when growth benefits are likely to be outweighed by predation or environmental costs.

Potential costs of remaining in the nest after development could be higher mortality as a result of severe freezing or flooding as well as the indirect cost of a loss in feeding and early growth. Potential costs of immediate emergence result primarily from high probabilities that hatchlings will encounter an unfavorable situation. These might include earlier exposure to predators, drying-up aquatic habitats or the onset of cold weather. These risks, when coupled with no enhancement of feeding opportunities, would result in immediate emergence being disadvantageous if the risks could be minimized by extending the date of emergence.

We conclude that species whose habitat associations result in high levels of uncertainty about the cost/benefit of emergence will be more likely to delay emergence whereas species in less variable and more predictable situations usually will not. Toward a specific model, we predict that delayed emergence will be least likely to occur in species in which: 1) The terrestrial terrain between nest and water and/or the features of aquatic environments which critically affect young turtles do not vary appreciably by season. 2) Food sources for juveniles have a high assurance of being favorable throughout the period of hatching. 3) Mortality of hatchlings which remain in the nest after hatching is likely to be greater than mortality of hatchlings which leave the nest and enter the typical juvenile habitat.

ALLIGATORS LIVING UNDER THE ICE HAVE BODY TEMPERATURES MONITORED BY NEW TECHNIQUE (E. A. Standora and I. L. Brisbin)

The severity of the past winter made it possible to observe alligators and monitor their body temperatures as they survived near their lethal thermal minimums. This was accomplished by inserting one end of a temperature sensor into each animal's cloaca and suturing it into position. The other end of the wire lead which was approximately four meters long was folded and placed into a flexible plastic tube mounted on the alligator's tail. This procedure required about one half hour and was performed in the field. The alligators were then outfitted with a radio transmitting collar and released. On subsequent days the animals were easily located by using a directional receiving antenna. Once the alligator was found, a hole was cut in the ice above the animal's tail. By reaching under water, the sensor lead was removed from its storage tube and attached to a telethermometer at the surface which provides a readout of body temperature. Because of the low water temperature the alligators were very sluggish and seldom moved during this measurement. If it was probable that the animal would move before the next measurement the sensor lead was reinserted into its storage tube.

With the surface ice melted but the water still cold, it was possible to attach a small float to the sensor lead after removing it from its protective tube and then quietly paddle to the float at frequent intervals to record body temperatures without disturbing the animal. One such instrumented alligator showed a drastic change in body temperature during February as it came out of 5.0°C water and basked for several hours in 8.5°C air, thus increasing the temperature of its 135 Kg (298 pound) body by 72% going from 6.4°C in the morning to 11.0°C at midafternoon.

Other field and environmental chamber heating and cooling experiments conducted on both living and dead animals have shown that large alligators heat in half the time it takes them to cool. These data imply the existence of a physiological control of thermoregulation; one possible mechanism might involve blood shunting. The geographic location of the Savannah River Plant at the northern limit of the alligator's range

and the occurrence of thermal effluent from production reactors provide us the unique opportunity of studying alligators which are stressed by both cold and hot temperature extremes.

TELEMETRY SYSTEM PROVIDES NEW CAPABILITIES FOR FIELD STUDIES OF AMERICAN ALLIGATORS (E. A. Standora and I. L. Brisbin)

During the past year efforts have been continuing to better understand the thermoregulatory behavior and physiological ecology of the American alligator. Temperature data from free ranging animals in the field were obtained with a newly developed eight-channel, temperature-sensing radio transmitter. Unlike the earlier sonic transmitters we used, this new system has made data acquisition possible even when the alligator is in dense vegetation or out of the water basking. This latter condition is very critical to the animals' thermal energy balance.

The transmitter (Figure 9) which operates at 164 MHz, is fitted around the animal's neck and has a varying pulse rate dependent on the value of resistive sensors, in this case thermistors. The transmitter samples eight channels sequentially for a period of 15 seconds each. Two of the channels are reserved for reference and calibration purposes to correct for battery voltage drop and thermal drifting of components. The remaining six channels accommodate thermistors which are attached to the alligator during a six hour operation. The thermistors are positioned at the head (supratemporal fossa), mid-dorsally (subcutaneous), mid-ventrally (subcutaneous), in the cloaca, in the tail musculature and at the tail surface. A lengthy calibration procedure gives individual exponential equations (interpulse period vs. temperature) for each sensor-channel pair and for the transmitter body at four different temperatures. The pulsed telemetry signal is received at a tracking boat, fed into a signal conditioner and then timed and displayed on a digital panel meter. These time intervals are then used in a computer program which corrects for transmitter drift and then converts them into temperatures.

In addition to the temperature data transmitter, a telemetering recapture block has also been successfully employed. The recapturing of a telemetered alligator has been difficult in the past for while the animal could be located with a minimum of difficulty using a directional antenna, the chance of successfully snaring the individual was highly variable dependent on the posture of the alligator and the likelihood of slipping the snare noose over its head before it would swim away.

With the new approach a small wooden block containing a radio beeper is attached to a collar around the alligator's neck by means of a short stainless steel wire tether. This transmitting block which floats several inches above the animal's neck provides an easily accessible and highly visible target for snaring. With the help of a plexiglas underwater viewer held over the side of a boat the brightly

colored recapture block can be easily seen and snared regardless of the alligators posture or density of aquatic vegetation.

This method of recapturing alligators has been used successfully both during the day and at night. With this technique perfected it is now possible to conduct studies on particular individuals within the population knowing that they can be recaptured at will. Such an approach is particularly applicable to studying growth rates throughout the year, to sampling blood at various times to determine the health status of a population, and to studying the reproductive cycles of particular individuals.

ALLIGATOR MULTICHANNEL TRANSMITTING COLLAR

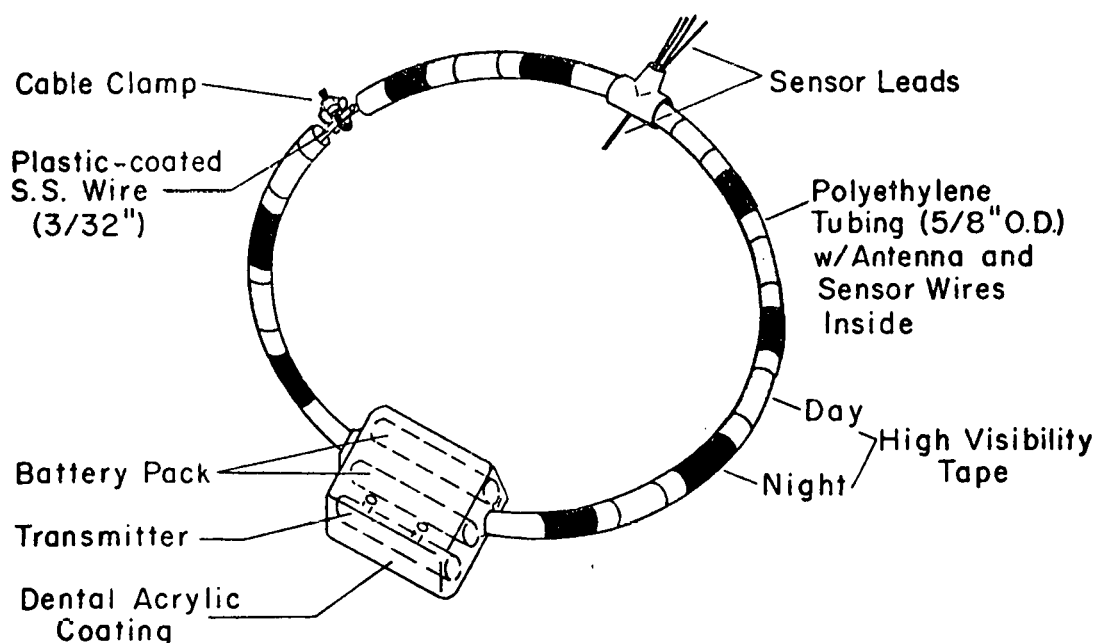


FIGURE 9. EIGHT-CHANNEL RADIO TRANSMITTER USED TO MONITOR ALLIGATOR BODY TEMPERATURES AND WATER TEMPERATURES AS ANIMALS SWIM IN HEATED RESERVOIR.

II. MINERAL CYCLING PROGRAM

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OBJECTIVES OF THE MINERAL CYCLING PROGRAM: IDENTIFICATION AND SCOPE

The mineral cycling program at SREL is an umbrella for subprograms studying cycling of heavy metals and other stable elements, radioisotopes, radioecology of transuranic elements, and the management of natural resources. These studies focus on the impact of energy production and other human activities on the natural and agricultural environments of the Savannah River Plant (SRP). The broad range of environments and habitat types of the SRP lead to the more general applicability of our studies to much of the southeastern U. S. Several of our studies are carried out in cooperation with DuPont's Savannah River Laboratory and the U. S. Forest Service which are also contract investigators on the SRP.

We have sought to maintain a balance between trying to uncover general principles of ecological functioning and elemental cycling on one hand and studying the behavior and effects of specific pollutants, their pathways to man and the impacts of specific technologies and management practices on the other. This duality is complementary; in studying principles we can achieve greater generality and applicability to specific energy production and management practices, whereas the applied studies allow us to test more general hypotheses and answer questions important to environmental planning, policy formulation and decision making. The studies reported herein reflect this philosophy.

In studying heavy metals and other stable elements, we have sought to identify and quantify the biotic and abiotic reservoirs of these elements in terrestrial and aquatic ecosystems on the SRP, the routes and rates of transfer between these reservoirs, and the factors controlling those rates. Studies designed to understand biological accumulation and magnification of heavy metal concentrations up food chains is one part of

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this general approach. The role of dissolved organic substances in the availability, toxicity and rate of accumulation of heavy metals by aquatic organisms is being investigated in detail. The differences in cycling of essential elements and those that are nonessential and potentially toxic are being investigated in both aquatic and terrestrial ecosystems in hopes that general principles of predictive significance will emerge. Cadmium is a specific heavy metal pollutant mentioned in several reports. By looking at this element in depth we expect to determine mechanisms of cycling and sublethal effects which may be common to other pollutant metals as well. Such discoveries would not likely result from a broad, less intensive, multi-element study. Coal fly ash is a second specific pollutant receiving much study. Here the effort has gone into determining which heavy metals associated with the fly ash may pollute aquatic or terrestrial ecosystems. In a stream-swamp system of the SRP, cadmium, lead, and chromium from fly ash seem to contribute to elevated concentrations in biota.

Radioisotopes have been used as tracers of ecological and elemental cycling processes. They are particularly useful in tracing elemental movements from known sources, identifying unknown sources, and determining biological availability and rates of transfer. Frequent advantages of using radioisotopes rather than stable elements in these studies have been in ease of quantification and a reduction in sampling variability. Cesium-137, long known as a major radioactive pollutant from nuclear reactors, has served as a useful tool in radioisotope tracer studies on the SRP because of the releases to streams here. Transfers to edible species such as waterfowl, deer, and fish, have been part of these ¹³⁷Cs studies.

Work in radioecology of transuranic elements has sought to determine critical pathways to man of the elements plutonium and americium. The nuclear fuel reprocessing plant on the SRP has provided us with a low-level source of these radioactive elements in the environment. We have looked at transport mechanisms and availability to biota. Our initial emphasis has been placed on the uptake of transuranics by agricultural crops and the effects of various agricultural practices on the transfer of these elements directly to man or to the animals on which he might feed.

Our program in the management of natural resources has sought to determine the impact of various environmental management practices of the SRP on ecosystem integrity. In the past we have studied how pine forest management programs of the U. S. Forest Service, for example, influence endangered species and their special habitats. In other studies the harvesting of "red straw" (pine needles) has been shown to remove significant quantities of nutrients from pine forests, with a possible effect on productivity. In other studies, the deer and feral pig populations on the SRP which are managed under a plan of controlled hunting have been examined in terms of their genetics, feeding and habitat adaptations.

COAL FLY ASH MAY BE A SIGNIFICANT SOURCE OF CD, CR, AND PB TO AQUATIC PLANTS (D. W. Evans and J. P. Giesy)

Coal fly ash contains elevated concentrations of some trace metals such as As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Se and Zn. If released to the natural environment, fly ash may transfer some fraction of these metals to biota, with unknown consequences. Elevated trace metal concentrations have been observed in soils and plants surrounding coal burning power generating facilities, presumably resulting from aerial fallout of fly ash. If, as an alternative to aerial dispersal, fly ash is removed from emission stacks and disposed of on land, localized but much higher trace metal elevations in biota are risked. This is particularly important in aquatic ecosystems which can accumulate fly ash-derived trace metals from more extensive terrestrial drainage areas. Moreover, chemical transformation of fly ash in aquatic environments can make associated trace metals more readily available or toxic to sensitive aquatic organisms.

Beaver Dam Creek is a small stream on the SRP which has received effluent from settling basins in which coal ash has been deposited for the last twenty years, and precipitator fly ash since December 1975. It has provided us with a site to study the fluxes, transformations and biological transfers of selected coal ash-derived trace metals. Our initial study approach, only partially completed, has surveyed trace metal concentrations in water, suspended particulate matter, periphyton, fish and selected aquatic and semi-aquatic plants along a downstream transect of Beaver Dam Creek. Hopefully, variations in metal concentrations grading away from the effluent source will indicate the extent of fly ash-derived trace metal transport and biological availability. Less extensive sampling in a similar stream not receiving coal ash effluents (Steel Creek) provides a comparison by which we may judge to what extent coal ash elevates trace metal concentrations in the ecosystem components of southeastern coastal plain streams.

Our sampling and analyses are still incomplete but several tantalizing patterns worthy of further exploration have emerged and can be pointed out: (1) Although not unexpected, metal concentrations in all ecosystem components are highly variable, even in samples from a single time and location. This requires collection of a large number of samples if statistically significant tests of concentration differences among stations within a stream, among different streams, or among different times are to be obtained. Rather than a broad survey, future research will focus on more specific aspects of biological availability and chemical transformations of fly ash metals. (2) Comparison of metal concentrations in plants from Beaver Dam Creek, which receives coal ash effluent, and Steel Creek, which does not, reveals that only Cd, Pb, and Cr, show elevated concentrations in Beaver Dam Creek. This is particularly evident for the cattail, Typha latifolia (Table 5). The other metals studied, Co, Cu, Fe, Mn, Mo, Ni, and Zn rarely showed any significant differences in concentration between the two streams. There are two potential explanations for this dichotomy in behavior: (a) Cadmium,

lead, and chromium are among the elements most concentrated in fly ash relative to bottom ash and natural soils and sediments; and (b) Cadmium, lead, and chromium are thought to be non-essential elements for plants, whereas the other metals are required in metabolic processes. As a result, it would be expected that plants would have evolved mechanisms to regulate their concentrations of the required elements, maintaining a degree of constancy in internal concentration despite variations in the metal concentrations in the external environment. Non-essential elemental concentrations in plants are expected to reflect variations in the external environmental concentrations. In the future, we might expect further elevations of Cd, Pb and Cr concentrations in plants in Beaver Dam Creek if these conclusions are true. Although this creek has received coal bottom ash for more than twenty years, bottom ash has trace metal concentrations that differ little from natural soils and sediments. In contrast, fly ash has relatively elevated concentrations of both essential and non-essential trace metals, and Beaver Dam Creek has been receiving fly ash removed from the stack electrostatic precipitators for less than a year. (3) Trace metal concentrations in plants are not constant among stations along a downstream transect in Beaver Dam Creek. Rather than a decrease in concentration for many of the metals, grading away the fly ash source upstream, we see lowest concentrations at intermediate stations and higher concentrations at the extreme upstream and downstream stations, especially the latter. This is particularly evident for Cd. The extreme downstream station is located in a small lake into which Beaver Dam Creek flows before entering the Savannah River. Here, fine suspended particulate matter can settle out, providing a substrate for rooted aquatic plants. The fine fly ash particles, enriched in certain trace metals, could be carried through the faster flowing upstream sections of Beaver Dam Creek and deposited in the lake to serve as the source of elevated metal concentrations in plants there. Alternatively, some as yet unknown chemical transformations or changes in bioavailability characteristic of the lake system could explain the elevated concentrations. (4) Metal concentrations in both periphyton (Table 6) and suspended particulate matter in Beaver Dam Creek are highly variable. But the extreme downstream station, as for the macrophytic plants, shows the highest concentrations for the metals analyzed so far. This adds further support to the hypothesis that the lake is a site of preferential accumulation of some trace metals. (5) Concentrations of the metals, with the exception of Mn, are very similar in periphyton and suspended particulate matter. This is to be expected since the biotic periphyton community effectivity traps suspended particulate matter, which is probably the dominant contributor to the total metal content in periphyton. As such, periphyton can be an important direct vector of metals in stream-borne fly ash to herbivores and detritivores. Higher Mn concentrations in periphyton than in suspended particulate matter, suggests that this element is significantly accumulated in periphyton by direct biological uptake, as well as by simple physical entrapment of suspended particulate matter. (6) Concentrations of Cr, Fe, and Zn are not significantly different in redbreast sunfish, Lepomis auritus, collected from both Beaver Dam Creek and Steel Creek. Manganese concentrations are higher in the latter. We are awaiting completion of analyses of the other metals under study. This species of fish migrates up and down the streams of the SRP from the Savannah River

and cannot be expected, therefore, to reflect entirely the trace metal environment of the location of capture. We have collected and are analyzing for metals the mosquitofish, Gambusia affinis, from stations in both Beaver Dam Creek and Steel Creek. These fish are non-migratory and should better reflect metal concentrations due to fly ash inputs.

TABLE 5. METAL CONCENTRATIONS IN TYPHA LATIFOLIA COLLECTED ALONG A LONGITUDINAL TRANSECT OF A STREAM (BEAVER DAM CREEK) RECEIVING EFFLUENT FROM A COAL FLY ASH SETTLING BASIN AND IN A CONTROL STREAM (STEEL CREEK). STATIONS 1 THROUGH 12 IN BEAVER DAM CREEK ARE PROGRESSIVELY DOWNSTREAM FROM THE EFFLUENT SOURCE. CONCENTRATIONS IN $\mu\text{g/g}$ DRY WEIGHT ARE SHOWN AS MEANS \pm 2 STANDARD ERRORS OF THE MEAN.

	Beaver Dam Creek				All Stations	Steel Creek
	Station 1	Station 4	Station 7	Station 12		
Cd	1.74 \pm 0.48	1.40 \pm 0.76			1.57 \pm 0.44	0.21 \pm 0.11
Co	0.87 \pm 0.14	2.52 \pm 0.90			1.70 \pm 0.58	1.16 \pm 0.48
Cr	2.9 \pm 0.7	1.9 \pm 0.5			2.4 \pm 0.5	0.08 \pm 0.07
Cu	11.2 \pm 2.8	12.3 \pm 4.0			11.8 \pm 2.4	5.3 \pm 1.2
Fe	320 \pm 120	360 \pm 190	NOT PRESENT		340 \pm 120	280 \pm 120
Mn	1900 \pm 500	800 \pm 200			1300 \pm 300	2700 \pm 1000
Mo	1.45 \pm 0.69	0.30 \pm 0.72			0.87 \pm 0.42	0.39 \pm 0.25
Ni	1.0 \pm 0.5	2.3 \pm 0.4			1.6 \pm 0.4	2.4 \pm 2.3
Pb	5.9 \pm 1.7	6.9 \pm 4.1			6.4 \pm 2.2	2.3 \pm 1.3
Zn	8 \pm 2	13 \pm 6			10 \pm 4	10 \pm 5

FOOD CHAIN STUDIES COMPARE UPTAKE AND RETENTION OF HEAVY METALS (J. B. Gentry)

A large coal ash storage basin is located near a coal burning electrical generator on the Savannah River Plant. Over the past 20 years major portions of the basin have been colonized by vegetation which now supports a dense and diverse invertebrate community. In a recent study (fall 1975), cadmium (Cd) behavior was described in an invertebrate food web in the ash basin system. The concentrations of Cd increased at each progressively higher trophic level in the terrestrial food web. A snail, functioning in the system as a detritivore or litter-eater, had unusually high Cd levels (16.9 ppm). The present report concerns the behavior of certain heavy metals in food chain relationships on the coal ash basin. It was felt that this approach would provide a more accurate comparison of uptake and retention of different elements.

TABLE 6. METAL CONCENTRATIONS IN PERIPHYTON COLLECTED FROM TWELVE STATIONS ALONG A DOWNSTREAM TRANSECT OF A STREAM RECEIVING COAL ASH EFFLUENT AND A SINGLE STATION IN A COMPARISON STREAM (STEEL CREEK).

Station	Cd μg/g	Cr μg/g	Cu μg/g	Fe mg/g	Mn μg/g	Ni μg/g	Zn μg/g
Beaver Dam Creek							
1	0.47 ± 0.13	49 ± 20	203 ± 36	78 ± 41	800 ± 500	(17 ± 14)	225 ± 85
2	0.64 ± 0.29	46 ± 7	178 ± 67	67 ± 30	2350 ± 2250	37 ± 11	219 ± 56
3	1.40 ± 1.77	33 ± 8	141 ± 166	35 ± 28	520 ± 290	1.9 ± 0.5	157 ± 92
4	2.5 ± 3.4	70 ± 26	211 ± 49	63 ± 12	1380 ± 1220	28 ± 36	321 ± 212
5	0.90 ± 0.56	62 ± 21	211 ± 34	56 ± 9	1590 ± 1190	8	282 ± 81
6	0.74 ± 0.04	130 ± 3	254 ± 69	65 ± 13	610 ± 300	41 ± 50	390 ± 259
7	0.88 ± 0.88	30 ± 10	131 ± 13	34 ± 10	4260 ± 650	43 ± 6	121 ± 4
8	1.42 ± 0.76	43 ± 1	220 ± 81	62 ± 28	21870 ± 19710	62 ± 20	325 ± 81
9	1.16 ± 0.81	39 ± 2	160 ± 33	37 ± 3	22730 ± 20730	55 ± 32	256 ± 72
10	2.8 ± 2.5	35 ± 4	226 ± 91	44 ± 21	38350 ± 27980	122 ± 59	320 ± 134
11	1.4 ± 1.0	47 ± 24	198 ± 10	45 ± 11	17560 ± 24530	65 ± 50	320 ± 226
12	4.8 ± 3.0	27 ± 16	201 ± 54	27 ± 9	74480 ± 62550	112 ± 130	405 ± 159
Steel Creek	1.7 ± 1.1	20 ± 8	49 ± 15	54 ± 34	63420 ± 54440	0.53	248 ± 102

Two food chain relationships were studied (Table 7). Samples from the first, a plant-aphid-ant system, were collected in the 1976 summer. Samples from the second, a plant-fall webworm system, were collected in the 1976 fall. Feces, which accumulate at the bottom of the webs spun by the larval webworms, were also collected. All samples were analyzed for copper (Cu) and Cd. Further analyses will provide values for zinc (Zn) and lead (Pb).

In food chain #1 (Table 7) the ants contained three to four times more Cu than the plants and aphids. Copper, a component of the blood pigment hemocyanin, is a required element of many invertebrates. Cadmium, on the other hand, is not considered an essential element, yet Cd concentrations were relatively high in the ants (two times higher than in the plants and seven times higher than in the aphids). A possible explanation may lie in a unique feature of food chain #1. Aphids have mouth parts especially adapted to pierce plant tissues and suck their juices. As large volumes pass through the aphids body, part of the sugar, amino acids and other nutrients are removed. The remainder is excreted. It is these excretions, obtained from the plant on which they were excreted or directly from the aphids, that provide the ant's food. Although it is believed that only slight alterations occur in the plant juices as they pass through the aphids system, there is the possibility that the process could cause significant alterations in elemental concentrations and thus be reflected in the next higher trophic level.

Food chain #2 is simpler and more direct than food chain #1. The webworm caterpillars are not plant juice suckers but derive their nutrition from direct consumption of the cottonwood leaves. Again, like the ants, Cu levels in the caterpillars were greater. The high Cu concentrations in the feces indicate that the rate of Cu uptake by the caterpillars may be quite rapid. Unlike the ants in food chain # 1, the caterpillars did not concentrate Cd.

Further elemental relationships in food chains are planned. Samples of a plant-tent caterpillar food chain have been recently collected. Caterpillars have been brought into the lab and allowed to pupate. In this particular food chain, we hope to describe the behavior of certain essential and nonessential trace elements from plant (wild cherry) through caterpillar, pupae and adult. Along the way certain by-products of the food chain (feces, cast larval skins, pupal skins and cocoons) will be analyzed and their role assessed in specific trace metal dynamics in food chains.

TABLE 7. MEAN (\pm SE) COPPER AND CADMIUM CONCENTRATIONS ($\mu\text{g/g}$) IN TWO TERRESTRIAL FOOD CHAINS FROM A COAL ASH BASIN.

FOOD CHAINS	Cu	Cd
FOOD CHAIN #1 (Samples Size = 12)		
<u>Populus</u> *	8.1 \pm 0.8	0.75 \pm 0.11
Aphids	6.7 \pm 0.9	0.24 \pm 0.11
Ants	27.2 \pm 7.6	1.69 \pm 0.31
FOOD CHAIN #2 (Samples Size = 21)		
<u>Populus</u> *	10.9 \pm 0.7	1.12 \pm 0.09
Caterpillars	26.7 \pm 1.3	0.52 \pm 0.07
Feces	10.4 \pm 0.6	0.92 \pm 0.10

* Cottonwood

ESSENTIAL AND NON-ESSENTIAL TRACE ELEMENTS FOLLOW DIFFERENT FREQUENCY DISTRIBUTIONS IN FISH POPULATIONS (J. P. Giesy and J. G. Wiener)

Processes affecting compartmentalization, biogeochemical cycling and transport of trace metals in ecological systems must be elucidated if the impact of trace metal releases on biota are to be understood. Because elemental cycles are often closely linked to hydrologic processes, freshwater and marine systems serve as both transport vectors and sinks for trace metals in the environment. Fishes may have major roles in elemental compartmentalization and flux in certain aquatic systems and can comprise a significant pathway for transport of toxic substances to man because of their importance in the human diet. Fishes may also act as integrators of environmental conditions in aquatic habitats. An understanding of frequency distributions in living organisms allows establishment of health standards for human consumption of food items, proper design of monitoring programs and estimation of contamination levels by sampling procedures. The major objectives of this study were to collect baseline information on whole body concentrations of various trace metals in representative fishes of different trophic levels and natural history and to examine frequency distributions of the concentration data. Frequency distribution data was fitted to the normal, one-parameter exponential, log-normal and Weibull distributions.

Mean whole-body concentrations of Par Pond fishes were similar to those of fishes from other areas not highly contaminated by industrial or mine effluents. Twenty of the 23 species-element groupings for whole body concentrations had positive skewness (g_1) values, while three of the groupings had negative skewness values. None of the negative skewness values were significant, whereas 13 of the 20 positive skewness values were significant. The lognormal, normal Weibull and exponential distributions had the best fit (lowest D) to 57, 17, 26 and 0 percent of the species-element groupings respectively (Table 8). The lognormal, normal and Weibull distributions were, respectively, suitable models of frequency distributions of trace metal concentrations for approximately 78, 57 and 78 percent of the total number of cases considered.

Even though we attempted to minimize effects of size and environment on metal concentrations by our selection of samples, trace metal concentrations were often highly variable and positively skewed. The "Law of Proportionate Effect" may offer an explanation for these positively skewed data. This law applies to processes of change in which the random variable at any step of the process is a random proportion of the previous value of the random variable. Random variables which are the result of such a series of proportional effects tend to be lognormal. Many probability functions are involved in trace metal accumulation and the net result of these functions is frequently a convergence toward a log-normal distribution. Lognormal distributions are also multiplicative.

The Central Limit Theorem states that addition of a large number of small independent random variables yields a quantity whose distribution tends toward normality. If the normal distribution does not occur, one or more of the following requirements for applicability of the Central

Limit Theorem are not satisfied: (1) variables are not random, (2) there is not a large number of random variables, and (3) the distribution of variables is extremely skewed or these effects are not simply additive, but combine in some other way.

Skewed distributions of trace metal concentrations can also be generated from underlying processes such as exponential elimination with time. Such uptake and elimination processes would seemingly produce skewed distributions for all trace elements; however, essential trace elements are less skewed than nonessential elements. Because concentrations of nonessential elements are more skewed, they are apparently under less homeostatic control than concentrations of essential elements. If required elements in tissues are actively concentrated and toxic elements are actively excreted to prescribed concentrations, frequency distributions of these elements should tend to normality. A constant elimination rate tending toward total elimination of a toxicant, rather than elimination to a prescribed concentration, would result in a positively skewed distribution. The mean and variance of the resulting normal distribution would be determined by uptake and elimination reaction kinetics.

Analysis of frequency distributions of body concentrations of trace contaminants may provide valuable insights concerning the mode of toxicity of chronic levels of these contaminants. For example, Zn is acutely toxic to fish at higher concentrations even though Zn is under homeostatic control and internal body concentrations are closely regulated. The mode of Zn toxicity is probably external to the organism involving direct effects on gill membranes or indirect effects due to production of mucus. Threshold concentrations for contaminants such as Zn in water can be established. However, it is more difficult to set maximum allowable concentrations in water for contaminants such as Cd, whose concentrations in biota are not strictly regulated by homeostatic mechanisms. Because certain toxicants can be accumulated to potentially toxic levels, allowable water concentrations must then be set with reference to rate of accumulation in relation to longevity of the organisms(s) in question. Use of fishes as integrative organisms for monitoring trace metal concentrations in ecological systems may not be appropriate for metals such as Zn and Cu which are homeostatically controlled.

In summary, certain factors should be considered when surveys of trace metal contamination in aquatic biota are conducted. For example, the distributions of trace metal concentrations in the populations being studied may be non-normal, highly skewed and outlier-prone. Therefore, sample sizes must be large and caution should be exercised in editing and statistical analysis of data. Presentation of a mean and measure of variability may not adequately summarize the data. Trace metal concentrations in fishes are the result of many complex probability functions describing input-output phenomena and homeostasis in the organism.

TABLE 8. BEST FITS TO THE FOUR DISTRIBUTIONS FOR ALL SPECIES-ELEMENT GROUPS. THE DISTRIBUTION WHICH PRODUCED THE LOWEST D (FROM LILLIEFORS' TEST) FOR EACH DATA SET WAS JUDGED TO HAVE THE BEST FIT.

Distribution	Number of Best Fits ^a	
	All Four Included	Weibull and Exponential Excluded
Lognormal	13 (3)	16 (4)
Normal	4	7 (1)
Weibull	6 (1)	-
Exponential	0	-

^a Numbers in parentheses indicate the number of significant departures from the best fit at $\alpha = 0.05$.

DIRECT ACCUMULATION FROM WATER IS THE MOST IMPORTANT SOURCE OF CADMIUM (CD) TO MOSQUITOFISH (J. P. Giesy)

Cadmium (Cd) is a biologically nonessential element which occurs naturally in the environment in trace quantities. Rapid mobilization of Cd into the biosphere may have adverse effects on organisms which have evolved in an environment containing low concentrations of this element. Cadmium, unlike required elements such as Zn and Cu, which are homeostatically controlled by organisms can be accumulated to toxic levels over a long period of time from very low concentrations in the environment. Cadmium toxicity to fish is cumulative. Therefore, to assess deleterious effects on fish populations and possible human health hazards due to biomagnification we need to be able to predict Cd levels that fish will accumulate from all sources. In addition, the mechanisms of Cd accumulation by fish must be understood before valid models of Cd flux through fish populations or biotic communities can be formulated and elemental cycling patterns predicted. Fish can potentially accumulate metals from both ingested food items and directly from water. For predictive purposes, if one vector or the other is unimportant, the effort required to collect information for predictive models can be reduced.

During the first six weeks of exposure, no Cd was accumulated from food by the mosquitofish, Gambusia affinis (Table 9). Only after eight weeks of exposure did food contribute significantly to Cd uptake. This may have been due to cumulative Cd effects resulting in an inability to restrict Cd entry from gastrointestinal sources.

TABLE 9. MEAN CD CONCENTRATION IN G. AFFINIS UNDER FOUR TREATMENT COMBINATIONS OVER TIME, C.I. = 95%, N = 5.

Week	Water	Response ($\mu\text{g Cd/g}$) *	
		Food	
		L	H
2	L	1.60 \pm 1.48	1.07 \pm 0.68
	H	14.67 \pm 2.33	18.14 \pm 17.07
4	L	1.48 \pm 0.64	0.93 \pm 0.17
	H	28.10 \pm 10.6	25.38 \pm 2.85
6	L	3.86 \pm 2.58	3.82 \pm 3.14
	H	30.72 \pm 14.16	25.88 \pm 2.86
8	L	0.41 \pm 0.12	0.61 \pm 0.22
	H	46.88 \pm 12.57	71.49 \pm 16.49

* Dry weight.

L = water < 0.02 $\mu\text{g/l}$ Cd

H = water 10 $\mu\text{g/l}$ Cd

L = food 0.1 $\mu\text{g/g}$ Cd

H = food 1.0 $\mu\text{g/g}$ Cd

CADMIUM INHIBITS LEAF DECOMPOSITION (J. P. Giesy)

Prior to human perturbation, most streams and rivers were densely covered with vegetation. Shielding from direct sunlight and the structure of stream channels fostered the development of a heterotroph based system. The dominant trophic energy source of small woodland streams is allochthonous input, such as leaf fall. But only a small portion of the energy contained in leaf material is directly available to aquatic animals. The animal and microbial components of the stream community have evolved to process these inputs, with the animal community relying on microorganisms

to partially decompose refractory plant tissues such as lignin and cellulose. Because of the importance of fungi and bacteria as intermediaries in leaf litter processing, their inhibition in streams would mean a drastic change in community structure and decreased secondary productivity. While the toxic and inhibitory properties of heavy metals to aquatic microbes have been studied, little is known about the effects of low levels of these toxicants on the colonization and leaf litter decomposition by natural communities.

Leaf material was placed in screen mesh holders and suspended in the channels of the U. S. EPA-ERDA stream microcosm facility. Cadmium at (10 µg/l) and below (5 µg/l) the present drinking water standards inhibited leaf litter decomposition (Table 10). Scanning electron microscopic examination of leaf surfaces exposed to cadmium for 13 weeks revealed an almost complete inhibition of microbial colonization. A variety of fungi and bacteria have been shown to be tolerant to high concentrations of heavy metals, relative to concentrations which are toxic to other organisms. By contrast, the results of this study indicate that low Cd concentrations can inhibit the functioning of decomposing microorganisms.

LOW LEVELS OF CADMIUM AFFECT STREAM MICROCOSMS (J. P. Giesy)

The direct toxicity of high levels of cadmium to both plants and animals is well documented. What happens when small amounts of this metal are introduced into aquatic ecosystems, however, is poorly known. The ubiquity of cadmium in the environment, and the fact that it does not degrade into harmless products, makes it necessary that we have a better understanding of the cycling of this metal in the aquatic environment.

The fate and biological effects of low levels of cadmium introduced into stream microcosms has been studied the past two years as part of an integrated comprehensive SREL research program in elemental cycling. Microcosms were used to bridge the gap between data derived from controlled laboratory systems with minimal biological complexity, and those derived from uncontrolled and unreplicable field studies.

The microcosms used for this work are part of an EPA facility located on the Savannah River Plant; funds for the work were provided by the EPA.

The microcosms facility consists of six channels approximately 300' long, 2' wide, and 1' deep with pools located at both ends of each channel.

In March 1976, cadmium inputs were initiated into four of the six systems to establish paired treatments at 5 and 10 µg Cd · l⁻¹. Two channels were maintained as controls. Routine sampling of channel components, which was initiated several months prior to the introduction of cadmium, is still on-going although the cadmium inputs were discontinued in March, 1977.

TABLE 10. EFFECT OF CD ON FINAL BIOMASS OF LEAF MATERIAL IN LEAF LITTER PACKS $\bar{X} \pm 2 S_{\bar{X}}$.

TREATMENT	DRY WEIGHT (g)		WET WEIGHT (g)
	TYPE I	TYPE II	
Control	2.3 \pm 0.18	1.1 \pm 0.08	
5 $\mu\text{g}/\ell$ Cd	4.0 \pm 0.06*	1.7 \pm 0.19 ⁺	
10 $\mu\text{g}/\ell$ Cd	4.0 \pm 0.73*	1.7 \pm 0.11 ⁺	
SPECIES			
TYPE I			
<u>Pinus taeda</u> L.			5.0
<u>Sassafras albidum</u> (Nutt.) Nees.			3.0
<u>Quercus nigra</u> L.			3.0
<u>Quercus laurifolia</u> Michx.			2.0
<u>Prunus americana</u> Marsh.			2.0
<u>Acer rubrum</u> L.			2.0
TOTAL			17.0
TYPE II			
<u>Acer rubrum</u> L.			3.0
<u>Quercus nigra</u> L.			3.0
<u>Prunus american</u> Marsh.			2.0
TOTAL			8.0

*,⁺ Not significantly different from one another, N = 4, α = 0.05.

The cadmium level of the biota and sediments in the microcosms reached equilibrium values 2 - 3 months after inputs were started. The concentrations at that time were proportional to water concentrations. Biological effects observed in the treated systems included: (1) reduced productivity, periphyton biomass and pigment concentrations relative to controls; (2) alterations in algae, microinvertebrate, and macroinvertebrate species composition; (3) reduced macrophyte growth; and (4) alteration of several nutrient cycling regimes. Current efforts are directed toward documenting recovery of the treated systems since the inputs were stopped.

CHARACTERIZATION OF CHEMICAL FORM OF TRACE METALS IN THREE SOFT WATERS (J. P. Giesy and L. A. Briesse)

Because the association of transition metals with organic and inorganic ligands can affect the availability and toxicity of these elements, a study was conducted to determine relative concentrations of free and bound metal in surface waters and the binding capacities of whole waters and various organic fractions. The relative concentrations of cationic and anionic species were determined using ion exchange resins. The binding capacities for Ca, Cu, Cd and Pb were determined with ion selective electrodes. Total metal concentrations were determined by flameless atomic absorption.

Calcium and Mg exist almost entirely as free divalent cations while Fe is almost entirely bound. The other elements studied were found to exist in both the bound and free forms. Binding capacities were greatest for Pb. Calcium did not compete favorably with other metals for binding sites. Competition studies indicated a generalized order of stability of metal-organic complexes to be $Al > Cd > Pb > Cu > Ni \approx Mg \approx Zn \approx Mn > Ca$.

FREEZING CHANGES NOMINAL MOLECULAR SIZE OF NATURALLY OCCURRING ORGANICS (J. P. Giesy and L. A. Briesse)

Naturally occurring colored organic compounds are found at varying concentrations in almost all surface waters and their properties are largely independent of their source. These refractory polyphenolic compounds are major constituents of soil and sediment organic matter and are thought to be the result of chemical polymerization and microbial decomposition of plant components such as lignin. The solubility of humic substances in water is dependent upon their size or degree of polymerization. Larger organics, such as humics, are colloidal in water. Thus, any change in the form of naturally occurring organics may affect the complex chemistry involving these organics.

Freezing may be important in determining the degree of humification which organics undergo. For instance, organics in northern aquatic systems generally exhibit a distribution of nominal molecular sizes skewed toward the larger species. Many studies have been conducted on organics isolated by freezing techniques. A study was conducted to determine the effects of freezing naturally occurring organics on their nominal molecular diameter.

Freezing changed the distribution of organic carbon among the four molecular weight fractions (Table 11). Sonification reduced the percentage of organics in the largest fraction but did not return the distribution to that of the unfrozen organics. Solubilization with 0.5 N NaOH also did not result in the same distribution as untreated samples.

TABLE 11. DISTRIBUTION OF TOC IN NOMINAL SIZE FRACTIONS BEFORE AND AFTER FREEZING AND SONIFICATION.

Ultrafilter	Pore Diameter	Nominal Molecular Weight Range	Unfrozen		Frozen			
			mg C/l	%	Unsonified mg C/l	%	Sonified mg C/l	%
XM-300 (I)	0.0183 μ m	F > 300,000	1.9	12.4	13.7	92	7.3	47.9
PM-10 (II)	0.0032 μ m	300,000 > F > 10,000	0.33	2.2	0	0	0.97	6.3
UM-05 (III)	0.009 μ m	10,000 > F > 500	3.0	19.1	0.022	0	1.8	12.0
UM-05 (IV)	0.009 μ m	F < 500	10.0	66.0	1.2	8	5.3	34.0
Total			15.2		14.9		15.4	

NATURALLY OCCURRING AQUATIC ORGANIC ACIDS AFFECT CADMIUM TOXICITY TO AQUATIC ORGANISMS (J. P. Giesy and G. J. Leversee)

The physical and chemical state of trace metals is dependent upon water quality and must be considered when availability and toxicity of metals to aquatic organisms are assessed. Metal toxicity is hardness dependent. The soft acidic waters of the southeastern United States have low inorganic ligand concentrations. Thus, inorganic complexation chemistry predicts that transition metals, such as Cd, introduced into these waters would exist mainly as free divalent cations, which are known to be more toxic than combined forms. Therefore, Cd introduced into southeastern surface waters may be more toxic to biota than predicted from experiments conducted in harder waters.

Surface waters of the southeastern coastal plain are highly stained with refractory organic compounds referred to as humic or fulvic acids. Such naturally occurring organic ligands can form soluble complexes and chelates with trace metals and affect their transport and availability in natural waters. Humic compounds can thus reduce toxicity to aquatic biota. Trace metal availability from organic complexes is dependent upon the size of the organic fraction with which it is associated. The molecular size distribution of humics can vary seasonally and temporally.

A study was conducted to compare the chelating capacity of dissolved organics to their effects on Cd toxicity to the invertebrate Simocephalus serrulatus (Daphnidae) and fish Gambusia affinis (Poeciliidae). Organics were fractionated into four nominal molecular diameter fractions, using ultrafiltration. Binding capacities were determined by titration using selective ion electrodes. The three largest nominal molecular weight fractions reduced Cd toxicity to S. serrulatus and bound added free Cd. The smallest nominal molecular weight fraction exhibited the greatest Cd binding capacity but increased Cd toxicity to S. serrulatus (Table 12). By comparison the binding capacity was not great enough to affect the acute toxicity of Cd to G. affinis but may significantly affect uptake from and toxicity of lower Cd concentrations.

NATURALLY OCCURRING AQUATIC ORGANIC FRACTIONS AFFECT UPTAKE OF TRANSURANICS BY ALGAE AND BACTERIA (J. P. Giesy)

Americium and plutonium are toxic manmade elements which have been dispersed in the environment as a result of the nuclear power and weapons industries. Cycling processes and biological uptake of Am and Pu must be understood prior to environmental releases if rational assessments of their hazards are to be evaluated. While much is known about transuranic chemistry and these elements have been monitored in the environment, little is known about the interaction of these elements with naturally occurring organic substances. Chelation and complexation of transuranic elements in the aquatic environment may immobilize elements of the actinide series or increase availability by maintaining a soluble available pool. A study was conducted to determine the effects of various nominal molecular weight fraction organics on uptake by algae and bacteria. Algae and bacteria are known to concentrate both Am and Pu greatly over aqueous concentrations and may function in trophic level biomagnification.

Organics were collected from surface waters on the SRP and fractionated into four nominal molecular weight fractions using ultrafiltration. Organics were added to culture medium at the concentration they occurred in nature and experimental flasks containing algae or bacteria, organics and Pu or Am incubated for 4, 6 or 96 hr. Cells were harvested from suspension and isolated from the medium into phthalate by centrifugation.

TABLE 12. EFFECT OF WATER QUALITY ON 48 HR LC-50 AND SLOPE OF THE PROBABILITY PLOT OF CD INDUCED TOXICITY TO SIMOCEPHALUS SERRULATUS. CONFIDENCE INTERVALS ($\alpha = 0.05$) FOR 48 HR LC-50 AND SLOPE VALUES ARE INDICATED IN PARENTHESES. VALUES WHICH ARE NOT SIGNIFICANTLY DIFFERENT FROM EACH OTHER ($\alpha = 0.05$) ARE DENOTED BY THE SAME LETTER, EITHER A, B OR C.

Test Solution	48 Hr LC-50 ($\mu\text{g}/\ell$)	Slope (Log {Cd} / % Mortality)
Well water	7.0 (5.4-9.1)	2.2 ^C (1.5-3.1)
Skinface Pond water	35.0 ^{**}	—————
Well water + F-I	8.6 ^A (6.7-11.1)	1.5 ^C (1.21-1.92)
Well water + F-II	16.5 ^B (12.8-21.3)	1.5 ^C (1.3-1.8)
Well water + F-III	12.0 ^{AB} (8.8-16.3)	2.4 ^C (1.5-3.7)
Well water + F-IV	3.5 (2.5-4.8)	2.1 ^C (1.2-3.7)

* F = Organic fraction isolated.

** Confidence limits not calculated.

Fractions I, II and III reduced ^{237}Pu availability to both algae and bacteria, while F IV increased ^{237}Pu availability (Table 13). Americium uptake by algae was unaffected by F I - F III after four hours but increased by F IV. Uptake after 96 hr was more complex due to biological dilution caused by algal growth. Humic and fulvic compounds also stimulate increased algal growth. This fraction dependent differential growth increased the difficulty of interpretation. Americium uptake by bacteria was increased by F IV after four hours incubation. After 24 hr incubation all four organic fractions significantly increased ^{241}Am uptake.

TABLE 13. EFFECTS OF FOUR ORGANIC FRACTIONS ON S. OBLIQUUS CELLULAR ^{237}Pu CONCENTRATION AFTER SIX HOURS.

Treatment	Final Cell Density (cell $\times 10^5$ ml $^{-1}$)	$^{237}\text{Pu}^{+4}$ Removed From Medium	
		Removed/Cell (10^{-5} pCi ^{237}Pu cell $^{-1}$)	% Total Removed
AAP	1.1 ± 0.18^A	32 ± 6^B	29
AAP + F I	1.6 ± 0.36^A	6 ± 2^C	9
AAP + F II	1.3 ± 0.22^A	12 ± 4^C	14
AAP + F III	1.3 ± 0.30^A	29 ± 6^B	34
AAP + F IV	1.3 ± 0.28^A	52 ± 7	62

* $n = 4$, $\alpha = 0.05$, confidence intervals $\pm 2 S_x$. Means which are not significantly different from one another are denoted by A, B or C.

AMERICIUM-241 UPTAKE BY RICE IS ENHANCED BY SYNTHETIC CHELATING AGENT AND BY WATER PLACEMENT (D. C. Adriano and G. D. Hoyt)

Rice is the most important cereal crop in the world because it is consumed by no less than 50% of its population. It is also an important cereal and export crop in the U. S., being extensively grown in Arkansas, California, Louisiana and Texas where it is generally grown in lowland areas (rice paddies) under flooded conditions.

Wastes from nuclear technology has been released both to terrestrial and aquatic environments. Wastes from reprocessing operations contain the transuranic elements including ^{241}Am . If disposed of on land, the nuclides from these wastes will either stay in the soil, or be displaced from the point of release through the processes of leaching or soil erosion. Erosion will transport these nuclides to low-elevation areas and eventually to waterways. If disposed of through the streams, somewhat similar fates might occur although they might spread away from the watercourse during flooding. Thus, lowland areas where rice is usually grown are potential recipients of these transuranic nuclides.

Elements dissolved in water are generally known to be more available to aquatic plants. They can find entry to the plants through the stems and leaves in addition to the root pathway. The availability of elements depends on several factors including their stability. If they are not precipitated or polymerized they are apt to be absorbed readily by the plants. Chelating agents, either natural or synthetic, make these elements stable and keep them in the dissolved form in the aqueous phase. Synthetic chelates (EDTA, DTPA, etc.) are used in agriculture to enhance the availability of trace metals. They are also used in the nuclear industry for waste treatment and management.

A study in the glasshouse was conducted to determine which method of placement (soil versus water application) and what period of growth would induce greater ^{241}Am uptake by rice. Specifically the effect of the chelator DTPA (diethylene triamine pentaacetic acid) on ^{241}Am availability was evaluated.

The soil or the standing water in the pots (5 kg of soil) was spiked with 2 μCi of ^{241}Am diluted in weak HNO_3 solutions. In the soil placement, only the top 1 kg of soil was spiked. In the water placement, ^{241}Am was added to the standing water (about 5 cm deep) in 50 mls of either water or 100 ppm DTPA. ^{241}Am was added to water during the following growth stages: ear-forming to booting stage (period 1); late-flowering stage (period 2); and maturity stage (period 3). All treatments had five replications. Pots were placed in water tanks where the temperature was regulated.

The plants were harvested, separated into various parts and counted in a NaI well crystal. The results indicate that where either chelated or non-chelated ^{241}Am was added to the standing water, ^{241}Am became more available in later stages of growth (Table 14). This was more pronounced with the addition of DTPA. The increase in ^{241}Am contents in the leaf blades with maturity was more pronounced in the dead than in the green blades especially with ^{241}Am -DTPA treatment. It should be pointed out that when the last spiking (period 3) was made there were only a few dead blades. In general, higher concentrations of ^{241}Am , by a factor of about ten occurred in the dead leaf blades where DTPA was applied. It appeared that ^{241}Am contents of the grain could be increased slightly with the addition of chelated- ^{241}Am to the standing water. The leaf sheaths accumulated very high concentrations of ^{241}Am especially with the DTPA treatment of standing water.

It appears that the method of ^{241}Am placement had an influence on ^{241}Am availability to the rice plants, with introduction to the standing water being more favorable. The soil "held up" the ^{241}Am ions from being absorbed by the plants. This is obvious from the leaf blades and leaf sheath data.

Of the total ^{241}Am absorbed by the rice plants, an insignificant fraction was translocated to the grain. Most of the ^{241}Am was accumulated in the dead leaf blades and leaf sheaths which accounted for about 90% of the total amount absorbed.

Results of this study imply that the availability of ^{241}Am to the rice plant can be enhanced by water placement of this nuclide, by chelate DTPA, and by application at later stages of growth of the plant.

TABLE 14. CONCENTRATIONS (pCi/g DRY WEIGHT) OF ^{241}Am IN VARIOUS PARTS OF THE RICE PLANT AS AFFECTED BY CHELATE, METHOD OF ^{241}Am PLACEMENT AND TIME OF APPLICATION OF ^{241}Am . VALUES ARE MEANS OF 5 REPLICATES \pm 1 STANDARD DEVIATION.

Plant Parts	S ¹ / ₂	+ DTPA ²			S	- DTPA		
		1	2	3		1	2	3
Grain	B.G. ² / ₂	B.G.	0.46 \pm 0.31	0.42 \pm 0.15	B.G.	0.02 \pm 0.26	0.27 \pm 0.20	B.G.
Stem	2.03 \pm 1.07	4.52 \pm 2.75	10.3 \pm 3.7	36.5 \pm 25.0	2.07 \pm 0.72	3.26 \pm 1.27	6.45 \pm 1.68	7.15 \pm 1.99
Green leaf blade	0.00 \pm 0.74	1.53 \pm 1.27	5.37 \pm 1.73	6.75 \pm 3.53	1.05 \pm 0.72	2.12 \pm 2.48	2.30 \pm 1.22	3.81 \pm 7.22
Dead leaf blade	11.2 \pm 2.6	109 \pm 123	260 \pm 222	631 \pm 476	9.81 \pm 2.13	18.6 \pm 11.8	19.3 \pm 32.9	169 \pm 309
Leaf sheath	9.6 \pm 3.9	443 \pm 254	1,581 \pm 607	3,715 \pm 2,248	4.17 \pm 1.86	42.0 \pm 32.7	89.0 \pm 90.0	406 \pm 280

¹/ S refers to soil application. Periods 1, 2, and 3 refer to water application and correspond to "ear-forming to booting" (period 1), "late flowering" (period 2), and "maturing" (period 3) stages.

² Chelated - on non-chelated ^{241}Am (in 50 mls of 100 ppm DTPA, as acid or water) was added to the standing water. In soil, DTPA was mixed in the whole pot to give 40 ppm.

UPTAKE OF SOIL-APPLIED ^{241}Am BY BAHIA GRASS IS INHIBITED BY LIME (G. D. Hoyt and D. C. Adriano)

Evaluating contamination of the environment and the health hazard to man by the wastes and by-products from the expanding nuclear power industry is a topic of extensive research at the SREL. Although much of the current interest has been focused on plutonium, the importance of americium-241 in biological systems has also gained considerable attention. One primary reason is the apparent increased mobility and availability of ^{241}Am relative to ^{238}Pu and ^{239}Pu in soil systems.

Over 52% of all farmland in the U. S. is currently being managed for the production of forage crops. Bahia grass is the most important grass species here in the humid southeast. It is imperative that a study be initiated on the transport of soil-incorporated ^{241}Am into the plant system.

Bahia grass was grown in spiked soil containing various lime and organic matter amendments. Lime increases the soil pH and changes the availability of nutrients to plants. Organic matter addition stimulates microbial activity, and eventually increases the fertility status of the soil. The natural chelators, fulvic and humic acids, are derived from decomposed organic matter. These chemical and biological manipulations in the soil-plant system may change ^{241}Am mobility and availability and help us evaluate how agricultural practices affect ^{241}Am transport.

Standing biomass was cut at three periods and analyzed for ^{241}Am . Concentration ratios (C.R.) from Table 15 show that bahia grass grown in Dothan sandy clay loam soil (pH 4.2) absorbed greater amounts of this nuclide than plants grown in the Troup loamy sand soil (pH 5.0). Lime, which increased the pH to 7.1 in the Dothan soil and to 6.6 in the Troup soil, considerably suppressed ^{241}Am uptake by bahia grass. In some cases, liming reduced ^{241}Am activity in the vegetation to background levels. These results indicate that soil pH plays an important role in the availability of this nuclide to plants.

Addition of organic matter suppressed ^{241}Am uptake and was more pronounced in the 5% than in the 1.25% level. The effect of organic matter alone in suppressing ^{241}Am uptake was not as great as lime amendment alone. However, when organic matter was added to limed soils, suppression of uptake was less or equal to lime alone. The time of cutting did not seem to affect the ^{241}Am uptake, although concentrations tended to be high in the first cutting. The slight reduction in uptake with organic matter addition was probably due to immobilization of ^{241}Am into soil microbial biomass.

The mobility and availability of ^{241}Am appears to be pH dependent. Liming, a common agricultural practice in the southeast where soil types are predominantly acidic and nutrient poor, could be instrumental in affecting the ultimate degree to which transuranics could be incorporated into agricultural food chains. Also, incorporation of organic matter into the soil may help contain and immobilize ^{241}Am , at least temporarily, from further uptake by agricultural crops. The eventual effect of organic matter addition on ^{241}Am availability to plants is the subject of continuing study in this laboratory.

TABLE 15. INFLUENCE OF LIME AND ORGANIC MATTER ON ^{241}Am CONCENTRATION RATIOS (PLANT TISSUES:SOIL)¹ FOR BAHIA GRASS GROWN IN TWO SOIL TYPES. VALUES ARE MEANS OF 5 REPLICATIONS \pm 1 STD. DEVIATION.

Treatment	DOTHAN SOIL			TROUP SOIL		
	1st Cutting	2nd Cutting	3rd Cutting	1st Cutting	2nd Cutting	3rd Cutting
Control	$1.2 \pm 0.4 \times 10^{-1}$	$8.0 \pm 2.7 \times 10^{-2}$	$5.9 \pm 2.3 \times 10^{-2}$	$4.0 \pm 1.2 \times 10^{-2}$	$7.5 \pm 2.7 \times 10^{-2}$	$3.0 \pm 1.4 \times 10^{-2}$
+ Lime	$0.8 \pm 1.2 \times 10^{-2}$	B.G. ²	B.G. ²	$3.8 \pm 3.0 \times 10^{-3}$	$6.2 \pm 3.0 \times 10^{-3}$	$0.9 \pm 1.4 \times 10^{-3}$
1.25 % O. M.	$1.5 \pm 0.3 \times 10^{-1}$	$6.9 \pm 1.5 \times 10^{-2}$	$3.7 \pm 1.4 \times 10^{-2}$	$2.0 \pm 0.7 \times 10^{-2}$	$2.4 \pm 0.7 \times 10^{-2}$	$1.3 \pm 0.3 \times 10^{-2}$
1.25 % O. M. + Lime	$1.4 \pm 1.4 \times 10^{-3}$	$1.2 \pm 1.0 \times 10^{-3}$	$3.3 \pm 2.6 \times 10^{-3}$	$3.0 \pm 2.6 \times 10^{-3}$	$5.0 \pm 2.8 \times 10^{-3}$	$1.3 \pm 1.3 \times 10^{-3}$
5.0 % O. M.	$5.0 \pm 1.9 \times 10^{-2}$	$2.8 \pm 1.1 \times 10^{-2}$	$2.8 \pm 0.8 \times 10^{-2}$	$9.0 \pm 3.6 \times 10^{-3}$	$1.5 \pm 0.6 \times 10^{-2}$	$1.0 \pm 0.2 \times 10^{-2}$
5.0 % O. M. + Lime	$1.7 \pm 2.1 \times 10^{-3}$	$3.0 \pm 3.2 \times 10^{-3}$	$0.8 \pm 1.4 \times 10^{-3}$	$5.6 \pm 6.8 \times 10^{-4}$	$2.4 \pm 1.4 \times 10^{-3}$	$1.5 \pm 0.8 \times 10^{-3}$

¹Concentrations in plant tissues can be calculated from the C. R. values and the ^{241}Am concentrations (500 pCi/g soil) of the potted soil.

²Activities were below the detection limit.

"AMERICIUM-241 TRANSLOCATED TO THE RICE GRAIN IS EQUAL TO OR SLIGHTLY HIGHER THAN BACKGROUND LEVEL" (D. C. Adriano and G. D. Hoyt)

Field conditions near SRP's reprocessing facilities do not provide the type of environment suitable to study incorporation of the transuranic elements in plant tissues through root uptake. This is because contamination of plants is primarily external in nature, from fallout deposition from the emission stack. Thus, studies were conducted in the glasshouse to evaluate the influence of common soil admendments on the ^{241}Am uptake by rice.

Americium is becoming an important topic of research because of its long physical half-life (460 years). Also, it appears to be more available to plants than plutonium and other transuranic elements. Its uptake and translocation to plants are dependent on a number of factors, including soil type, pH, soil amendment, etc.

The two soil amendments tested were the chelating agent DTPA (diethylene triamine pentaacetic acid) and organic matter. Synthetic metal chelates are often used in agriculture as a form of micronutrient for crops. The nuclear industry also accumulates chelating agents in radioactive wastes and these wastes are often stored in chelated forms. Fertility of the soil is often indicated by its organic matter content. Organic matter in agricultural fields come from crop residues or from addition of domestic animal wastes like poultry, cow, and swine manures. Sewage sludge is also a possible source of organic matter in farms.

The glasshouse procedures entailed spiking the top layer (1 kg) of the potted soil (5 kg dry soil per pot) with 2 μCi of ^{241}Am diluted in weak HNO_3 solutions. The $^{241}\text{Am}(\text{NO}_3)_3$ solution was further diluted in solutions of the Na - salt of the chelating agent, DTPA. Spiking was carried out by adding the solution into thin soil layers until the whole 1 kg soil was spiked. The pots were incubated prior to planting and grown in water tanks where temperature was regulated.

The rice plants were grown to maturity and separated into various parts: grain, stem, green leaf blades, dead leaf blades, and leaf sheath. ^{241}Am activity of dried plant parts was counted for 100 minutes using a NaI well crystal interphased to a multichannel analyzer. Minimum detectable activity was 1.4 pCi/g plant material for 100 minutes.

There was very little translocation of ^{241}Am to the mature grain (Table 16). In the flood variety the activity in the grain was equal to background level. The activities in the grain of the non-flood variety were also equal to the background level. There was also little translocation from mature (dead leaf blades) to younger (green leaf blades) leaves. The chelator DTPA slightly reduced ^{241}Am uptake, apparently caused by the chelator level (40 ppm) in the soil which appeared harmful to the rice plants, resulting in retarded and reduced growth. Organic matter did not have a clear-cut effect although it tended to suppress the uptake by the non-flood variety. In general,

the plant tissues of the non-flood variety have higher ^{241}Am levels, probably caused by physiological differences in the two varieties or soil conditions. Some of these differences, for the same plant part, were not statistically different, however.

Of the total ^{241}Am taken up by the rice, only about 1% was translocated to the grain in the non-flood variety, and 0% in the flood variety. At least 50% of the total ^{241}Am in the plant was contained in dead leaf blades and leaf sheaths.

In rice, the only part which is of economic importance is the grain. The foliage is seldom used for animal feed. Since ^{241}Am does not appear to be readily translocated to the grain its health hazard to man is minimized.

TABLE 16. CONCENTRATIONS (pCi/g DRY WEIGHT) OF ^{241}Am IN VARIOUS PARTS OF THE RICE PLANT AS AFFECTED BY WATER REGIME AND SOIL AMENDMENT. VALUES SHARING THE SAME LETTER IN THE SAME PLANT PART COLUMN ARE NOT STATISTICALLY DIFFERENT AT THE 5% LEVEL OF TUKEY'S TEST.

Plant Parts	Flooded ^{1/}			Non-Flooded		
	Control ²	+ DTPA	+ O.M.	Control	+ DTPA	+ O.M.
Grain	B.G. ^{a3}	B.G. ^a	B.G. ^a	0.28 ^a	0.10 ^a	0.11 ^a
Stem	2.07 ^{ab}	2.03 ^{ab}	2.66 ^b	5.58 ^c	0.010 ^a	4.07 ^{bc}
Green leaf blade	1.05 ^a	0.082 ^a	3.31 ^{bc}	10.6 ^d	1.15 ^{ab}	5.89 ^c
Dead leaf blade	9.81 ^a	11.2 ^a	5.97 ^a	23.8 ^b	8.73 ^a	6.58 ^a
Leaf sheath	4.27 ^{ab}	9.65 ^c	3.66 ^{ab}	7.72 ^{bc}	2.93 ^a	2.56 ^a

^{1/} The flood variety was dwarfed "Miracle rice" variety from Southeast Asia and was ponded with water all the time. The non-flooded variety was also from Asia, was not ponded, and was taller than the flood variety.

² The treatments were control (only ^{241}Am); 40 ppm DTPA as acid; and 5% ground pasture hay, on dry weight basins.

³ B.G. indicates that the activity was equal to background level.

CONTRIBUTION OF ROOT UPTAKE TO PLUTONIUM CONTAMINATION OF FIELD CROPS FROM NUCLEAR FUEL REPROCESSING APPEARS MINIMAL (D. C. Adriano, K. W. McLeod, J. E. Pinder, III and G. D. Hoyt)

Crops have been grown in an agricultural area adjacent to a nuclear reprocessing facility (H-Area). Because of the chronic releases of the Pu-bearing particles at low levels (usually less than 5 mCi/year) from the emission stack, it is impossible to determine the plutonium uptake by the crops from the soil through the root pathway. Thus, whatever

amount of plutonium is translocated to the plant foliage from the soil would be obscured by external deposition and retention from stack fallout. Therefore, glasshouse studies were conducted to determine the amount of plutonium translocated to the aerial portions of the plants from the soil.

Soils used in the glasshouse were obtained from the surface layer (0 - 20 cm) of the south field where crops were grown and had an average total Pu content of 1.96 pCi/g dry weight with 21% ^{238}Pu .

Results are presented in Table 17. Wheat straw (less grain) harvested in June 1975 from the south field had an average total Pu content of 88 ± 65 S.D. fCi/g (N = 18). The glasshouse grown wheat had only 3.0 ± 1.5 fCi/g (N = 10). Thus, the glass house value is only 3% of the field value. This value from the glasshouse study is considered to be due to root uptake.

Total Pu content of field soybeans (harvested in fall of 1975) was 52.2 ± 17.0 fCi/g (N = 14), compared with only 5.6 ± 1.6 fCi/g (N = 10) for glasshouse grown soybeans. This gives an 11% root uptake. No plausible explanation can be offered at this time for this difference. Additional glasshouse studies are being conducted to confirm this difference.

Field corn (harvested in fall of 1976) had total Pu content of 33.5 ± 14.5 fCi/g (N = 10), compared with only 1.1 ± 0.4 fCi/g (N = 6) for glasshouse-grown corn. This gives a 3% root uptake, the same as for wheat.

The results are considered preliminary since additional glasshouse and field studies are being conducted to verify these data. It appears, however, that plant uptake of plutonium via roots is minor since the values obtained, ranging from 1.1 ± 0.4 fCi/g for corn leaves to 5.6 ± 1.6 fCi/g for soybean foliage, are close to the detection level (~ 1 fCi/sample) by alpha spectrometry.

A MODEL OF PLUTONIUM CYCLING IN WINTERWHEAT IS CONSTRUCTED (D. C. Adriano, K. W. McLeod, D. Paine, J. E. Pinder; in conjunction with the Savannah River Laboratory)

Plutonium (Pu) releases to the atmosphere from fuel reprocessing facilities are an important aspect of the breeder reactor technology, and the impacts of low-level aerial releases on a winter wheat crop have been evaluated. Figure 10 summarizes the impacts predicted for a winter-wheat field receiving $260 \text{ pCi } ^{239}\text{Pu} \cdot \text{m}^{-2} \cdot \text{yr}^{-1}$ of aerial deposition and has been constructed from data collected in a cooperative SRL-SREL project that involved studies of several wheat fields growing in the vicinity of a fuel reprocessing facility. An input rate of $260 \text{ pCi } ^{239}\text{Pu} \cdot \text{m}^{-2} \cdot \text{yr}^{-1}$ may be expected within 1 km of a reprocessing facility that releases approximately $0.5 \text{ mCi} \cdot \text{yr}^{-1}$. The transfers illustrated in Figure 10 are those expected in the 21st year of operation after 20

TABLE 17. COMPARISON OF TOTAL PLUTONIUM CONTENTS (fCi/g DRY WT.) OF VEGETATIVE MATERIALS FROM CROPS GROWN ON A FIELD (SOUTH FIELD) NEAR A REPROCESSING FACILITY (H-AREA) AND IN A GLASSHOUSE. THE SOILS USED IN THE GLASSHOUSE WERE FROM THE TOP LAYER OF THE SOUTH FIELD.

Vegetative Material	South Field	Glasshouse	Difference	% External Content ¹
Wheat Straw	88.0	3.0	85.0	97
Soybeans, Whole plants	52.2	5.6	46.6	89
Corn leaves ²	33.5	1.1	32.4	97

¹ Calculated from the equation:

$$\frac{\text{Field content} - \text{Glasshouse content}}{\text{Field content}} \times 100$$

² Fiberglass mats were placed on top of the soil in the corn pots in the glasshouse to prevent plant contamination by resuspension.

years of constant input. Some transfers are based on work by D. A. Cataldo at Pacific Northwest Laboratories and R. C. Dahlman at Oak Ridge National Laboratory.

Transfers occurring in the field and those occurring in the combine during harvesting are shown in separate subsystems (denoted by dashed lines). The major source of ^{239}Pu in vegetation is surface deposition of current releases. Root uptake and resuspension of soil ^{239}Pu by wind and raindrop splash accounts for less than 1% of the total. Grain, which is not exposed in the field, receives $10^{-3} \text{ pCi } ^{239}\text{Pu} \cdot \text{m}^{-2} \cdot \text{yr}^{-1}$ from root uptake and a second $10^{-3} \text{ pCi} \cdot \text{m}^{-2} \cdot \text{yr}^{-1}$ due to mobilization and translocation of surface deposited ^{239}Pu . The major transfer to grain occurs during combining. This transfer, $0.2 \text{ pCi } ^{239}\text{Pu} \cdot \text{m}^{-2} \cdot \text{yr}^{-1}$ results in a ^{239}Pu concentration of $2 \times 10^{-3} \text{ pCi } ^{239}\text{Pu} \cdot \text{g}^{-1}$ in the harvested grain. More than 99% of this concentration is the result of surface deposition and may be lost during subsequent milling and refining operations.

After 20 years of input, the soil would have accumulated about $5200 \text{ pCi } ^{239}\text{Pu} \cdot \text{m}^{-2}$, yet the impact of this soil ^{239}Pu on total grain concentrations would still be small. ^{239}Pu contained in grain is possibly more mobile and more likely to be assimilated than surface ^{239}Pu . The contribution of soil ^{239}Pu to the internal concentration after 20 years

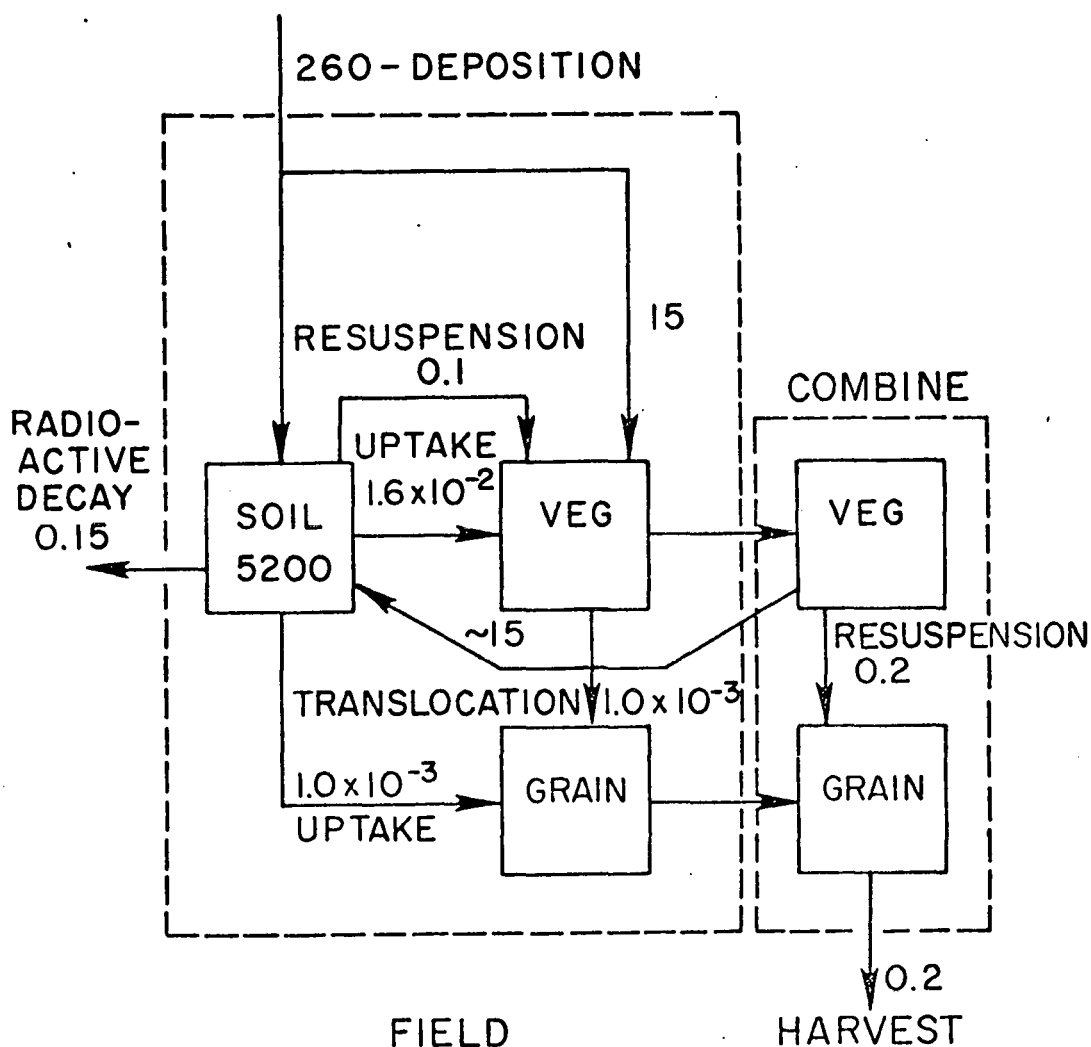


FIGURE 10. THE PREDICTED TRANSFERS OF ^{239}Pu IN A WINTER WHEAT FIELD THAT HAS RECEIVED $260 \text{ pCi } ^{239}\text{Pu} \cdot \text{m}^{-2} \cdot \text{yr}^{-1}$ FOR THE PAST 20 YEARS. VEG REFERS TO THE NON-GRAIN ABOVE-GROUND PORTIONS OF THE WHEAT PLANTS. NUMBERS ASSOCIATED WITH ARROWS REPRESENT FLUXES DURING THE 21st YEAR OF INPUT AND HAVE UNITS OF $\text{pCi } ^{239}\text{Pu} \cdot \text{m}^{-2} \cdot \text{yr}^{-1}$. NUMBERS IN BOXES ARE AMOUNTS ACCUMULATED AFTER 20 YEARS AND HAVE UNITS OF $\text{pCi } ^{239}\text{Pu} \cdot \text{m}^{-2}$. DATA ARE COMPUTED FOR A $130 \text{ g} \cdot \text{m}^{-2} \cdot \text{yr}^{-1}$ SINGLE HARVEST YIELD OF GRAIN AND NO INITIAL ^{239}Pu CONTAMINATION OF THE SITE. SEPARATE SUBSYSTEM (THE LARGE AREAS ENCLOSED BY DASHED LINES) ARE EMPLOYED TO MODEL THE TRANSFERS OCCURRING IN THE FIELD AND THOSE OCCURRING IN THE COMBINE DURING HARVESTING OPERATIONS. SLIGHTLY CONSERVATIVE (i.e., POSITIVELY BIASED) ESTIMATES OF TRANSFERS HAVE BEEN USED. FOR PREDICTIONS AT OTHER INPUT RATES, OUR RESULTS SUGGEST THAT FLUXES WITHIN THE SYSTEM ARE ROUGHLY PROPORTIONAL TO YEARLY INPUT.

input would be roughly equivalent to that translocated from surface deposition on leaves and stems. Moreover, the calculations in Figure 10 assume no initial soil ^{239}Pu content although global fallout has resulted in soil contents of approximate $2000 \text{ pCi } ^{239}\text{Pu} \cdot \text{m}^{-2}$. Thus, soil ^{239}Pu would not be greatly elevated over fallout levels.

Wheat grown in more arid regions may show greater transfers from soil to plant via resuspension. A ten-fold increase in resuspension would not be unexpected. Consequently, Figure 10 is probably valid only in humid regions. Inclusion of loss terms which are representative of soil erosion have little impact on the fluxes in Figure 10.

Assuming ICRP standards, the wheat obtained from this field could be safely consumed on a regular basis. Consumption of 50 kg wheat per individual would result in a 70-year bone dose of approximately 1 mrem, which is well below that received from natural sources in a similar time period.

TREE CANOPY ACTS AS A NATURAL FILTER IN INTERCEPTING AND RETAINING PLUTONIUM FROM ATMOSPHERIC FALLOUT (D. C. Adriano and J. E. Pinder, III)

Long-term environmental studies of the transuranic nuclides at SRP are focused to evaluate potential environmental and health risks associated with the release of plutonium (Pu) and americium (Am) from chemical reprocessing or irradiated reactor products. Plutonium is known to be highly toxic and some radionuclides have long half-lives ($\sim 24,000$ years for ^{239}Pu and ~ 90 years for ^{238}Pu). Their fate and behavior in the environment are not yet well understood.

One aspect of studies at SRP concern dispersal of Pu from reprocessing (chemical separations) facilities to terrestrial environments and the resulting contamination of the biota. This release closely represents an industrial source of Pu.

Forests are the dominant terrestrial ecosystems at SRP. The two reprocessing facilities are surrounded by coniferous forests. Thus, forest canopies will play an important role in intercepting radionuclides released from these facilities. This study examined the concentrations and distributions of the radionuclides $^{239,240}\text{Pu}$ and ^{238}Pu in the organic and soil mineral layers in pine and hardwood stands adjacent to an SRP reprocessing facility. It also evaluated the effect of stem flow water on the downward movement of Pu particles in the soil mineral layers.

The sampling sites are within 1 km north of a 62 m-tall stack emitting approximately 2.4 mCi (usually less than 5 mCi) of Pu per year from a reprocessing facility at the H-Area. This reprocessing facility has been in operation since 1955 to reclaim Pu. Although current Pu releases are 95% ^{238}Pu , releases prior to 1968 were predominantly $^{239,240}\text{Pu}$. Release rates were also higher in the past. Other radionuclides being reclaimed include ^{244}Cm , ^{252}Cf , and ^{60}Co .

Two pairs of loblolly pine (Pinus taeda) were sampled in the summer of 1975. In the first pair, the trees were 7.5 m apart. One had a DBH (diameter breast high) of 32 cm and height of 15 m; the other had a DBH of 27 cm and height of 17 m. In the second pair, the trees were 13 m apart. One had a 43 cm DBH and a 16 m height; the other had a 40 cm DBH and a 16 m height. No intermingling of crowns occurred in either pair. The crowns of these four trees were at about the same level as those of adjacent pine trees. Ages of the trees averaged 31 years. Three hardwood trees were also sampled in the vicinity of the pine trees. One was a southern red oak (Quercus falcata), estimated to be 33 years old with a 38 cm DBH and a 14 m height. The other two were mockernut hickories (Carya tormentosa); one was 115 years old with a 40 cm DBH and a 17 m height; the other was 55 years old with a 22 cm DBH and a 14 m height. There was also no intermingling of crowns between the pine and hardwood trees. All trees were in existence prior to reprocessing operations so that Pu was not deposited on the soil prior to accumulation of litter.

Litter, organic matter and soil samples were collected from the following locations relative to each tree: outside the canopy, midway between the canopy edge and trunk, and two samples from the opposite sides of the base of the trunk. Litter (Aoo or L layer) and organic matter (Ao or F layer) samples were collected from a 0.1 m² quadrat. Samples of the soil mineral layers were composited from two soil cores, obtained using a 3.5 cm diameter split-barrel soil corer operated manually. The soils were classified as Troup sandy loam (Grossarenic Paleudults) where the Ao and the A (soil) layers could be easily separated. Bark slices were collected from the trunk at the crown, 1 m above the ground, and midway between the ground and crown in a hardwood trees. In pines, samples were collected from the top 1/3 of the crown and at the 1 m level. Only a limited number of samples were obtained because of the expense involved in analyzing these for Pu concentrations.

Litter and organic matter samples were oven-dried to a constant weight at 65°C and weighed to the nearest 0.1 g. Total contents of Pu were calculated from mass and concentration data of the various samples. A bulk density of 1.3 g/cm³ was assumed for soils. The samples were analyzed for ²³⁸Pu and ^{239,240}Pu by alpha spectrometry.

The results indicated that tree crowns intercepted fallout Pu (Pu-bearing particles) and produced higher Pu concentrations in the organic matter and soil under tree crowns. Higher ^{239,240}Pu concentrations were found under pines than under hardwoods. Plutonium concentrations in the Aoo (litter) and Ao (organic matter) layers were higher than those in mineral soil, but most of the Pu was contained in the mineral soil. Higher contents of ^{239,240}Pu were observed near the tree stems than in locations outside of the tree crowns. In pines these values were 163 and 80 nCi ^{239,240}Pu/m² and in hardwoods, 122 and 80 nCi ^{239,240}Pu/m², for the respective locations, from the litter to the 15 cm depth (Figure 11).

The proportion of ^{238}Pu contained in foliage, litter and organic matter was greater than for $^{239,240}\text{Pu}$. However, the latter radionuclides had a greater proportion contained in the mineral soil. This observation is consistent with the more recent releases containing a higher percentage of ^{238}Pu from reprocessing operations. Plutonium concentrations in the 5 to 15 cm depth indicated limited Pu mobility in soil, but $^{239,240}\text{Pu}$ concentrations at this depth were higher near tree stems suggesting greater mobility perhaps as a result of stem flow.

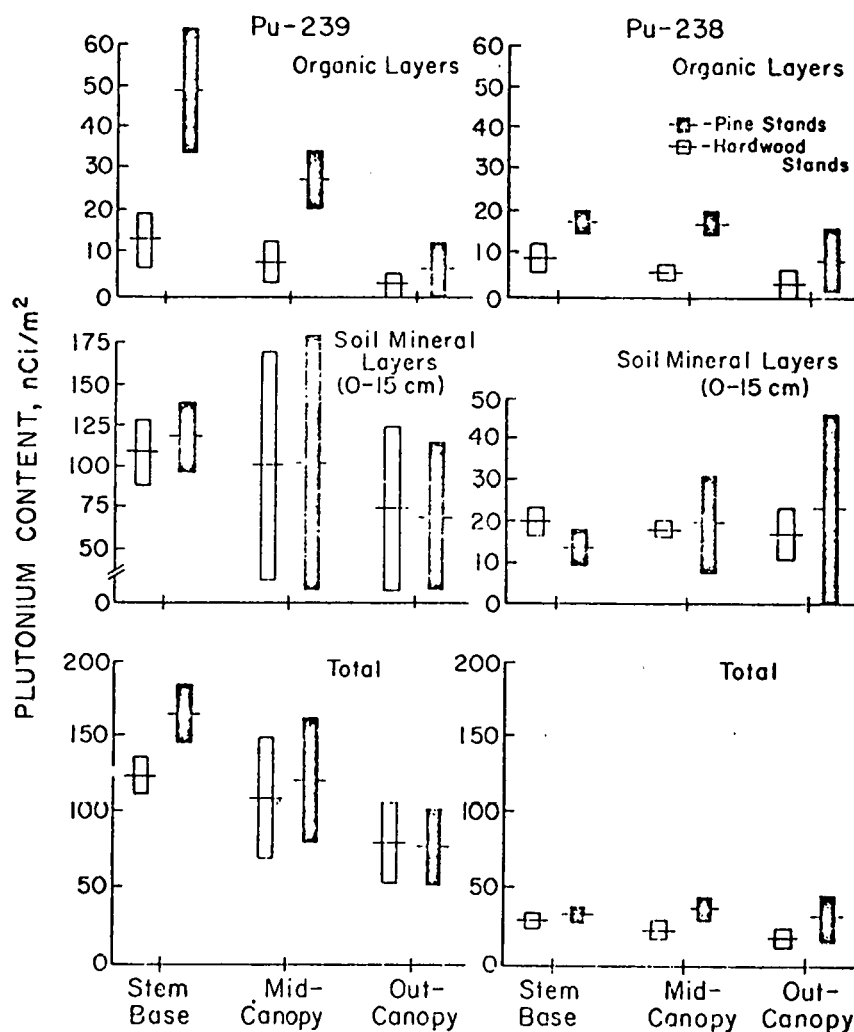


FIGURE 11. TOTAL ACCUMULATED AMOUNTS OF $^{239,240}\text{Pu}$ AND ^{238}Pu IN THE ORGANIC AND MINERAL SEGMENTS OF THE SOIL PROFILE IN MIXED PINE AND HARDWOOD STANDS CLOSE TO A NUCLEAR FUEL REPROCESSING PLANT. THE TOTAL CONTENTS ARE THE SUM OF THE TWO SEGMENTS. VALUES ARE MEANS \pm 1 STANDARD ERROR.

CESIUM-137 APPEARS TO BE MORE MOBILE IN THE SOIL PROFILE THAN EITHER ²³⁹Pu OR ²³⁸Pu AERIALY DEPOSITED IN MIXED FOREST STANDS FROM NUCLEAR FUEL REPROCESSING (D. C. Adriano, G. D. Hoyt and J. E. Pinder, III)

The reprocessing operation at SRP, which started in the early 1950's, has been releasing some transuranic nuclides to the atmosphere from its emission stack. The releases has been at chronic low levels (usually less than 5 mCi/year). The bulk of the total release (3.6 Ci to the atmosphere) occurred in the late 1960's when the sand filter failed to operate.

The mode of transport and eventual fate in the environment of these radiologically important elements from atmospheric deposition needs to be determined. Other radionuclides, including ¹³⁷Cs, are also being released to the atmosphere. There have been numerous studies conducted on the fate and behavior of fallout radiocesium in terrestrial ecosystems, but none has been done where a reprocessing facility is the point source. Thus, a study on the transport and fate of ¹³⁷Cs from reprocessing activity in terrestrial environment was conducted. This study was in conjunction with the Pu deposition study in mixed forest stands (see Adriano and Pinder) where the experimental techniques and procedures have already been described.

Unlike plutonium, ¹³⁷Cs was about equally concentrated in the litter (Aoo) and organic matter (Ao) layers under the canopies. ²³⁹Pu was more concentrated in the Ao layer, whereas there was greater proportion of ²³⁸Pu in Aoo layer. These differences were due to the release events, where earlier releases were primarily ²³⁹Pu and recent releases were primarily ²³⁸Pu.

Results in Table 18 indicate that ¹³⁷Cs occurred in greater proportion than either ²³⁹Pu or ²³⁸Pu in the litter (Aoo) and in the 5 - 15 cm depth. This is expected if ¹³⁷Cs behaves chemically similar to potassium and hence ¹³⁷Cs is used as a K analog. Potassium is a soluble cation, as has been shown in numerous stemflow and throughfall studies indicating that this element was being leached from the canopy.

Cesium-137 concentrations in the organic and mineral soil layers were 2 - 3 times greater than the ¹³⁷Cs concentrations in control sites, where the only source of this radionuclide is global fallout.

Because of the expense involved in Pu analysis by alpha spectrometry, a few researchers have proposed other radionuclides as environmental tracers for Pu. It appears from this study that the use of ¹³⁷Cs as a Pu tracer in ecosystem studies is highly questionable.

TABLE 18. RATIOS OF $^{239,240}\text{Pu}$, ^{238}Pu , AND ^{137}Cs DEPOSITED FROM NUCLEAR FUEL REPROCESSING IN THE ORGANIC AND MINERAL SOIL LAYERS OF MIXED FOREST STANDS.

Layer	Location	Pine Stand			Hardwood Stand		
		^{239}Pu	^{238}Pu	^{137}Cs ^{1/}	^{239}Pu	^{238}Pu	^{137}Cs
Aoo	Base ^{2/}	1	1.6	6.9	1	1.6	14.5
	Mid	1	2.3	9.9	1	1.3	19.0
	Out	1	1.5	8.9	1	0.7	4.7
Ao	Base	1	0.4	1.3	1	0.6	4.5
	Mid	1	0.5	1.6	1	0.7	3.5
	Out	1	1.2	—	1	1.0	4.2
0-5 cm	Base	1	0.1	3.7	1	0.2	3.6
	Mid	1	0.2	2.4	1	0.2	2.2
	Out	1	0.3	4.2	1	0.2	3.3
5-15 cm	Base	1	0.2	8.9	1	0.2	5.8
	Mid	1	0.3	14.9	1	0.1	3.3
	Out	1	0.5	14.4	1	0.5	13.9

^{1/} All concentrations are in pCi/g dry weight.

^{2/} For location description see accompanying report by Adriano and Pinder.

HARVESTER ANTS MAY SERVE AS INDICATORS OF ENVIRONMENTAL CONTAMINATION
(J. B. Gentry)

Pogonomyrmex badius, the Florida harvester ant, is an important granivore (seed-eater) in the old-field ecosystem of the southeastern United States. Colonies are distributed in a regular spatial pattern which, even though seasonal nest relocations occur, remains fairly constant from year to year. These data, along with preliminary information on foraging patterns, indicate that the ants have adopted a foraging strategy which allows for more efficient utilization of available food resources. Similar behavior has been documented in certain harvester ant species of the western United States. Such a strategy may be closely related to resource distribution. If so, P. badius may represent an excellent model to gain valuable information concerning the more general question of foraging strategies and resource distribution among different animal groups. Such information might be valuable in a more practical aspect if applied to problems of environmental contamination. In other words, the Florida harvester ant may exhibit unusual value as an indicator of the levels of chemical contamination in our environment.

Therefore, before experiments can be designed to test the value of P. badius as an indicator species, other basic questions need to be answered. For example, (1) Do P. badius workers forage in specific territories? (2) How do foraging territories change with nest relocation? (3) How large are foraging territories? (4) Are the territories discrete or do they overlap?

A study designed to answer these specific questions was conducted in the summer of 1976. Ten adjacent colonies were selected from a large old field abandoned since 1951. Several hundred foraging ants from each colony were individually marked on the thorax with a small drop of paint. Varying colors of paint were used in order to observe separately the ants from each colony. Wire flag markers were used to mark the location of any foraging marked ant. After an adequate number of locations were indicated, a compass and metric tape were used to gather the information necessary to transfer each marked forager location to a map. By connecting the most distal forager locations for each colony, the foraging ranges were illustrated. Foraging territories were determined in August and again in October after eight of the ten colonies had relocated their nests to distances ranging from 1.4 to 3.7 meters.

As illustrated in Figure 12 (solid lines) the foraging territories in August were tight with very little overlap. Foraging area for individual colonies ranged from 66 to 186 m² and averaged 136 m². The October foraging territories (dashed lines, Figure 12) shifted consistently in the same direction as nest relocation. Colonies 215 and 302, which did not relocate their nests, shifted foraging territories in response to the movement of adjacent colonies. With the exception of colonies 259 and 269, the distance of the foraging territory shift following nest relocation was essentially the same as the distance of the nest relocation. The territory shift of colony 269 was in response

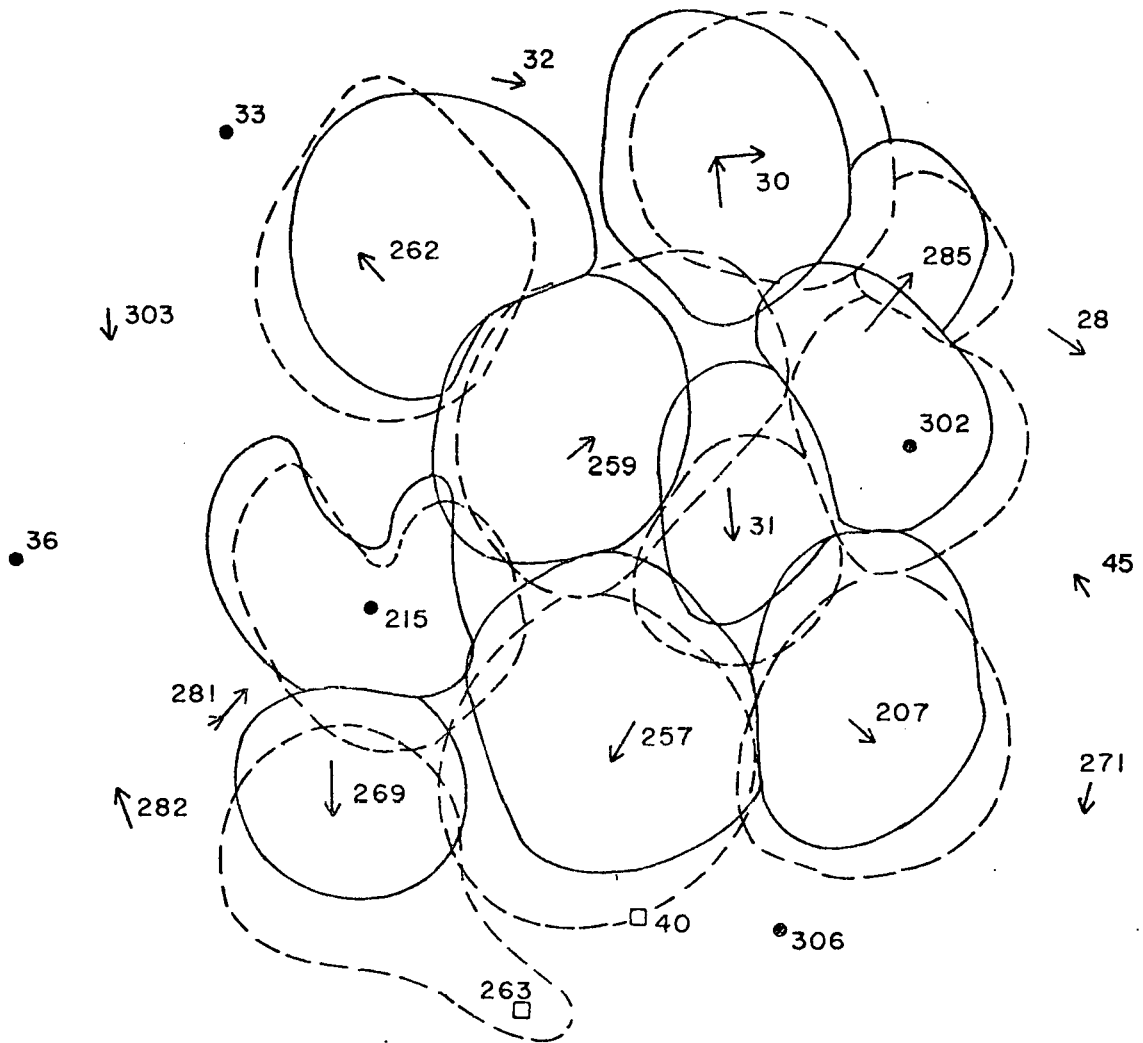


FIGURE 12. FORAGING TERRITORIES OF THE FLORIDA HARVESTER ANT (*P. badius*) IN A SOUTH CAROLINA OLD FIELD. SOLID LINES INDICATE TERRITORY IN AUGUST; BROKEN LINES INDICATE TERRITORIES IN OCTOBER AFTER NEST RELOCATIONS. (ARROWS = DISTANCE AND DIRECTION OF NEST RELOCATION; SOLID CIRCLES = COLONIES WHICH DID NOT RELOCATE THEIR NEST; OPEN SQUARES = COLONIES WHICH HAVE EITHER DIED OUT (#263) OR BEEN EXCAVATED (#40); SCALE: 1 INCH = APPROX. 7 M).

to the death of colony 263 during the previous summer. The major shift for colony 256 apparently occurred during a previous move since its foraging territory now includes the vacant site of colony 40 which was excavated during the summer of 1974.

There were changes in the area foraged following nest relocation. The area for colonies number 207, 262 and 285 remained unchanged. However, the foraging area for colonies 30 and 302 declined by 17 and 19 m² respectively. Increasing in area foraged following nest relocation were colonies 31, 215, 257, 259 and 269. The increases ranged from 21 to 81 m² and averaged 37 m². It is uncertain whether the changes in area of foraging territories following nest relocation are a result of that phenomenon or some form of seasonal shift.

As we learn more about the behavior of the Florida harvester ant, it becomes apparent that it is very efficient in this utilization of food resources. This is possible because of a foraging strategy (combined with seasonal nest relocations) which results in regular spacing of the colonies and very distinct and seldom overlapping foraging territories. The real scientific mystery is how these territories are maintained with almost a complete absence of intraspecific interaction between adjacent colonies. Possibly specific pheromones are used to define each territory. From an academic viewpoint it is important that future investigations take into account the mechanisms by which such distinct foraging territories are maintained. From a more practical viewpoint future investigations should be designed to elucidate the role played by the Florida harvester ant in elemental cycling in grassland ecosystems. So far, our knowledge of the food habits and foraging behavior of this unique ant species indicates an important role. Thus, the prediction that P. badius may serve as an indicator of environmental contamination appears valid.

RADIONUCLIDE ACCUMULATION RATES OF FREE-LIVING WOOD DUCKS MAY BE
ACCURATELY PREDICTED FROM LOSS-RATES CALCULATED FOR CAPTIVE BIRDS
(T. T. Fendley and I. L. Brisbin)

Waterfowl, because of their high mobility and harvest by man, have a high potential to serve as vectors to man of radionuclides from otherwise secured waste disposal areas, seepage basins or other aquatic habitats contaminated with such waste materials. In order to quantify and predict the role of waterfowl in this regard, information is needed to describe the rate at which such radioactive materials may be accumulated once a bird enters a contaminated area and the rate at which these materials are later eliminated after the bird has left the area.

The determination of contaminant uptake and elimination rates by wildlife has been complicated because most available information has been calculated for birds held for study under captive conditions. It is possible that where the bird is free to accumulate body burdens through natural food chain processes rather than through artificially contaminated commercial feeds, accumulation and elimination rates may differ strikingly. Studies have therefore been designed to determine radionuclide uptake and elimination rates by free-living waterfowl as well as to evaluate the accuracy with which the rates of these processes may be predicted from loss-rate parameters calculated for birds held under captive conditions.

Radiocesium elimination rates were determined for ten male and ten female wood ducks which has accumulated natural body burdens of this isotope in the contaminated Steel Creek swamp delta of the SRP. Biological half lives for these birds averaged 5.57 days and elimination constants for the isotope were linearly related to body weight, with heavier birds losing their body burdens at proportionally slower rates. The relationship between elimination rate and body weight was then used to predict elimination rates for an additional uncontaminated male and female wood duck which were then equipped with radio-transmitters and released in the contaminated Steel Creek swamp delta after having flight feathers removed from one wing. Both birds were recaptured at 5 - 10 days intervals with hand nets and whole-body counted to determine radiocesium uptake rates under field conditions. The "Davis-Foster" equation, which predicts the time required to attain 90% of equilibrium body burden ($0.90 Q_e$) as a function of elimination rate-constant, was used to predict the time required to attain $0.90 Q_e$ after correcting for body weight differences. The average time of $0.90 Q_e$ was calculated to be 20.9 days compared to an observed value of 23.4 days, based on a visual inspection of the actual uptake curve (Figure 13).

The results indicate that elimination rate-constants derived from studies of captive waterfowl may be used to closely calculate the time required to accumulate equilibrium body burdens in the field. While the studies described here have used radiocesium, emphasis is on attaining more accurate predictive ability and quantification of cycling-rate processes in these animals with respect to all contaminants, including such substances as heavy metals, pesticides and transuranics.

Preliminary results of similar studies with American coots suggest, for example, that while rate-constants of captive birds may serve as accurate predictors of uptake and elimination rates under field conditions in a variety of species, there may be important differences between species with respect to the rate constants themselves.

CAPTIVE-REARED WOOD DUCKS FROM THE RADIOACTIVELY CONTAMINATED STEEL CREEK SWAMP DELTA SHOW ACCELERATED GROWTH PARAMETERS IN COMPARISON TO SOME CONTROL POPULATIONS (T. T. Fendley and I. L. Brisbin)

There is little evidence that elevated levels of radionuclide contamination in the Steel Creek swamp delta have had significant impacts upon resident wildlife populations. An attempt was made, however, to determine whether sigmoid growth curve characteristics of captive-reared wood ducks hatched from eggs laid in the Steel Creek habitat showed differences from similar parameters for ducklings from nearby control habitats contaminated only by world-wide atmospheric fallout. The logistic growth curve was a better approximation of the growth of all birds than either the Gompertz or von Bertalanffy growth models.

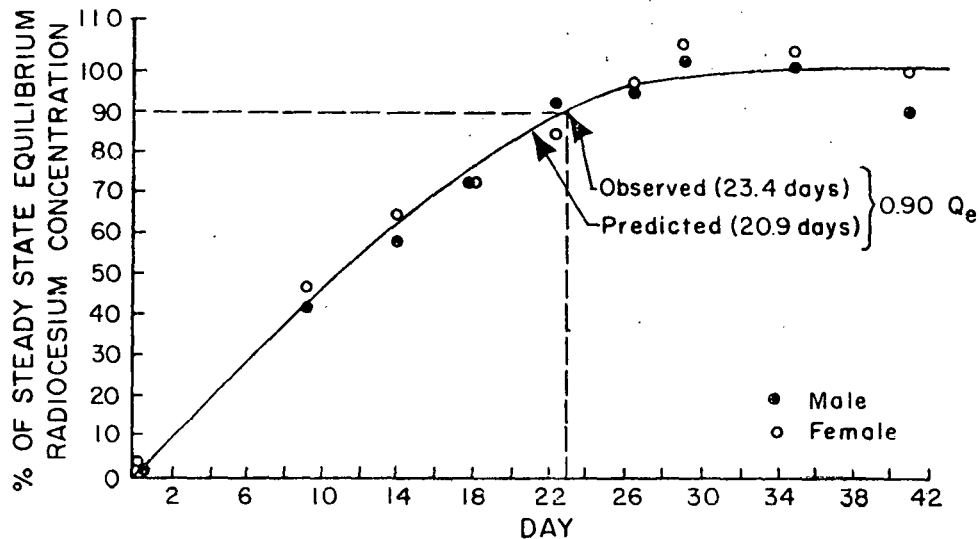


FIGURE 13. RADIOCESIUM UPTAKE BY TWO FREE-LIVING WOOD DUCKS EQUIPPED WITH RADIOTRANSMITTERS AND RELEASED INTO THE CONTAMINATED STEEL CREEK SWAMP DELTA OF THE U. S. ERDA SAVANNAH RIVER PLANT. PREDICTED TIME OF 90% EQUILIBRIUM ($0.90 Q_e$) WAS CALCULATED FROM THE "DAVIS-FOSTER" EQUATION USING ELIMINATION RATE-CONSTANTS DERIVED FROM CAPTIVE BIRDS. OBSERVED TIME TO $0.90 Q_e$ WAS PLOTTED FROM A VISUAL INSPECTION OF THE FITTED UPTAKE CURVE.

Using the logistic model, no significant differences were shown in the magnitude of asymptotic weight attained by birds from any of the three study populations. However, ducklings from Steel Creek showed an earlier attainment of the point of inflection and 99% of asymptotic weight and had a higher overall growth-rate ("k") constant than did ducklings raised from eggs collected near Columbia, South Carolina, approximately 100 km to the northeast of the Steel Creek study area. The growth characteristics of birds from the Fort Gordon military reservation near Augusta, Georgia, about 40 km to the west of Steel Creek, did not differ significantly from those of either the Steel Creek or Columbia birds, and were intermediate in value between the two (Figure 14).

It is possible that genetic differences in growth characteristics may have been responsible for the differences observed in this study, especially since birds raised in captivity under identical conditions showed these differences. Such genetic differences are difficult to support in light of the high mobility of waterfowl and the documented tendency for juvenile birds to disperse over long distances and to breed

far from the habitat in which they originally hatched. Such movement patterns would make development of isolated gene pools between populations less than 100 km apart extremely unlikely. Nevertheless, future genetic studies are planned to study the extent of gene flow among these three populations.

At the present time, the biological significance of the accelerated growth of the Steel Creek birds is not known. In general, little or no difference can be observed in the health and vigor of the birds raised from any of the three study areas. Literature references are available which indicate that other species of animals, particularly invertebrates, often tend to show accelerated growth rates following low level radiation stress in laboratory experiments.

Perhaps the most significant aspect of these studies is the development of sigmoid growth-curve analyses as a tool to compare functional differences between animals under standardized captive conditions, using birds from different populations and habitats. The usefulness of this technique is emphasized in the significant differences shown between the growth curve characteristics of the Steel Creek and Columbia populations, even though other more standard comparative indicators (e.g., single determinations of hatching weight, weights of incubating hens or clutch sizes) did not differ significantly among populations.

SOIL PROFILES IN SWEETGUM STANDS HAVE RICHER NUTRIENT STATUS THAN SOIL PROFILES IN OTHER TREE SPECIES (D. C. Adriano and T. G. Ciravolo)

Productive agricultural fields need continuous external inputs (fertilizer, plowing, etc.) to counter the physical and chemical stress placed on the soil system by cropping. If these external inputs are withheld, encroachment on these fields by weeds and grasses will occur. With time, old fields will succumb to invading coniferous species and finally, if not protected by fire, to a deciduous forest climax. Chemical and physical modification of the soil system accompanies these successional steps. The objective of this investigation was to study some chemical changes of the soil system in old field succession in the southeastern United States.

Six forest sites were selected according to the predominant tree species, which include loblolly, mixed loblolly and sweetgum, sweetgum, longleaf, mixed longleaf and oak, and oak stands. For comparison, a two-year old agricultural field and an old field were included.

Litter (A₀₀) and organic matter (A₀) samples were obtained from six 0.25 m² quadrats from each stand. Soil samples (composite of two soil cores) from the mineral layer were taken from each quadrat at each stand at depths of 0 - 5, 5 - 15, 15 - 30, 30 - 45, and 56 - 60 cm. The samples were air dried, ground, and determined for pH, total C, total N, and total S. For brevity, data are averaged for the two organic layers (A₀₀ and A₀) and for the five mineral layers (0 - 60 cm).

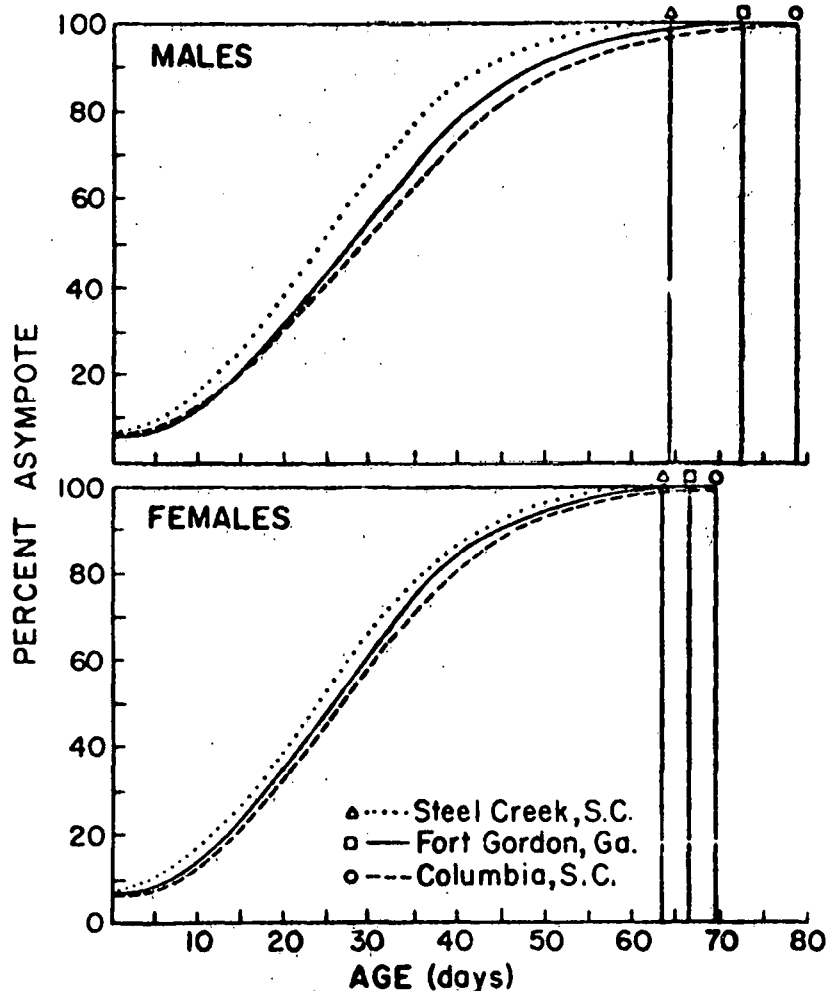


FIGURE 14. LOGISTIC GROWTH CURVES OF CAPTIVE-REARED WOOD DUCKS FROM A CONTAMINATED (STEEL CREEK) AND TWO UNCONTAMINATED HABITATS IN SOUTH CAROLINA AND GEORGIA. VERTICAL LINES REPRESENT THE TIME TO ATTAINMENT OF 99% OF ASYMPTOTIC WEIGHT.

The largest standing crop of nitrogen (47 g/m^2) in the organic layer was found at the loblolly stand (Table 19), the lowest at the old field. Other stands are intermediate, ranging from 12 g/m^2 of N in the mixed loblolly and sweetgum to 33 g/m^2 of N in the longleaf stand. Litter in coniferous forests tends to accumulate due to its high resin contents, resisting rapid decomposition. Litter accumulation in the old field is low as the decomposition rate is relatively rapid. Thus, nutrients in the organic layers in an old field are expected to be lower because of their more rapid turnover rates.

In the mineral layers (0 - 60 cm) the lowest standing crops of carbon and nitrogen were found in the longleaf and oak stands. Although differing in carbon and nitrogen standing crops, the various forest stands have similar C:N ratios ranging from 22 in the mixed loblolly and sweetgum to 28 in the sweetgum stands. The agricultural field was expected to have the lowest C:N ratio with 12, followed by the old field with 17. However, examining the individual soil profiles, the C:N ratio varied with depth depending on the moisture status. In sweetgum where the profile is usually saturated with water, the C:N ratio ranged from 19 for the 0 - 5 cm depth to 36 for the 45 - 60 cm depth. In loblolly and longleaf, it was just the opposite, where the C:N ratios were highest at the surface layers. In loblolly, the C:N ratios were 32 for the 0 - 5 cm and 21 for the 45 - 60 cm. In longleaf, the ratios were also 32 for the 0 - 5 cm and 13 for 45 - 60 cm depth. In the other stands the ratios remain similar throughout the soil profiles.

In the sweetgum stand, the waterlogged conditions in the soil profiles caused the slow decomposition, especially at deeper depths, resulting in very high C:N values. The surface layers are subject to aeration and drying cycles where lower ratios were obtained. Also the high C:N values in deeper layers could have been caused by denitrification of nitrate to nitrogen gas.

The C:N ratios give an indication of N availability. Nitrogen is required by the decomposing organisms which will compete with higher plants if N is limiting. High C:N will retard decomposition and slow the rate of N mineralization. In general, a C:N of 10 or lower is required for crop production. Higher values may result in N deficiencies caused by temporary N immobilization and by slow rates of N mineralization. Due to their more extensive root system, forest species can tolerate a slower rate of N mineralization.

Prior to leaf fall, leaching of nutrients from senescent leaves prime the forest floor for decomposition. Deciduous leaves with higher base concentrations are more rapidly decomposed. Soil organisms are numerous in the forest floor, enriching it with excreta and mixing the organic matter into the mineral layer. The high standing crop of N found in the sweetgum mineral layer could be a result of this active faunal and microbial activity.

SIGNIFICANT QUANTITIES OF NUTRIENTS REMOVED IN "RED" STRAW HARVESTING (K. W. McLeod)

The management practices of our national forests must be evaluated so that we may objectively determine the value of such practices. A management practice currently being employed at the Savannah River Plant (SRP) is the removal of "red" straw from longleaf pine plantations. This straw is of commercial value for mulching and decorative purposes. Its removal potentially poses several problems. First, the litter contains large quantities of nutrients which normally would be returned to the

TABLE 19. STANDING CROPS OF TOTAL CARBON AND TOTAL NITROGEN IN VARIOUS FOREST SITES COMPARED WITH AN OLD FIELD AND AN AGRICULTURAL FIELD. VALUES ARE MEANS OF SIX REPLICATES.

Site	Organic Layer		Mineral Layer	
	N, g/m ²	C, Kg/m ²	N, g/m ²	C:N ¹ / _—
Agricultural field	—	2.8	229	12
Old Field	9	2.7	137	17
Loblolly	47	6.3	289	26
Loblolly and sweetgum	12	11.7	557	22
Sweetgum	21	19.6	728	28
Longleaf	33	1.9	85	23
Longleaf and oak	24	3.4	127	26
Oak	22	3.3	120	27

¹/_— Ratio of percent total C and percent total N in the dry soil.

soil as the litter decomposes. Second, the necessity for the operation of heavy equipment within the plantation can mechanically damage the trees. Third, pine litter is recognized to inhibit the understory vegetation. Removal of the litter could cause understory releases; increasing competition for water and nutrients and increasing the management needed to control the understory. Finally, the reduced depth of litter could increase the evaporative loss from the soil surface and lead to moisture stress in the trees. All of these factors can potentially decrease productivity of the pine plantations.

As reported in last year's Annual Report, a growth reduction was observed following the "red" straw removal. To examine the impact of this removal on the nutrient status of the plantations, elemental concentrations were determined for the litter components, soil and tree foliage.

In the "red" straw removal process, 392 kg/ha of litter are removed. This represents 25% of the total litter biomass. The removed litter contains 2.1 kg/ha N, 0.22 kg/ha P, 1.6 kg/ha K, 1.4 kg/ha Ca and 0.12 kg/ha Mg. These quantities represent from 17 to 30% of the

elemental content of the total litter. This type of litter removal significantly reduces the total inventories of these elements in the litter system.

The concentrations of these same elements in the soil proved to be highly variable and did not show any reduction in the elemental concentration in the litter removal areas. This is not totally unexpected as these soils were collected three years following the removal and hence have had considerable time to "recover" from a nutrient loss. Since the soils are very sandy the elemental content of the soil is probably strongly controlled by current releases of elements from decomposing litter.

Elemental concentration of the foliage was also determined. The foliage concentrations were not significantly different between the control and litter removal areas. The samples suffer from the same time lag and consequent problems in sampling as the soil samples. These samples are serving as beginning samples for a reapplication of this treatment.

We conclude that significant quantities of nutrients are removed from the pine plantation ecosystem due to this management practice. This may be responsible for the reduction in growth but has not been observed to reduce elemental concentration in the soil or foliage after a three year time lag. Elemental concentrations of these components are being more rigorously sampled in the current study.

FERAL SWINE STUDIES DISCOVER A RACE OF PIGS WITH SMALLER BODY SIZES THAN THE LABORATORY MINIATURE PIG (I. L. Brisbin, M. J. Vargo, R. A. Geiger, and J. E. Pinder)

Generations of selective breeding in pigs have attempted to produce an animal with a small body size for use in laboratory biomedical studies. To date, these programs have not produced animals which mature at weights of less than 60 - 80 kg. Recently, morphological studies of two populations of free-ranging feral swine in the southeast have disclosed that swine of Ossabaw Island, Chatham County, Georgia may be among the smallest pigs ever studied in the world: 95% confidence limits for body weights of mature boars and sows do not exceed 30 kg (Table 20).

Morphological studies form part of a program designed to determine the structural and functional properties of swine populations as components of southeastern ecosystems in which these animals may serve as both important agents of environmental damage and as prized game animals. The two populations receiving the greatest attention at this time are those of Ossabaw Island, Georgia and the resident feral swine herd of the Savannah River Plant (SRP). These populations differ strikingly in their developmental histories and in the length of time they have existed in the feral state, free from introduction of domestic genes. The SRP population has existed in a feral state for less than 30 years, although occasional escaped domestic animals may cross onto the plant property

TABLE 20. COMPARISONS OF TOTAL BODY LENGTH AND WEIGHT AND BODY HAIR LENGTHS^a OF TWO POPULATIONS OF FERAL SWINE.

Locality	Sex	Number	Total Length (cm)		Weight (kg)		Average Hair Length (cm)	
			Mean	95% Confidence Interval	Mean	95% Confidence Interval	Spinal Hair	Flank Hair
Ossabaw Island	Males	20	95.8	88.8-102.8	21.9	17.6-26.2	6.8	4.0
	Females	20	96.3	89.1-103.5	24.0	19.4-28.6	6.2	3.7
Savannah River Plant	Males	20	127.8	121.4-134.2	67.1	52.6-81.6	4.3	3.0
	Females	20 ^b	121.9	111.4-132.4	55.3	40.1-70.5	5.0	3.5

^a Hair length values were calculated as the averages of measurements taken at the shoulder, mid-body, and hip, along the spinal and flank midlines, respectively.

^b Number = 12 for hair length data.

and enter this population. The insular habitat of the Ossabaw Island population (OIP) has prevented any significant input of domestic genes since the population first entered the feral state well over 400 years ago. Comparative studies of these populations indicate that OIP swine are not only smaller than those of the SRP but that their body proportions, normalized for differences in absolute body size, are also different, with OIP animals tending to stand taller at the shoulder than those of the SRP. Ossabaw Island population animals also have longer body hair than those of the SRP and this may be viewed as a trait which would be adaptive to animals which were required to endure cold winter temperatures without the benefit of shelter and heat usually provided domestic stock.

Frequencies of various color phenotypes have also indicated striking differences between the two populations. The black and spotted phenotypes were by far the most common in each study area, comprising 94.5% and 75.0% of the OIP and SRP populations, respectively. Low frequency of the rarer color phenotypes (red, brown, agouti and white) is found in the OIP. Of particular interest is the absence of white animals from the OIP since the color is known to be a genetic dominant, and historical records indicate that white animals were occasionally introduced into this area. This suggests that there has been some form of selection against the white phenotype in this habitat. Selection due to predation is not likely. It is possible that white animals are at a selective disadvantage in this habitat due to their susceptibility to sun-scalding, resulting in cracking and blistering of the skin which creates a site for infection and parasite entry. On Ossabaw Island feral swine often spend the summer months feeding in the open salt marshes where they are exposed to the full rays of the sun, while Savannah River swine spend much of their time in a deeply shaded bottomland swamp forest. In the SRP population, over nine percent of the animals are white while on Ossabaw Island none of over 400 sightings were of white animals.

Morphological studies suggest that the QIP population shows characteristics which are a result of selection for survival ability in the natural habitats of the southeast. Genetic drift and founder effect, however, may have played an important role in the OIP insular habitat, and comparative genetic studies are also underway to investigate these phenomena. Plans are also being made to bring animals from both the OIP and SRP populations into captivity for maintenance under conditions of optimal nutrition and ad libitum feeding to determine what differences in body size and weight between the two groups is caused by genetic as opposed to environmental factors.

SEVERAL BREEDS OF CHICKENS SHOW DIFFERENCES IN GROWTH AND MORTALITY RESPONSES TO ACUTE RADIATION STRESS (B. E. Lattimer and I. L. Brisbin)

In the concluding phase of a program studying the responses of poultry to acute gamma radiation stress, a comparative study has been conducted of the growth and mortality responses of five breeds of chickens. The breeds used were commercial broiler chickens (Cobb X Cobb line), white leghorns, Athens random-breds, feral bantams and white leghorn bantams. All birds were exposed to a ^{60}Co gamma source at an average dose rate of approximately 5 R/min between two to three days after hatching. Although there were no statistically significant differences between the $\text{LD}_{50/30}$ levels of the five breeds studied, as shown in Figure 15, the broiler chickens, as reported in previous studies in our laboratory, again showed the highest $\text{LD}_{50/30}$ level (1580 R), while the lowest level was shown by the white leghorn bantams (980 R).

Thirty-day growth rates indicated that body weights were proportionately more depressed following radiation exposure than were the sizes of various body structures such as the culmen, tarsus, middle toe and longest primary wing feather. Of these structures, the culmen seemed least affected in all breeds studied. On a scale of absolute size, growth-rate decrease with increasing radiation exposure was greatest for Cobb broilers (-0.86 g/day per 100 R of exposure), over twice that of any other breed. Comparable values for other breeds ranged from -0.35 to -0.14 g/day per 100 R for white leghorns and white leghorn bantams, respectively.

There were differences among breeds in the total depression of growth rate which could be tolerated before exhibiting a given level of mortality. At levels below the $\text{LD}_{50/30}$, for example, feral bantams were able to tolerate greater levels of growth-rate depression than any other breed, before exhibiting specific levels of mortality. This would be of obvious advantage to birds living in a feral state where unpredictable shortages of food or inclement weather might cause temporary growth-rate depressions. Domestic breeds, however, would seldom be exposed to such conditions. The smaller body size of the feral bantam was not responsible for this phenomenon as suggested by the observation that white leghorn bantams of similar body size showed the least tolerance of growth-rate depression of any breed. At higher exposure levels the larger Cobb broilers showed the greatest tolerance of growth-rate

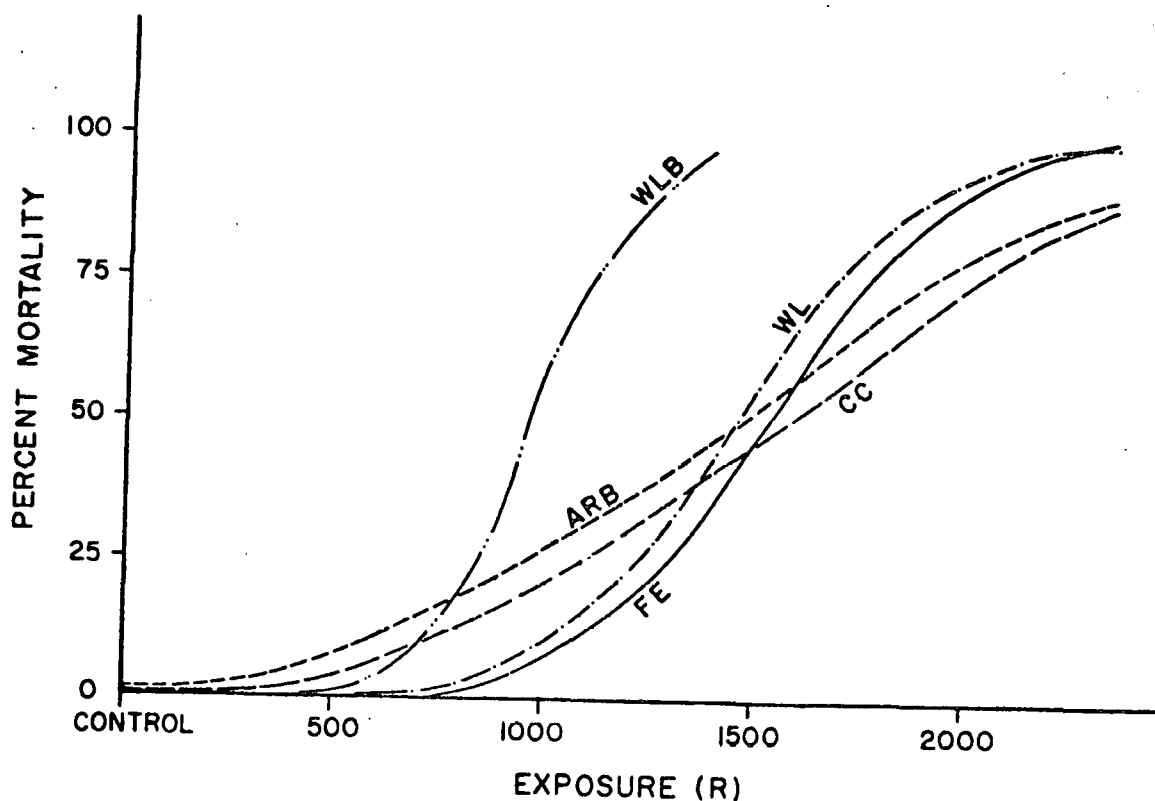


FIGURE 15. THIRTY-DAY MORTALITY RESPONSES OF FIVE BREEDS OF CHICKENS FOLLOWING EXPOSURES TO VARYING LEVELS OF GAMMA RADIATION STRESS AT TWO DAYS OF AGE. CURVES WERE FIT BY PROBIT ANALYSIS. WLB = WHITE LEGHORN BANTAMS, WL = WHITE LEGHORNS, ARB = ATHENS RANDOM-BREDS, CC = COBB-STRAIN BROILERS, AND FE = FERAL BANTAMS.

depression. This phenomenon may be related to the selection of broilers for rapid and efficient growth, thus making them capable of rapidly and efficiently repairing any damage sustained from radiation exposure.

With respect to body measurements, Athens random-bred chickens exhibited the lowest percentage tolerance to morphological distortion, particularly at exposure levels below the $LD_{50/30}$. The feral bantams again showed the greatest proportional levels of depression in size of both culmen and longest primary wing feather before exhibiting given levels of mortality.

Both a well-formed culmen for food gathering and well-developed wing feathers for flight would provide survival advantage to feral bantams in the wild state. It can be suggested that those traits for which a breed has been most highly selected under domestication may be those which tend to exhibit the greatest proportional decreases following radiation stress. An easily testable prediction of such a hypothesis would be that of all of the breeds studied, the white leghorn would show the greatest proportional decrease in egg laying following sublethal exposure to radiation stress.

Although studies of responses to acute gamma radiation stress are currently being phased out of many nuclear energy research programs, the work reported here indicates that such studies can still provide useful information concerning the interrelationships of basic biological phenomena such as growth, mortality and reproduction. The selection of various breeds of poultry for one or more of such traits make these birds particularly well suited for studies of such interrelationships.

QUALITY OF DIET VARIES SEASONALLY IN WHITE-TAILED DEER (M. H. Smith and I. L. Brisbin)

Concentrations of 14 elements (Ca, K, Mg, N, P, Na, Al, B, Cu, Mo, Sr, Zn, S and ^{137}Cs) were measured in the rumen contents of 194 white-tailed deer collected throughout the year on the Savannah River Plant. The crude fiber content and percent ash in the diet and an estimate of the amount of body fat (kidney fat indices) were also measured. Statistical analysis has been completed for crude fiber, percent ash, percent nitrogen, percent sulfur and kidney fat index. The complete analysis will be finished soon.

The identified sources of variation were sex, locality (swamp versus upland) and season. Only adult deer (> 1 yr of age) were used in the analysis. Because of the occurrence of numerous significant first and second order interactions between the variables, the analysis was conducted on the data for each sex separately as well as combined. The quality of diet definitely varies seasonally and according to locality (Table 21).

Crude fiber and percent nitrogen are both thought to be good indicators of dietary quality. Both sexes show significant differences in crude fiber content across seasons with the highest values generally occurring during the fall and the lowest during the summer or spring. The crude fiber content is correlated with percent nitrogen ($r = -0.37$ and $P < 0.01$), percent sulfur ($r = -0.26$ and $P < 0.01$), kidney fat index ($r = 0.19$ and $P < 0.01$) but not with percent ash ($r = 0.04$). The only other significant correlations ($P \leq 0.05$) involving these five variables were percent nitrogen-percent sulfur ($r = 0.63$) and percent sulfur-kidney fat index ($r = -0.16$). The only relationship that had a fair degree of predictability associated with it was that involving sulfur and nitrogen. Since both of these elements form an integral part of protein, this relationship should not be surprising but it still accounted for less than 50 percent of the variation between the variables.

TABLE 21. F-VALUES FOR TWO WAY ANALYSES OF VARIANCE FOR THE EFFECTS OF SEASON, LOCALITY (SWAMP VERSUS UPLAND) AND THE INTERACTION OF SEASON X LOCALITY, UPON CERTAIN SELECTED CHARACTERISTICS OF RUMEN CONTENTS AND KIDNEY FAT INDICES OF 194 WHITE-TAILED DEER FROM THE SAVANNAH RIVER PLANT NEAR AIKEN, SOUTH CAROLINA.

	SEASON			LOCALITY			SEASON X LOCALITY		
	MALES	FEMALES	BOTH	MALES	FEMALES	BOTH	MALES	FEMALES	BOTH
Crude Fiber	3.03**	9.41**	7.70**	0.18	6.93**	3.39	2.32	3.11*	4.85*
Percent Ash	0.56	4.44**	3.30*	0.09	0.03	0.02	0.10	0.08	0.16
Percent Nitrogen	69.00**	91.89**	152.52**	3.78*	6.91**	10.08**	7.85**	2.57*	9.73
Percent Sulfur	9.91**	24.94**	30.50**	0.75	2.60	2.86	0.80	0.23	0.82
Kidney Fat Index	1.09	9.04**	6.10**	0.05	>0.01	0.02	1.32	0.89	1.29

* $P \leq 0.05$

** $P \leq 0.01$

It is surprising that the kidney fat index varies as a negative function of percent sulfur, as a positive function of crude fiber and is not related to the percent nitrogen. As crude fiber content goes up, dietary quality is thought to decrease and the opposite is true for protein content. Body condition should go up as quality of diet increases other things being equal. However, other physiological variables may change in response to dietary quality. For example, reproductive activity may increase with dietary quality and thus change the pattern of energy partitioning within individual deer.

Percent sulfur is lowest during the fall and increases through the winter and spring and then starts decreasing during the summer season. Deer rumens collected from areas around coal burning power facilities on the Savannah River Plant will be examined for higher sulfur content and our other data for sulfur may form a baseline for dietary sulfur in wildlife populations. Percent nitrogen is highest in the spring and declines through the summer and fall and starts increasing in the winter. The nitrogen-sulfur ratios are not consistent in different protein types and our data may indicate a dietary shift in the type of proteins consumed during different seasons.

Previous studies of species composition in the diet of swamp and upland animals revealed no striking differences in their diets. However, percent nitrogen is generally higher in the rumens of upland compared to those of swamp animals and the differences are significant for both sexes (Table 21). The pattern is somewhat complicated by the significance of the interaction between season and locality. At any rate, these data should be of interest to the deer management program on the Savannah River Plant.

BIOLOGICAL RESOURCES SHOW TEMPORAL CHANGES IN GENE FREQUENCIES
(R. Baccus and M. H. Smith)

Small mammal populations have been documented to vary genetically with time. Such long term studies on larger mammals are decidedly scarce. This scarcity is due in part to the longer generation times of the animals involved and to the difficulties of manipulating population structure. The deer herds on the Savannah River Plant have expanded rapidly since the initiation of the project 20 years ago. At that time, the deer herd was limited to a very narrow strip of swamp land directly adjacent to the Savannah River. Since that time, the population has extended itself to cover almost the entire area of the Plant. Such rapid expansion would be expected to greatly alter the population structure. Since 1971, the deer herd has been sampled periodically by unrestricted hunting during the fall deer season. Tissue samples collected from deer sampled during the fall of 1974, 1975 and 1976 were analyzed electrophoretically.

Preliminary evidence indicates that the deer population on the Savannah River Plant varies through space and time. The common allele frequency of the B-hemoglobin (Table 22) ranges from 47% to 83%. Analysis of the data showed significant variation ($P < 0.05$) in allele frequency between years in addition to that observed through space within each year. The deer population is subdivided into a number of smaller breeding units and these units are changing through time. The identification of these smaller units may form the basis of a redefinition of the management units (hunt compartments) on the Savannah River Plant. The management of this biological resource should recognize the dynamic nature of population structure even at the genetic level of organization. These data mean that genetic changes in populations affected by industrial activities must be interpreted with care.

TABLE 22. COMMON ALLELE FREQUENCIES OF B-HEMOGLOBIN IN WHITE-TAILED DEER POPULATIONS ON THE SAVANNAH RIVER PLANT. DATA ARE GIVEN ONLY FOR HUNT COMPARTMENTS THAT CONTAINED AN ADEQUATE SAMPLE SIZE (IN PARENTHESES) FOR STATISTICAL ANALYSES.

Compartment	Year [*]		
	1974	1975	1976
15	0.77 (44)	0.68 (36)	0.60 (61)
17	0.68 (25)	0.69 (18)	0.63 (50)
18	0.71 (29)	0.70 (27)	0.68 (39)
20	0.76 (25)	0.65 (29)	0.59 (40)
25	0.72 (43)	0.67 (18)	0.59 (33)
29	0.47 (17)	0.71 (29)	0.57 (34)
32	0.64 (35)	0.69 (18)	0.82 (30)
40	0.83 (18)	0.68 (17)	0.60 (31)
42	0.66 (25)	0.64 (22)	0.73 (66)
44	0.71 (44)	0.59 (28)	0.65 (76)
46	0.81 (39)	0.61 (28)	0.73 (33)
48	0.66 (28)	0.69 (13)	0.57 (54)
Mean	0.74	0.66	0.64

* Overall F = 4.24 and P < 0.05.

III. SREL-ERDA LABORATORY COOPERATIVE PROGRAMS

R. R. Sharitz	K. W. McLeod
D. C. Adriano	D. H. Nelson
I. L. Brisbin, Jr.	R. I. Nestor
D. W. Evans	D. Paine
J. B. Gentry	J. E. Pinder, III
J. W. Gibbons	D. H. Smith
J. P. Giesy	M. H. Smith
R. W. McFarlane	J. H. Thorp, III

The goal of the SREL-ERDA Laboratory Cooperative Programs is to provide research experience, perspective, and advice for student and faculty investigators from other colleges and universities who wish to utilize the unique field and laboratory facilities of SREL and the Savannah River Plant (SRP) to conduct ecological studies. Through these programs, a visiting scientist is able to conduct full-time research in the company of SREL individuals working in related areas. The ultimate objective of the effort is the completion and publication of significant ecological findings.

Research at SREL is centered around Thermal Ecology and Mineral Cycling studies with an emphasis on heavy metals and transuranic isotopes as well as on stable elements. The SRP provides unequalled opportunity for studies in these fields because of the spectrum of thermally altered aquatic habitats and the variety of radioisotopic releases resulting from the operation of nuclear production reactors.

Research in these programs is carried out by a highly qualified and experienced staff, and projects of visiting faculty and students are designed to fit into the context of one of the major programs and the on-going research of current staff workers.

Faculty, undergraduate, and graduate student research participation has been an important effort at SREL for many years. A summer undergraduate program has been active since 1967 and has expanded in recent years to include support for students during the non-summer months. Through the end of FY 1976 and the transition quarter, 189 undergraduates have participated in the program. More than 47 graduate students have completed thesis or dissertation research at SREL, and 19 had degree programs in progress at the laboratory in FY 1976. These students conduct studies which are complimentary to or can be coordinated with the activities of a member of the SREL research staff. SREL has also supported more than 25 faculty research participants since 1967. Four visiting faculty members conducted research at SREL during 1976 and four additional participants are expected during the summer of 1977.

The following student and faculty investigators participated in SREL-ERDA Laboratory Cooperative Programs during FY 1976 and the transition quarter.

A. Undergraduate Research Participation:

K. S. Ammerman	SUNY-Cortland
I. F. Ashley	Paine College
P. M. Burke	Beloit College
B. Carducci	University of Pittsburgh
L. A. Clapsaddle	St. Mary's College of Maryland
M. M. Cooper	Paine College
W. F. Crawford, Jr.	Furman University
A. V. Daniely	Paine College
J. M. Dunbar	Michigan State University
M. A. Fierstine	Iowa State University
J. D. Gordos	Ohio State University
N. E. Herr	University of California-Irvine
C. E. Hill	Paine College
A. M. Johnson	Paine College
R. G. Keeney	Furman University
L. G. Lewis	Paine College
J. R. Lovvorn	University of Georgia
C. E. Martin	University of California-Santa Barbara
L. T. Mayweather	Paine College
L. Roberts	Paine College
C. L. Scott	SUNY-Buffalo
S. P. Skinner	Duke University
J. P. Skorupa	University of Redlands
P. A. Sloat	Michigan State University
S. K. Thieme	Denison University
D. R. Williams	Drury College
J. W. Yokel	Colorado State University

B. Laboratory Graduate Participation:

J. M. Aho	M.S.	Wake Forest University
E. J. Christy	M.S.	University of Georgia
A. H. Domby	M.S.	University of Georgia
J. D. Felley	M.S.	University of Georgia
T. T. Fendley	Ph.D.	Utah State University
D. W. Foltz	Ph.D.	University of Michigan
T. P. Graham	M.S.	University of Georgia
T. C. Hazen	Ph.D.	Wake Forest University
L. W. Hersloff	Ph.D.	Colorado State University
E. M. Iglich	Ph.D.	University of Georgia
T. F. Mueller	Ph.D.	SUNY-Stony Brook
K. K. Patterson	M.S.	Wake Forest University
L. Scudder	M.S.	Boston University
R. D. Semlitsch	M.S.	University of Maryland
E. A. Standora	Ph.D.	University of Georgia
J. R. Suda	M.S.	University of Georgia
W. R. Teska	Ph.D.	Michigan State University
D. H. Wood	Ph.D.	University of South Carolina
J. G. Wiener	Ph.D.	University of Georgia

C. Faculty Research Participation:

J. C. Avise	University of Georgia
S. W. Duvall	University of Georgia
G. J. Leversee	Nasson College
E. H. Liu	University of South Carolina

The success of these programs can best be measured in terms of the accomplishments of the participants. During FY 1976, 11 manuscripts authored or co-authored by undergraduate participants, 16 by graduate students and 7 by visiting faculty members were published in professional journals or symposium volumes. These studies are related directly to one of the major research programs of the laboratory, and many are included in the data presentations in the Thermal Ecology or Mineral Cycling sections of this report.

Publications of the University of Georgia Institute of Ecology under Contracts AT(38-1)-301, AT(38-1)-310, AT(38-1)-708, AT(38-1)-819, and EY-76-C-09-0819 between April 1, 1976 to March 31, 1977. Numbers preceding the citation refer to the Institute publication numbers.

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