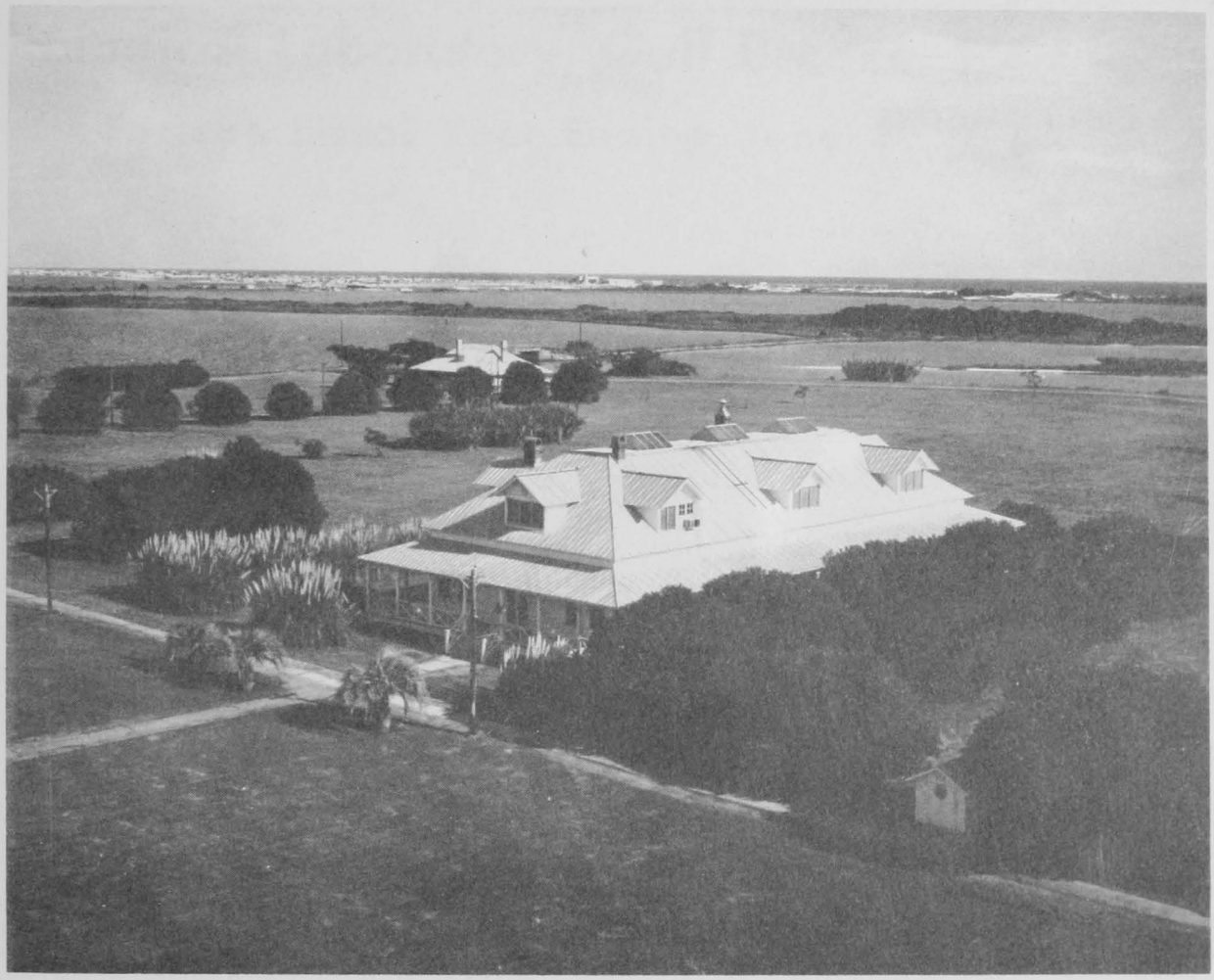
Annual Report of the Bureau of Commercial Fisheries Biological Laboratory, Gulf Breeze, Fla.

For the Fiscal Year Ending June 30, 1965



UNITED STATES DEPARTMENT OF THE INTERIOR

FISH AND WILDLIFE SERVICE

BUREAU OF COMMERCIAL FISHERIES

Circular 247

UNITED STATES DEPARTMENT OF THE INTERIOR
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nual Report of the Bureau of Commercial Fisheries Biological Laboratory, Gulf Breeze, Florida

For the Fiscal Year Ending June 30, 1965

PHILIP A. BUTLER, Laboratory Director

Circular 247

Washington, D.C.

May 1966

CONTENTS

over illustration: Main laboratory building on Government-owned Sabine Island, an artificial allast rock island in Santa Rosa Sound, 8 miles south of Pensacola, Fla. The Gulf of Mexico is isible in the distance.

Annual Report of the Bureau of Commercial Fisheries Biological Laboratory, Gulf Breeze, Florida

For the Fiscal Year Ending June 30, 1965

INTRODUCTION

The Bureau of Commercial Fisheries Biological Laboratory at Gulf Breeze, Fla., was established in 1938 to investigate the estuarine factors affecting the commercial production of oysters. The program was broadened in 1958 to include other estuarine animals having importance to commercial fisheries, and to study especially the effects of pesticides on these animals and their food supply.

The research program has stressed three principal objectives in order to define the effects of synthetic organic pesticides on commercial fisheries.

1. The determination of the acute and chronic effects of commercial pesticides on specified animals under controlled laboratory conditions. Progress towards this objective is summarized under the descriptions of Projects I, II, III, and IV.
2. The documentation of the present status of the biota in the Pensacola estuary, and analysis of the population dynamics of selected species there in order to be able, at a later date, to identify major changes attributable to man. Progress in these studies is summarized in Projects V, VI, VII, and VIII.
3. An evaluation of the present extent of pesticide pollution on the Atlantic, Pacific, and Gulf coasts. Progress in this study is described in Projects IX and X.

LABORATORY DIRECTOR'S SUMMARY

During the year, there have been significant achievements in the research program that resulted primarily from the increased knowledge and experience of project leaders and staff, many of whom have only now completed their second year in the program. This, coupled with analyses of data accumulated since 1958, made it possible to identify particular problems and gaps in our knowledge of pesticides where research needed to be stressed. In addition, two former residences on the laboratory island have been modernized to add more than 2,000 ft.^{2} (square feet) of urgently needed work space.

Tests for the objective evaluation of the toxic effects of pesticides on marine fauna have been accepted by the chemical manufacturing industry for use in obtaining registration of new chemicals.

The importance and usefulness of oysters as indicators of polychlor pesticide pollution in the estuary have been demonstrated. Tests are being developed for the detection of pollution by organophosphorus compounds.

A method has been discovered for handling and shipment of biological samples without refrigeration. This technique has made practical the establishment of a nationwide system for the detection of organochlorine pesticides in estuaries. By the end of the fiscal year, 6 of 10 proposed contracts had been entered into with State conservation agencies and universities to monitor pesticide pollution in estuaries important to commercial fisheries.

STAFF

Philip A. Butler, Director

Administration and Maintenance:
- Cynthia M. Herndon, Clerk-Typist
- Kenneth H. Herndon, Caretaker
- Hughey L. Jones, Fishery Methods and Equipment Specialist
- Anice M. Reynolds, Administrative Clerk

Pesticides Program:
- James J. Barklow, Jr., Physical Science Aid
- Nelson R. Cooley, Fishery Biologist
- David L. Coppage, Fishery Biologist
- Kenneth L. Echternacht, Fishery Aid
- David J. Hansen, Fishery Biologist
- Hugh T. Holland III, Fishery Biologist
- James M. Keltner, Jr., Fishery Technician
- Johnnie Knight, Fishery Aid
- Jack I. Lowe, Fishery Biologist
- Joy F. Morrill, Fishery Biologist
- Alan J. Rick, Fishery Biologist
- Donald C. Speed, Fishery Biologist
- Alfred J. Wilson, Physical Science Technician
- Robert R. W. Witte, Fishery Biologist

Resignations and Separations:

Donald S. Bush, Jr., Fishery Aid
Robert P. Hannah, Physical Science Aid
Allen A. Wolman, Fishery Biologist
William T. Young, Fishery Biologist

Summer Employees:

1964

John A. Couch, Fishery Biologist
Joseph K. Haburay, Fishery Biologist
James M. Keltner, Jr., Fishery Aid
James P. McVey, Fishery Aid

1965

Herbert L. Cash, Summer Trainee
Robert W. Hastings, Fishery Aid
Stephen L. Nall, Summer Trainee
Erwin H. Schroeder, Fishery Aid

TRAINING

P. A. Butler attended the Civil Service Commission Executive Leadership Institute in Washington, D.C., June 7-11, 1965.

Alfred J. Wilson attended a basic gas chromatography course and a workshop on the application of gas chromatography to analysis of pesticide residues offered by Wilkens Instrument and Research, Inc., Walnut Creek, Calif., June 14-18, 1965.

Donald Speed was on leave for 3 mo. (months) taking biology courses at Montana State University.

Employees from two other marine laboratories spent time at the Gulf Breeze laboratory for indoctrination in pesticide residue sampling methods.

MEETINGS

(Name of attendee in parentheses)

National Shellfisheries Association, New Orleans, La., July 13-16, 1964. (Butler.)

Gulf States Marine Fisheries Commission, Mobile, Ala., Mar. 17-19, 1965. (Butler.)

Gulf States Marine Fisheries Commission, Brownsville, Tex., Oct. 15-16, 1964. (Butler.)

American Fisheries Society, Atlantic City, N.J., Sept. 13-15, 1964. (Butler.)

Southern Weed Conference, Dallas, Tex., Jan. 21, 1965. (Butler.)

First Gulf Conference on Mosquito Suppression and Wildlife Management, Lafayette, La., Nov. 16-18, 1964. (Lowe.)

WORK CONFERENCES

Meeting of the Biological Laboratory Directors of the Bureau of Commercial Fisheries at La Jolla, Calif., Jan. 11-14, 1965. (Butler.)

Red Tide Symposium, St. Petersburg, Fla., Oct. 28-30, 1964. (Moderated by Butler.)

Interdepartmental Research Work Conference on Pest Control, University of Maryland, College Park, Md., May 13-15, 1965. (Butler.)

Pesticide Research Conference, Executive session of Gulf States Marine Fisheries Commission, Mobile, Ala., Mar. 17, 1965. (Butler.)

Bureau of Sport Fisheries and Wildlife Pesticide Program Review, Patuxent, Md., Oct. 6-8, 1964. (Butler.)

NAS Pesticide Research Review Committee, Denver, Colo., Nov. 19, 1964. (Butler.)

National Agricultural Chemicals Association Pesticides-Wildlife Committee Meeting, Washington, D.C., Sept. 21, 1964. (Butler.)

Workshop to develop needs for pesticide data and storage retrieval, Wildlife Research Center, Denver, Colo., Apr. 20-22, 1965. (Lowe.)

Conference on analytical methods for determining chlorinated hydrocarbons, Nashville, Tenn., Oct. 27, 1964. (Wilson.)

Conference of subcommittee on analytical procedures for determining chlorinated hydrocarbons, Dallas, Tex., Feb. 23, 1965. (Wilson.)

MISCELLANEOUS ACTIVITIES

The annual marine exhibit for area schools was held in April when P. A. Butler gave 35 lectures on marine biology to about 5,700 students visiting the Laboratory in the 10-day period. Also during the fiscal year, he gave eight lectures on marine biology to about 400 student officers in the Naval Air Training Command, and one lecture on vocational guidance at the high school; held a science seminar on oceanography for superior high school students; discussed the Laboratory program at meetings of two Chambers of Commerce; and served as judge at a local Science Fair.

Staff members assembled collections of 44 marine animals from the local estuary and presented them to the science department of 12 area junior high schools. The specimens were labeled, and information given as to each animal's classification and habits.

Figure 1.--High school science class attending a demonstration of local marine fauna. One-hour lectures are given each spring for area schools. Annual attendance for the 2 weeks is over 5,000.

PESTICIDE PROGRAM, RESEARCH PROJECTS

LABORATORY BIOASSAYS

Jack I. Lowe, Project Leader

Screening of new pesticidal chemicals is a continuing function of the program as manufacturers of pesticides provide us with additional samples of their products. Each compound received is evaluated in short-term bioassays on oysters, shrimp, and fish. During the past 4 yr. (years) we have screened most of the commonly used insecticides, fungicides, and herbicides. Certain candidate materials are selected for more intensive study in chronic-toxicity experiments.

The development of uniform bioassay techniques has been an important part of our laboratory research. Currently, all of the acute toxicity tests are conducted in flowing sea water. Separate populations of test animals are exposed to several concentrations of each pesticide, and 48- or 96-hr. (hour) median

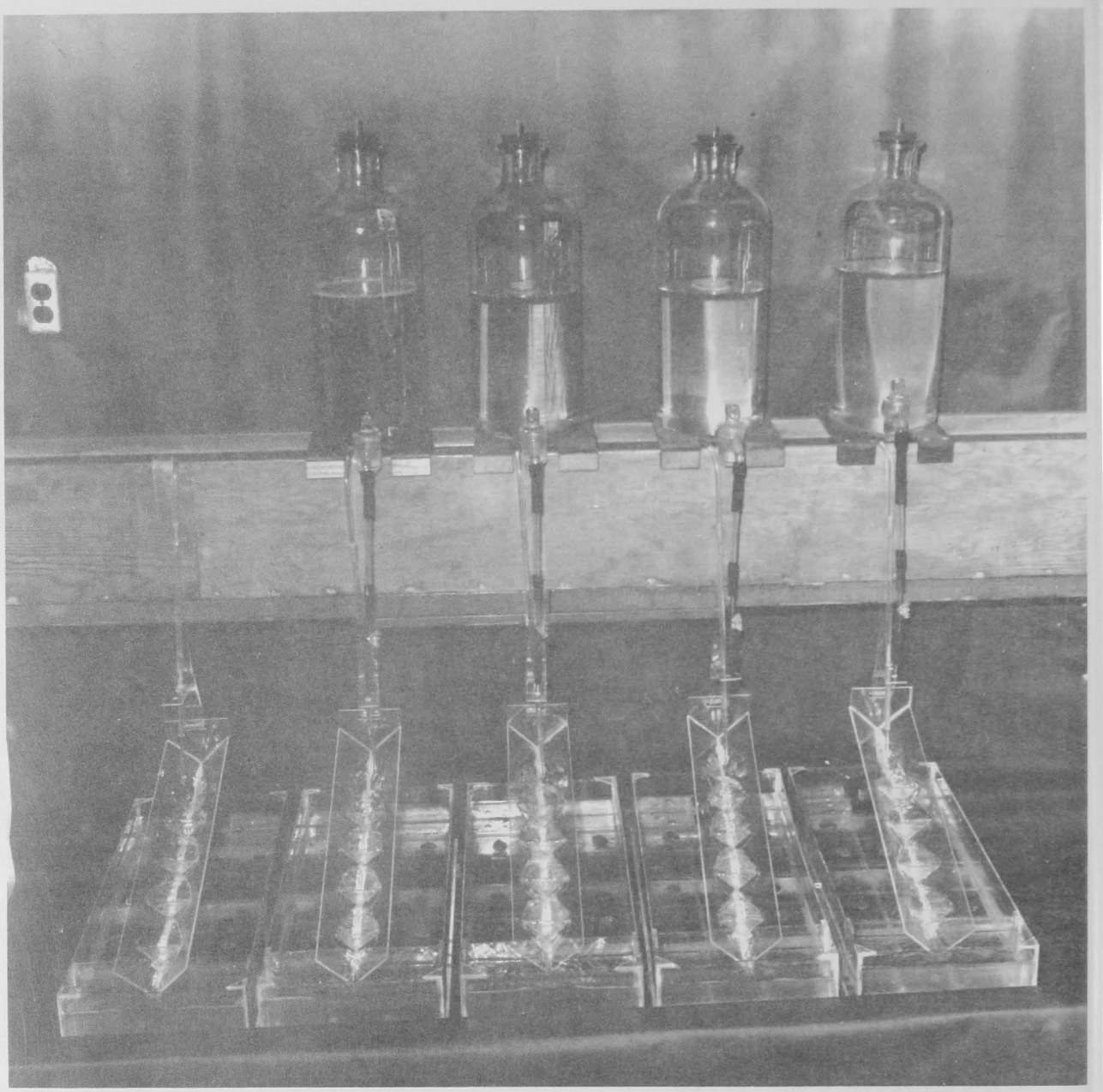

Figure 2.--Typical screening test to evaluate effects of a pesticide on oysters. Four dilutions of the pollutant are dripped into trays of oysters supplied with running sea water. Control oysters are in left-hand tray.

toxicity values are determined. Since various phyla of marine organisms are used as bioassay animals, biological response is diverse. Reduction of shell growth, loss of equilibrium, and death are used as criteria of toxicity in oysters, shrimp, and fish, respectively.

Short-term bioassays have shown that many of the commonly used pesticides are very toxic to estuarine organisms. Concentrations as low as 1.0 p.p.m. (parts per million, milligrams per liter) may cause a significant mortality within a short period under experimental conditions. Since the pollution level of pesticides in estuaries is likely to be less than this but may be persistent, the effects on estuarine fauna of long-term exposures to low levels are important.

Several studies were completed during the year, and others are in progress to determine some of these chronic effects of pollution. Commercially important species of fish and crustaceans are used as bioassay animals. We perform these experiments in a laboratory where flowing sea water is available for continuous-flow systems.

In one study, a population of juvenile spot, Leiostomus xanthurus, was reared to sexual maturity in sea water containing a sublethal

concentration of 0.05 p.p.b. (parts per billion, micrograms per liter) of the insecticide, endrin. The fish exhibited no symptoms of poisoning during 8 mo. (months) exposure in the endrin-polluted water. Mortality and growth rates were about the same as in a similar group of control fish. In preliminary screening tests, endrin was lethal to spot at a concentration of only 0.1 p.p.b. after 5 days of continuous exposure.

This critical threshold of sensitivity was further evaluated by histological examination of pesticide-exposed fish. We found no pathology in spot exposed to 0.05 p.p.b. of endrin for 7 mo., whereas a 3-wk. (week) exposure to a near-lethal concentration (about 0.075 p.p.b.) produced marked pathology, characterized by systemic lesions in the brain, spinal cord, liver, kidneys, and stomach.

Fish surviving long-term exposures to endrin were not affected by subsequent physiological stresses such as rapid salinity change and extended periods of starvation.

Chronic exposure of fish to low concentrations of chlorinated hydrocarbon insecticides does not increase their tolerance to these chemicals. The fish sometimes appear to become even more sensitive to the insecticide. This is not to say that resistant populations cannot be produced through the selective action of insecticides.

We are now studying the effects on fish of long-term exposure to malathion, an organophosphorus insecticide. A population of spot has survived 16 wk. of continuous exposure to 0.01 p.p.m. of malathion. The 96-hr. LC_{50} (concentration at which 50 percent of the fish died in 96 hr. was 0.22 p.p.m., and 0.05 p.p.m. affected spot after 14 days of continuous exposure. Specimens from the 0.01-p.p.m. exposure are killed every 2 wk. to determine any change in brain cholinesterase levels.

Since insecticides are designed to kill terrestrial arthropods, we are quite concerned about the effects of these chemicals on marine crustaceans such as crabs and shrimp, which are also arthropods. These animals are the basis of valuable commercial fisheries and spend much of their lives in shallow estuarine waters that are occasionally polluted with

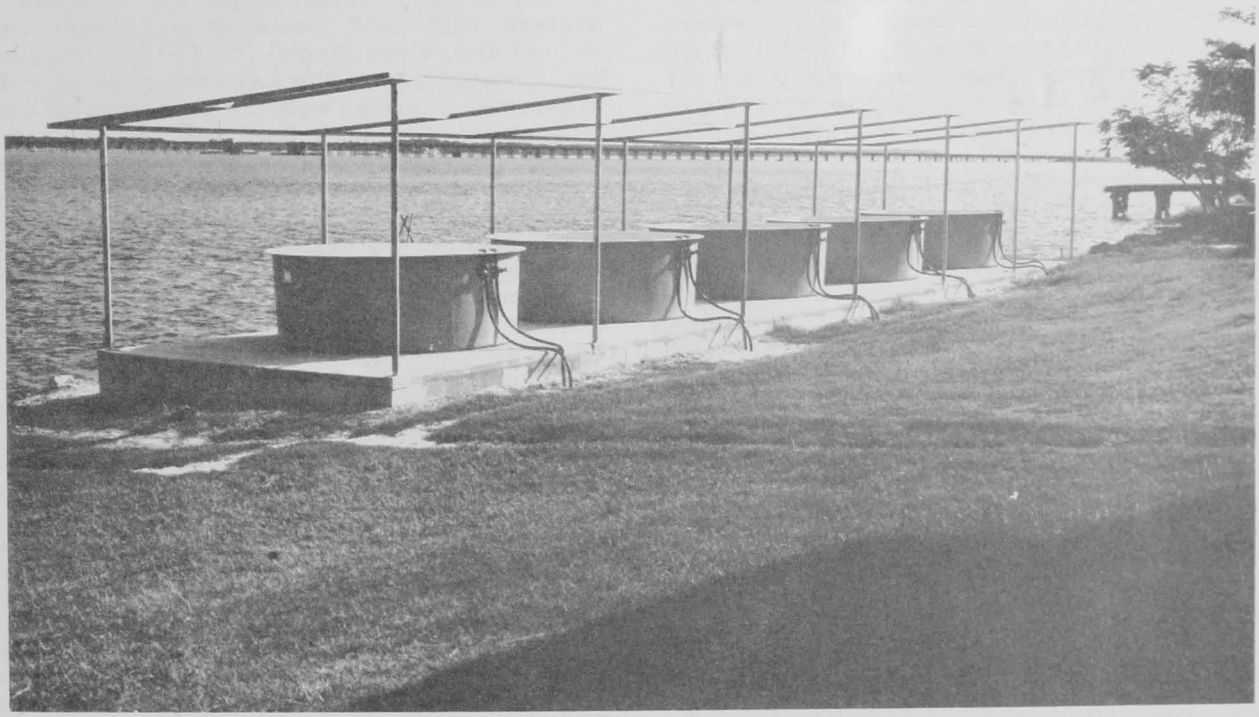

Figure 3.--Fiberglass tanks used for holding shrimp and fish for bioassay projects.

insecticides. Early in our pesticide studies we found that shrimp and crabs were extremely sensitive to most of the chlorinated hydrocarbon insecticides and to many of the newer phosphate compounds.

Studies during the year show that although the crustaceans can tolerate very low concentrations of some insecticides in their environment for extended periods, like fish, they have extremely critical toxicity thresholds. Juvenile blue crabs, Callinectes sapidus, were reared in flowing sea water containing sublethal concentrations of DDT. Crabs fed, molted, and grew for 9 mo. in sea water containing 0.25 p.p.b. of DDT but could survive only a few days in water containing DDT in excess of 0.5 p.p.b. All crabs surviving the DDT exposure molted as frequently as the controls. A small percentage of a population of juvenile brown shrimp, Penaeus aztecus, was able to tolerate a very low concentration (0.025 p.p.b.) of endrin for 60 days; shrimp survive only a few days at an endrin concentration greater than 0.05 p.p.b.

The contamination of shellfish, such as oysters and clams, by pesticides presents problems somewhat different from those encountered with fish and crustaceans. Shellfish are essentially sedentary organisms, unable to move if the surrounding waters become polluted, but they are able to close their shells and protect themselves to a certain degree from toxic substances in the environment. The filter-feeding habit of oysters make them particularly susceptible to toxic substances such as pesticides, which may be adsorbed on particulate matter in the water. These mollusks are able to store in their tissues relatively high concentrations of chemicals that exist in very low concentrations in the surrounding sea water; this is particularly true of the insecticide DDT.

Since oysters are used for human food with little or no processing, we need to know the rate of uptake and retention time of pesticides. Our laboratory studies showed that three species of commercial oysters concentrate many of the commonly used insecticides during a few days exposure to low concentrations. Residues of some of the organochlorine insecticides remain in oyster tissues after 60 days of flushing in unpolluted water but are eventually removed. The organophosphate insecticides, as a group, are much less stable than the chlorinated hydrocarbon compounds, but some may persist for 30 days as residues in oysters that have been removed to clean water.

CHEMICAL ASSAYS

Alfred J. Wilson, Jr., Project Leader

Majority of the assays we made were requested by project leaders engaged in laboratory and field studies at Gulf Breeze. More than 840 samples were analyzed for residues of one or several of the organochlorine insecticides. This is more than a twofold increase over last year. State and Federal agencies requested several analyses. The procurement of a gas-liquid chromatograph greatly increased the precision of our residue analyses.

To initiate a nationwide monitoring program of pesticides in estuarine waters, we undertook studies to: (1) determine the optimal number of animals per sample to reflect changes in the environment, and (2) develop simple methods of preserving fish and shellfish tissues so that they could be shipped unfrozen from the field to the laboratory for residue analysis.

Studies were made to determine the minimum number of animals (shellfish or fish) needed in a sample to obtain statistically reliable data. Shellfish were exposed to DDT under laboratory conditions, and fish known to have significant DDT residues were collected locally. We analyzed individual specimens and small groups. Analyses of pooled lots of 10 individuals provided average data that fell within the range of variation inherent in the precision of the analytical methods.

We felt that the success and continuity of a nationwide monitoring program would be more assured if we could find a method for holding and shipping samples without refrigeration. In exploratory tests, duplicate samples of oysters with DDT residues were preserved in formalin, ethyl alcohol, and isopropyl alcohol. Comparison of the analyses of chemically preserved samples with the refrigerated control samples showed that loss of residues in each preservative was erratic and significant. Samples preserved with the desiccant sodium sulphate had no residue loss in 7 days at room temperatures. This success led us to expose oysters to eight of the common chlorinated hydrocarbon pesticides under laboratory conditions in flowing sea-water aquariums for 10 days. The tissues were then homogenized with anhydrous sodium sulphate and held at room temperature. Subsamples were analyzed at 10-day periods for 30 days. We detected no loss of residues in the 30-day samples as compared with the refrigerated control samples. This procedure has been adopted for shipment of all monitor samples to Gulf Breeze and has resulted in considerable savings in time and money.

Table 1 shows the amount of residual pesticide in p.p.m. found in oyster tissues after 10 days' exposure to the chemicals used in the above study.

Table 1.--Amount of residual pesticides found in oyster tissues after 10 days exposure to certain chemicals, Gulf Breeze, Fla.

Chemical	Exposure concentration	Residue concentration	Biological magnification
	P.p.m.	P.p.m.	
Toxaphene.....	0.05	146.	2920X
Methoxychlor.....	.05	289.	5780X
Lindane.....	.05	3.	60X
Chlordane.....	.01	73.	7300X
Heptachlor.....	.01	176.	17600X
Endrin.....	.001	1.	1000X
Dieldrin.....	.001	1.	1000X
DDT + metabolites.	.001	15.	15000X

MICROBIOASSAYS

Joy F. Morrill, Project Leader

During fiscal year 1964-65, renovation of an existing building provided laboratory space for expanding the pesticide program to include culture and testing of marine micro-organisms. When determining the various effects pesticides may have on commercially important species, we must consider not only immediate effects upon a particular species, but also indirect effects on its food supply and possible effects on its embryological and larval development. The best way at present to evaluate such effects is to observe under laboratory conditions those plants and animals known to be involved in the intricacies of the food web.

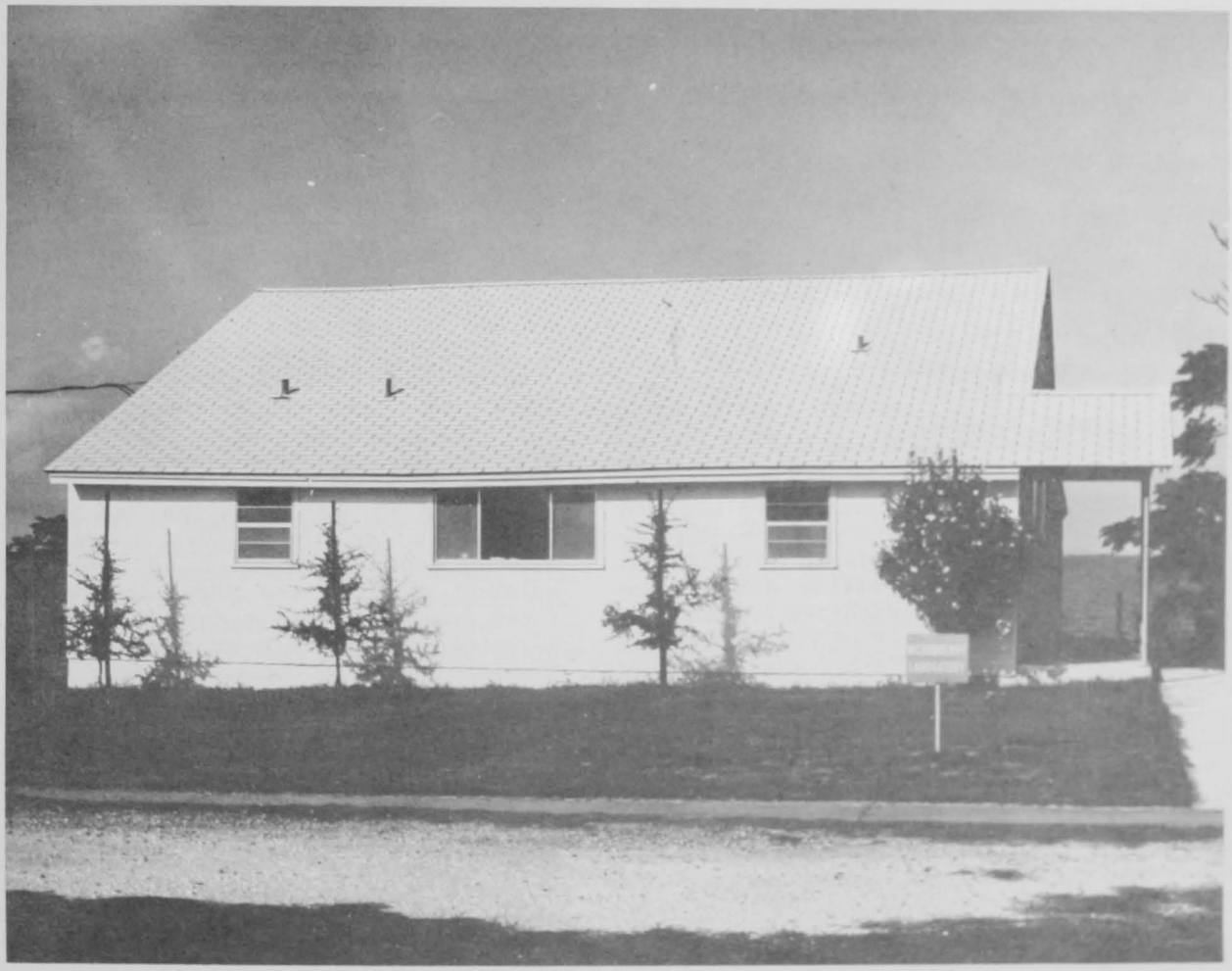

Figure 4.--Recently modernized building that adds 1,600 ft.^{2} of air conditioned work space for the culture of micro-organisms.

Culture of Phytoplankton

Unialgal cultures obtained from collections of the Bureau of Commercial Fisheries Biological Laboratory at Milford, Conn., and from the Woods Hole Oceanographic Institution, Woods Hole, Mass., are maintained in stock. Mass cultures used as food for larvae and for pesticide screening are grown in aerated 5-gal. (gallon) carboys in a constant-temperature cold room. Outside the building, wild natural cultures are grown in tubs filled with standing sea water enriched with commercial fertilizers. Various resultant algal blooms have been the source of certain warm-water species that we feel it advisable to seek. These local estuarine species will be used both for screening pesticides and for feeding oyster larvae. Local warm-water species are especially desirable for the latter purpose, because microalgae now available from culture collections are North Sea isolates, which do not thrive at the temperatures necessary for the growth and setting of oyster larvae in Florida.

Culture of Oyster Larvae

Larvae are obtained by excising gametes or by inducing specially conditioned parent stock oysters to spawn by means of sudden temperature change. Such gametes are easily obtained, and larvae readily develop. Larvae are grown in filtered sea water sterilized with ultraviolet light for use in bioassay tests to determine the effects of common pesticides on their growth and mortality.

Bioassay of Plankton

by Allen A. Wolman

In earlier studies of the effects of pesticides on plankton, natural samples were exposed to serial dilutions of the pesticide in the presence of radioactive carbon ($BaC^{14}O_3$). The amount of labelled carbon used by the phytoplankton could be accurately measured and indicated the toxicity of the pesticides. These tests lasted only 4 hr.--probably too short a period to be valid. Currently, similar samples are distributed among light and dark bottles, and the production of oxygen over a 24-hr. period is measured to evaluate pesticide toxicity. By using appropriate dilutions of the pollutant, it is possible to estimate the amount causing a 50-percent decrease in the production of oxygen during the exposure period (EC_{50}).

We have screened a broad array of pesticides and have made certain generalizations. Curiously, more than half of the herbicides evaluated have EC_{50} values in excess of 1.0 p.p.m., i.e., they are not very toxic. A few are extremely toxic--e.g., the EC_{50} of Ametryne is 0.0016 p.p.m. Some of the fungicides are equally harmful. Casoron, however, is a herbicide that stimulates oxygen production at moderate concentrations. The chlorinated hydrocarbons (dieldrin, DDT, etc.) have EC_{50} values in the range of 0.03 to 0.3 p.p.m. Even with these tests there is some question about what part of the plankton population is being affected. All future tests will be made with pure cultures of algae.

ARTIFICIAL SELECTION OF FISH

Hugh T. Holland III, Project Leader

Genetic resistance to pesticides in insects has been known for many years, but only recently has this aspect of pesticide research been investigated in vertebrates. Workers have developed a DDT-tolerant strain of laboratory mice, and naturally occurring populations of fishes in Mississippi were found to be markedly resistant to many insecticides. These fish populated areas known to be grossly contaminated by insecticides through direct application and agricultural runoff. They continued to produce resistant offspring when transferred to uncontaminated water. The fact that this resistance was shown to persist through three generations, suggests that these fish possessed genotypes which somehow enabled them to survive pesticide concentrations lethal to fish from uncontaminated waters. The selective agent and number of generations required to produce a resistant population are unknown.

Studies were initiated during the year to determine if genetic resistance to pesticides in sheepshead minnows (Cyprinodon variegatus) could be demonstrated by testing the F_1 generation of survivors of DDT concentrations that killed 90 percent of the fish tested. All tests were conducted in 20-l. (liter) (5.3-gal.) plastic aquaria to which an acetone stock solution of DDT was added to give the desired concentration of toxicant. Tap water, adjusted to a salinity of 4 p.p.t. (parts per thousand) with artificial sea salt, was used. Water temperature was 21 $\pm$ 1° C. during the tests. Control groups received acetone only. Adult fish surviving these tests were transferred to breeding pools similar to the natural habitat of this species so that possible pesticide resistance in their young could be investigated.

Offspring of sheepshead minnows that had survived DDT exposure in 1964 were more sensitive to both DDT and endrin than offspring of control fish; however, fish of the F_1 generation of brood stock selected in 1965 were more resistant than controls to the same pesticides. We need continuing studies to

Figure 5.--Application of rotenone to eliminate undesirable fish prior to introduction of resistant breeding stock.

answer the questions arising from these data and to help elucidate the mechanism of pesticide resistance in fishes.

It is possible that the selection of resistant individuals in nature takes place during embryonic development or during larval life. These are periods in the life history of fish when they are particularly vulnerable to an adverse environment, but few studies of this type have been reported.

The effects of various pesticides on hatching of eggs of the longnose killifish (Fundulus similis) were studied by stripping mature fish in the laboratory and placing the fertilized eggs in petri dishes containing different concentrations of DDT, endrin, Dibrom, and malathion. We changed the solutions daily. Hatching was first observed on the 15th day after fertilization, and continued for 1 wk. The pesticides apparently had no effect on hatching but in some instances killed the fry. All fish hatching in the malathion (0.01 p.p.m.) died within 48 hr., as shown in table 2.

Table 2.--Percentage of fertilized eggs of longnose killifish that hatched, and subsequent mortality, in water containing various pesticides, Gulf Breeze, Fla.

Pesticide	Concentration	Hatching rate	Mortality
	P.p.m.	Percent	Percent
Control.....	--	32.5	0
DDT.....	0.01	55.0	0
DDT.....	.001	32.5	0
Endrin.....	.001	40.0	6
Endrin.....	.0001	45.0	6
Dibrom.....	.01	47.5	11
Malathion.....	.01	47.5	100

Figure 6.--Research vessel Dolphin with otter trawl used for sampling fish populations and for collection of bioassay animals used in laboratory screening project.

INDICATOR ORGANISMS - FISH

David J. Hansen, Project Leader

Natural fluctuations in the abundance, feeding, growth, and movements of estuarine fishes are extensive. To determine how much pesticides or other pollutants may alter the life history of a species of fish, we must understand better these natural variations.

This study began in summer 1963. Two of the most abundant species of fish, pinfish (Lagodon rhomboides) in high-salinity areas and Atlantic croaker (Micropogon undulatus) in low-salinity areas, are being studied. Thirty 15-min. (minute) trawl hauls at the two pinfish stations and 10 30-min. hauls at the two croaker stations are being made monthly with a 16-ft. trawl that has 1-in. (inch) stretched mesh.

Mean numbers and volumes of fish per trawl haul varied monthly and yearly. Pinfish were most abundant in July. Because all except the smallest fish from the last spawning leave the estuary in the fall, pinfish are least abundant in the winter. They return to the grass flats in the spring; the newly hatched fish appear to enter the estuary slightly earlier.

Croakers leave the estuary in late summer and early fall and are least abundant during the winter. Very few ever re-enter the estuary. More were caught in the late spring and early summer than at any other time. In winter 1963, young of the year did not appear at the stations sampled until late February, but in 1964 they were found in the upper estuary in December.

A total of 11,000 pinfish were fin clipped this year. Recoveries indicate that once pinfish have entered the estuary and found a suitable grass flat they rarely leave until the fall migration. We tagged 290 pinfish with Atkins tags. Only two have been recaptured. One fish had been at large for 2 mo. and was recaptured within 100 yd. (yards) of the point of release. The other fish was recaptured 5 mo. after tagging during the fall migration and had moved about 2 miles towards the Gulf.

About 3,000 croakers were marked this year, but too few have been recaptured to

permit any conclusions about their movements. Trawling data indicate that young croakers enter the mouth of the bay and move into the upper estuary. As they grow they begin to move down to areas of higher salinity; this causes a "length-stratification" within the estuary. Samples of fish from high- and low-salinity areas have separate distinct peaks in their length-frequency distributions that might lead to erroneous conclusions when aging the fish.

Measurements of 11,800 pinfish indicated that there was very little difference in the monthly average standard lengths of the two year classes at the two stations sampled. One-yr.-old fish averaged about 47 mm. (millimeters) (1 3/4 in.) longer than young of the year. Growth rates were highest in the spring--about 9 mm. (3/8 in.) a month; slowed slightly in the summer to 6 mm. (1/4 in.) a month; and were negative in the fall with an apparent loss of 4 mm. (3/16 in.) a month (caused by the migration of larger fish out of the estuary).

Only 15 of 24,000 croakers captured during this study could be identified as 1-yr.-old fish. When croakers leave the estuary in the fall they average about 100 mm. (4 in.), standard length. The smallest croaker captured was 15 mm. (3/5-in.) long.

The condition factor indicates how heavy a fish is in reference to its length. Condition factors for both pinfish and croakers were highest in the summer and lowest in the winter.

A total of 849 pinfish stomachs and 1,409 croaker stomachs from fish captured this year have been analyzed. Stomachs from fish of each 25-mm. (1-in.) length range were pooled, and percentage by volume of the various food types and mean total volume per stomach were determined. In general, the percentage of each type of food in the stomachs varied with the size of fish and season: In the fall, pinfish stomachs contained relatively fewer crustaceans and more fish and plant material as size increased; plants made up 65 to 100 percent of the total volume of the contents of the stomachs. In the winter, 65 to 100 percent of the total volume was crustaceans; polychaetes and fish ranked next in abundance. Pinfish stomachs were fullest in the summer whereas croaker stomachs contained more food in the spring. Large decreases in mean volume of stomach contents coincided with times of migration of pinfish. Croaker movements to high-salinity areas and mean volumes of stomach contents seem to be related. It is therefore possible that migration is related to decreased food supply or decreased hunger.

Monthly pesticide residue analyses have been made for fish from all four stations. Because of the individual variability in the amount of DDT present in the tissue, we pooled 10 fish for residue analysis. In general, the amount of DDT present is related to the location of the sampling station, age of the fish, and time of year. Throughout the year all resident species of fish sampled in low-salinity areas had DDT residues, which were never higher than 0.27 p.p.m. At the high-salinity stations, DDT residues ranged up to 1.1 p.p.m. for a pooled sample and up to 13.7 p.p.m. for an individual fish. Residues for each species of fish sampled were rarely under 0.25 p.p.m. DDT during this period. In general, 1-yr.-old fish had twice as much DDT in their tissues as the young of the year.

One hundred pinfish and 50 croakers were exposed to 1.0 p.p.b. and 0.1 p.p.b. DDT in running-water aquariums to determine rates of uptake. Similar numbers of each species were held as controls. Results from this experiment indicate that DDT uptake is rapid and a threshold is usually reached after 2 wk. Residues are lost slowly after the fish have been removed from the presence of DDT in solution. After 2 wk., pinfish exposed to 1.0 and 0.1 p.p.b. DDT had residues of about 11.5 and 3.8 p.p.m. DDT, respectively. Further exposure caused no increase of pesticides in their tissue. After removal to clean water, levels dropped to 7.1 p.p.m. in 4 wk. in the group exposed to 1.0 p.p.b., and to 1.1 p.p.m. in 6 wk. in the group exposed to 0.1 p.p.b. Croakers held for 5 wk. at 0.1 p.p.b. had residues of 2.0 p.p.m.; after 6 wk. of recovery the concentration had dropped to 1.4 p.p.m.

ESTUARINE PRODUCTIVITY

Nelson R. Cooley, Project Leader

This project has established hydrographic and biological baselines for the Pensacola Estuary from which we can measure the effects of future environmental changes due to man's activities including pesticide pollution. Hydrographic baselines include monthly changes in salinity, water temperature, pH, and visibility index, and seasonal trends in salinity and water temperature at stations in areas of high, intermediate, and low salinity in the estuary. Biological baselines include studies on primary productivity in high-, intermediate-, and low-salinity areas of the estuary, and a faunal and floral inventory of the estuary.

Hydrography

Monthly observations were made for 2 1/2 yr. Salinity ranged from 11.6 to 32.8 p.p.t.; it was highest in November, fell steadily during the spring rainy season until April, and generally increased thereafter. Water temperature ranged from 4.4° C. in January

to 32° C. in July. Average pH levels, generally ranged between 8.1 and 8.4, were less in winter and early spring than during the rest of the year, and may be related to major seasonal salinity changes. Large-scale destruction of organic matter during dredge-and-fill operations from October 1959 to February 1960 depressed pH levels to 7.8 through April, but pH returned to normal by midsummer 1960. Visibility index was greatest in winter and early spring.

Trends in water temperature and salinity were established by means of seasonal studies of hourly changes in surface and bottom values during a single maximum-amplitude tidal cycle; observations were made simultaneously at stations in areas of high, intermediate, and low salinity in the estuary. Seasonal trends reflect the greater variability of the low- and intermediate-salinity areas, which are shallower and more distant from the Gulf of Mexico than is the high-salinity area.

Primary Productivity

by Nelson R. Cooley

In fortnightly determinations over a 15-mo. period, the standing crop of surface phytoplankton rose to a peak in May and again in July or August before declining to winter levels. We observed no pronounced "blooms." During 6 mo. of study, photosynthesis rates generally declined from highs in December to lows in March. Photosynthesis rates and standing crops of surface phytoplankton in the same water samples were not correlated.

Primary productivity data at surface water collection stations in areas of high, intermediate, and low salinity showed: (1) similar trends in phytoplankton standing crop and photosynthesis rates at both ends of the salinity gradient, although absolute values were generally higher and varied more widely at the station in the low-salinity area; (2) there is only very poor correlation between Chlorophyll A and either salinity, temperature, or photosynthesis rates; and (3) hourly variation in Chlorophyll A values may be as great as fortnightly variation.

by Allen A. Wolman

From September 1964 through May 1965, three water samples were collected from each of 10 stations in Pensacola Bay, at 28-day intervals, to observe trends in primary productivity. Sampling was discontinued at 10 additional stations in Escambia Bay after analysis of data indicated no statistically significant differences between the two areas.

Phytoplankton samples from three photic depths at each station were dispensed into light and dark replicates, and incubated in running sea water at photic intensities of 100, 55, and 19 percent, respectively. After the samples were incubated for 24 hr., we analyzed the dissolved oxygen and converted the results to organic carbon produced at each depth. Station values were calculated from production vs. depth data to give productivity values in grams carbon per meter^2 per day. An average of data for all stations is shown in table 3.

Table 3.--Primary productivity trends in Pensacola Bay, 1964-65

Date	Mean productivity[1]	Surface light intensity
1964	G. C/m.^{2}/day[2]	Foot-candles[2]
Oct. 12.....	0.066	3,060
Nov. 4.....	.094	2,940
Dec. 7.....	.150	3,030
Dec. 29.....	.053	1,950
1965		
Jan. 25.....	.054	2,505
Feb. 23.....	.045	1,815
Mar. 23.....	.178	1,324
Apr. 10.....	.199	4,020
May 24.....	.158	3,975

[1] Mean of all samples from three photic depths of 100, 55, and 19 percent at each of 10 stations sampled in random order on the same day.

[2] Gram, carbon per square meter per day.

Faunal Inventory

by Nelson R. Cooley

This study is a first attempt to conduct a systematic inventory of the Pensacola Estuary, to establish a checklist of species occurring there, and to assemble information on their seasonal occurrence, abundance, and habitats.

Standard seasonal samples of pelagic and bottom species were collected at stations in areas of low, intermediate, and high salinity in the estuary. Sampling devices used were: a 16-ft. otter trawl; an Ekman dredge[1] for use on deep water mud bottoms; a 0.1-m^2. frame with scoop for use on shallow-water sand bottoms; and sand-filled wooden protected-habitat boxes with hardware-cloth covers, placed on the bottom near stations with a sandy bottom, for collection of rare species not usually found because of predation. We took these samples during the same period that seasonal water samples were collected so that the changes in the population densities could be

[1] Trade names referred to in this publication do not imply endorsement of commercial products.

correlated with seasonal trends in salinity and water temperature.

A checklist of fauna in the Pensacola Estuary has been compiled from specimens collected during this study and in earlier years. The list includes 603 species in 327+ genera and 249+ families. Chief animals in the estuary are polychaete annelid worms, gastropod and pelecypod mollusks, crustaceans, and fishes. The numbers and kinds of animals are much greater at the high-salinity end of the estuary than elsewhere. Greater numbers and kinds of animals, especially fishes, appeared in spring and summer than in fall and winter.

Inventory of Area Marine Plants

by Joy F. Morrill

To establish the extent of change in an environment, whether due to pesticides, industrial pollution, or natural population variation, we must first learn which species exist in an area at some given time. This survey was made because no earlier work had been done here with seaweeds or with higher marine plants. At present it is possible to report 181 species, with notes concerning their relative abundance, time of year in which they are present, reproductive season when known, and substratum.

Examples of each species are stored as dried herbarium specimens, microscope slides, and wet-stack preservations, so that data and specimens are available for future study. Of the algae examined, about 20 percent were blue-greens, 18 percent chrysophytans, 20 percent greens, 14 percent browns, and 28 percent reds. Five genera of marine grasses are found in this area.

USE OF PESTICIDES TO CONTROL PREDATOR POPULATIONS

Philip A. Butler, Project Leader

Research at the Bureau of Commercial Fisheries Biological Laboratory, Milford, Conn., demonstrated the usefulness of Polystream, a mixture of chlorinated benzenes, in the control of snail predators on New England oyster beds. Since similar snails cause serious damage to Gulf oysters, we undertook studies to evaluate the usefulness of this chemical here.

With the cooperation of the University of Alabama and the Alabama Department of Conservation, plantings of reef oysters and oareshell cultch were made on 10 quarter-acre plots in Mobile and Pensacola Bays. The two areas selected were suitable for oysters but had none because of the snails present. The Pensacola area has a sand bottom, and the Mobile Bay area had a mud bottom.

Preliminary tests in the laboratory showed that other marine animals were unaffected by application of the pesticide at the recommended rate. The plots of oysters and shells received a single treatment in July; suitable plots were left untreated as controls. During the remainder of the summer and in the following spring, biological samples were removed from the plots at regular intervals.

The results in both areas were similar and essentially negative. The treatment had no effect in controlling drills or causing any other observable effect on the population dynamics of any of the bottom fauna. We believe that there was sufficient siltation in both areas to cover the deposit of the granular pesticide and render it ineffective. The project was terminated, and a final report is being prepared.

POPULATION DYNAMICS OF SEDENTARY FAUNA

Philip A. Butler, Project Leader (Acting)

Major fluctuations in the population density of estuarine forms are frequently noted, but the minor changes occurring seasonally and perhaps in longer cycles are difficult to identify. We have found that the setting index of some sedentary animals, including protozoa, polychaete worms, hydroids, bryozoa, mussels, oysters, and barnacles provides an objective criterion of population fluctuations.

Consequently, as a continuing project, and for the past 15 yr., such data have been recorded at 7-day intervals at the Laboratory island in conjunction with continuous records of changes in salinity, air and water temperature, tides, and precipitation.

We anticipate that with these records as a foundation, we will be able to document population changes and explain their causes as being due to "normal" cycles or as resulting from physical changes in the environment or chemical changes caused by domestic, industrial, or pesticidal pollution.

FISH PHYSIOLOGY

Hugh T. Holland III, Project Leader

Though use of organophosphorus pesticides has increased in recent years, development of techniques for the detection of residues of this group of chemicals has lagged. Methods are available, but their low sensitivity (generally about 0.1 p.p.m.) and time-consuming cleanup procedures (which vary for each compound) make them impractical for routine laboratory analyses. On the other hand, enzymatic methods based on the in vivo inhibition of fish brain acetylcholinesterase (AChE) have

a sensitivity of less than 0.001 p.p.m., and significant decreases in enzyme activity have been demonstrated within a few hours at concentrations of 0.01 p.p.m. In addition, regeneration of normal AChE levels following sublethal exposure occurs slowly, thus making it possible to detect not only current but past pollution.

The feasibility of using assay of AChE levels in fish brain as a means of measuring organic phosphate pollution is being studied. The method used for assay of enzyme activity is as follows: after the brain is excised and weighed, the tissue is homogenized in a phosphate buffer. The homogenate is then incubated with acetylcholine (ACh), and the residual ACh is determined colorimetrically. Activity is reported as micromoles of ACh hydrolyzed per mg. of brain tissue per hr.

During the year, work was directed toward establishment of normal AChE activity for several common estuarine fishes. Our data show that there is a decrease in activity per unit weight as the brain weight increases, but variation in a species for a particular brain weight is generally less than 10 percent. Consequently, normal enzyme activity can be estimated from the brain weight, and deviations (expressed as percentage of normal) are indicative of pollution.

The effects of various organophosphorus pesticides on fish brain AChE were studied by exposing fishes in running sea water. One of these chemicals, phorate, significantly inhibited fish brain enzyme within 48 hr. at concentrations of 0.0005 p.p.m., and several others had similar effects at a concentration of 0.001 p.p.m. We found that a particular pesticide may be species specific. For example, Guthion reduced the brain AChE activity of sheepshead minnows by 90 percent in 24 hr. at a concentration of 0.01 p.p.m., but had no significant effect on spot under the same conditions. Recovery to normal levels following exposure was erratic and varied with species, pesticide, concentration, and length of exposure. In general, recovery required periods in excess of 1 wk.

Studies to determine if AChE activity for a particular species varies with the time of year or locality are incomplete, but it appears that no significant changes occur in the species examined. It may be possible, therefore, to use one species to measure organophosphorus pollution over the entire geographical range of the fish.

PESTICIDE POLLUTION IN ESTUARIES

Philip A. Butler, Project Leader (Acting)

It was apparent from laboratory experiments that to detect the low levels of pesticide pollution capable of causing harm in the environment, biological monitors could be more instructive than chemical tests. Various species of the native fauna were periodically examined for pesticide residues. We found in the course of repeated monthly examinations in a relatively unpolluted estuary such as Pensacola Bay, that plankton, mollusks, and fish more often than not had chlorinated hydrocarbon pesticide residues, and that these residues fluctuated, apparently erratically. In the laboratory under controlled conditions, oysters and mussels were found to be much more efficient in storing residues than clams, gastropods, crabs, shrimp, and fish. Oysters stored pesticides at levels proportionate to the amount present in the environment, and these residues were flushed out when the environment was "clean." Casual samples of fish and oysters from other coastal areas indicated that residues of the chlorinated hydrocarbon pesticides might be widely but unpredictably distributed.

On the basis of these facts, it became obvious that a nationwide coastal survey was essential to assess the extent of this pollution. We needed to know its seasonal occurrence and centers of concentration so that its sources could be identified and, if possible, eliminated. In view of the magnitude of the problem from the point of view of manpower and our lack of knowledge of potentially critical areas along the coast, we called upon State and private agencies to assist in evaluating the problem.

To ensure success of a coastwide survey, it seemed necessary that uniform sampling methods be adopted, that the samples be collected at regular intervals, that they be chemically analyzed by one agency to ensure comparability of data and, finally, that the program be financially sound so as to ensure participation by well-trained personnel and sufficient continuity to permit review of the data with some perspective.

We have devised a program that meets these criteria, methodology has been standardized and is sufficiently simplified to avoid technical difficulties, and contracts were in effect at the close of the year with State and private agencies in Virginia, South Carolina, Mississippi, Texas, California, and Washington to collect the necessary samples and send them to our Laboratory for analysis. In addition, Federal Laboratories in Alaska, Michigan, and Florida are cooperating in the program.

We anticipate that agreements will soon be reached with additional State and Federal Laboratories so that coastal surveillance of pesticide residues will extend from Maine to Alaska. We believe that such data will be informative in pinpointing trouble areas after 12 mo., but we feel the program must continue for several years before we can assess the true importance of the problem of pesticide pollution in coastal estuaries.

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