REPORT OF THE BUREAU OF COMMERCIAL FISHERIES BIOLOGICAL LABORATORY, GULF BREEZE, FLORIDA

Fiscal Year 1966

UNITED STATES DEPARTMENT OF THE INTERIOR

FISH AND WILDLIFE SERVICE

BUREAU OF COMMERCIAL FISHERIES

Circular 260

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

Fiscal Year 1966

Philip A. Butler, Director
Robert F. Johnson, Assistant Director

Contribution No. 73, Bureau of Commercial Fisheries
Biological Laboratory, Gulf Breeze, Fla. 32561

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Cover illustration: An aerial view of the Biological Laboratory located on Government-owned Sabine Island, an artificial ballast rock island in Santa Rosa Sound, 8 miles south of Pensacola, Fla. The Gulf of Mexico is visible in the distance.

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

Fiscal Year 1966

ABSTRACT

The program of the Laboratory has been concerned since 1958 with evaluating the effects of pesticides--primarily synthetic organic chemicals--on marine plants and animals. Projects are designed to determine existing pollution levels, concentrations causing acute and chronic effects, and the translocation of residues in the biota and environment.

REPORT OF THE DIRECTOR

HIGHLIGHTS OF THE YEAR IN REVIEW

The appointment of Robert F. Johnson as Assistant Laboratory Director and Program Leader enabled the Director to devote additional time to developing the national program to monitor organochlorine pesticide pollution in estuarine fish and shellfish populations. From a modest beginning of 20 stations in two States, the program now covers a major part of our Atlantic, Gulf, and Pacific coasts. Samples are collected monthly at more than 150 stations in 13 States and sent to this laboratory for analysis.

The techniques for identifying organophosphorus pesticide pollution in fish have been standardized, and nine stations on the Atlantic and Gulf Coasts are routinely monitored.

Areal and seasonal inventories of local estuarine fauna have been completed, and final reports are in preparation.

A 2-yr. (year) study of the population dynamics of two species of fish is complete, and the final report is in preparation.

Studies of the effects of pesticides on marine bacteria and the metabolism of pesticides by bacteria have been initiated.

Laboratory facilities have been remodeled to provide a 300-square-foot laboratory for studies with radioactive pesticides; eight plastic holding tanks with a total volume of 16,000 gal. (gallons) have been installed for the bioassay program.

PUBLIC RELATIONS

The laboratory does not maintain public exhibits or a museum. To inform the general public of the laboratory program and to provide appropriate scientific information on local marine biology to area school students, the laboratory holds an open house for 2 wk. (weeks) each spring. As many kinds of fish and invertebrates as possible are collected from the local estuary and displayed in tanks of flowing sea-water. The relationships of the animals to each other and to man are explained in a 1-hr. (hour) lecture and demonstration. Classes have from 25 to 300 students; in the 1966 series, about 7,000 attended. These included about 5,000 students from the local grade and high schools, 800 Scouts, 350 college students (biology classes from a college

Figure 1.--Students have opportunity to "feel" marine animals at annual exhibit.

in Alabama and one in Illinois regularly schedule field trips to include this exhibit in their itinerary), and about 800 area adults (fig. 1).

During the year Bureau biologists Butler, Cooley, and Johnson presented lectures on oceanography and the laboratory program to eight local schools and civil groups. Staff members Cooley and Morrill served as judges at the Regional Science Fair.

TRAINING

Philip A. Butler attended the U.S. Civil Service Commission's Executive Seminar "Skills and Goals of Management" at Kings Point, New York.

Jack I. Lowe attended a course on Management for Supervisors at the BCF Technological Laboratory at Pascagoula, Miss.

James J. Barklow and Hugh T. Holland attended a course in The Use of Radioisotopes in Research at Oak Ridge, Tenn.

Chemists from two laboratories visited the laboratory at Gulf Breeze for training in and discussion of our gas-chromatography analytical methods.

Seminars are scheduled weekly throughout the year to enable the staff to keep abreast of current scientific literature and to discuss the progress of our own research. These seminars are part of the program to train our staff in the presentation of scientific reports and enable visiting scientists to discuss their programs with us.

MEETINGS

The indicated staff members presented research reports at the following:

NATO Advanced Study Institute, Monks Wood, England, July (Butler).

Combined Gulf and Atlantic States Marine Fisheries Commission Meeting, Miami, Fla., October (Bulter).

American Fisheries Society, Tulsa, Okla., October (Lowe).

American Fisheries Advisory Committee, San Pedro, Calif., October (Butler).

Florida Academy of Science, St. Petersburg, Fla., March (Johnson).

31st North American Wildlife and Natural Resources Conference, Pittsburg, Pa., March (Butler).

Interagency (Florida) Pesticide Research Meeting, Jacksonville, Fla., April (Butler).

The indicated staff members attended the following:

Gulf and Caribbean Fisheries Institute, Miami, Fla., November (Johnson).

Sea Nettle Research Conference, Chesapeake Bay Institute, Solomons Island, Md., December (Butler).

Pesticides Research Conference, University of California, Berkeley, Calif., October (Butler).

Conference on Pollution and Marine Ecology, Galveston, Tex., March (Butler).

PUBLICATIONS

Papers Published

BCF Laboratory, Gulf Breeze, Fla. Contribution No.

56 — 1965. Lowe, Jack I. Chronic exposure of blue crabs, Callinectes sapidus, to sublethal concentrations of DDT. Ecology 46(6): 899-900.

58 — 1966. Holland, Hugh T., David L. Coppage, and Philip A. Butler. Increased sensitivity to pesticides in sheepshead minnows. Trans. Amer. Fish. Soc. 95(1): 110-112.

59 — 1965. Butler, Philip A. Book Review. THE OYSTERS OF LOC MARIAQUER. By Eleanor Clark. Pantheon Books, A Division of Random House, New York. 1964. 203 p., illus. Trans. Amer. Fish. Soc. 94(3): 283-284.

60 — 1965. Butler, Philip A. The effects of pesticides on fish and wildlife, Commercial Fishery Investigations. U.S. Fish Wildl. Serv., Circ. 226: 65-77.

61 — 1966. Butler, Philip A. Pesticides in the marine environment. J. Appl. Ecol. 3: 253-259, Suppl.

62 — 1966. Staff. Editor, Philip A. Butler. Annual report of the Bureau of Commercial Fisheries Biological Laboratory, Gulf Breeze, Florida for the fiscal year ending June 30, 1965. U.S. Fish Wildl. Serv., Circ. 247: 15 p.

69 — 1966. Butler, Philip A. Book Review. THE LIFE OF FISHES. By N. B. Marshall. The World Natural History Series published by The World Publishing Co., 402 p. Pensacola News-Journal, June 12, 1966.

Manuscripts Approved or in Press

BCF Laboratory, Gulf Breeze Contribution No.

63 Lowe, Jack I. Some effects of endrin on estuarine fishes. Proc. Southeastern Game Fish Comm., 1965. (12 Ms. p.)

65 Forbes, Milton L. Generic differences in prodissoconchs of Gulf of Mexico oysters. Bull. Mar. Sci. (17 Ms. p.)

67 Butler, Philip A. Fixation of DDT in estuaries. Trans. 31st N. Amer. Wildl. Natur. Resourc. Conf. (11 Ms. p.)

68 Holland, H. T. and Jack I. Lowe. Malathion: chronic effects on estuarine fish. Mosquito News. (8 Ms. p.)

70 Butler, Philip A. Bureau of Commercial Fisheries pesticide monitoring program: a progress report. Nat. Shellfish. Ass., June 1966. (8 Ms. p.)

STAFF

Philip A. Butler, Director
Robert F. Johnson, Assistant 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:

Robert F. Johnson, Program Leader
James J. Barklow, Jr., Biological Technician
Nelson R. Cooley, Fishery Biologist
David L. Coppage, Fishery Biologist
Jerrold Forester, Physical Science Aid
David J. Hansen, Fishery Biologist
Hugh T. Holland III, Fishery Biologist
James M. Keltner, Jr., Biological Technician
Johnnie Knight, Biological Aid
Jack I. Lowe, Fishery Biologist
Alan J. Rick, Fishery Biologist
Donald C. Speed, Fishery Biologist
Alfred J. Wilson, Physical Science Technician.
Paul D. Wilson, Biological Technician
Robert R. W. Witte, Fishery Biologist

Resignations:

Joy F. Morrill, Fishery Biologist

Cooperative Program, University of South Florida:

Frank N. Darby III, Biological Aid
Russell J. Watrous, Biological Aid

Part-time Employees:

Henry T. Meredith III, Biological Aid
Jerry H. Sansom, Biological Aid

Summer Employees:

1965

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

1966

Thomas Glenn, Jr., Student Aid
Betty Anne Odom, Conservation Aid
Thomas S. Whitaker, Student Aid
Carl F. Whitaker, Student Aid

Figure 2.--Before and after pictures of the laboratory island showing results of landscaping by college students employed in the President's Youth Opportunity Campaign.

LABORATORY BIOASSAYS

Jack I. Lowe, Project Leader

Laboratory bioassays of commercial pesticides continued during the year with major emphasis on the chronic exposure of marine fish and crustaceans to selected compounds. We continued to use oysters, shrimp, fish, and pure cultures of marine phytoplankton as bioassay organisms for studies of acute toxicity, at 48- or 96-hr. exposures.

New pesticides screened included herbicides, fungicides, acaricides, and a few new insecticides. Most of the commonly used insecticides have been tested in previous years. The insecticides, as a group, are more acutely toxic to marine animals than are the other categories of pesticides. There are exceptions, however. Some of the newer herbicides and fungicides are similar in chemical structure to the insecticides and are extremely toxic to certain marine organisms.

We completed a 6-mo. (month) study of the effects of Malathion,[1] an organophosphorus insecticide, on juvenile spot, Leiostomus xanthurus. Length of exposure is critical in determining a sublethal concentration; in earlier experiments 50 p.p.b. (parts per billion, micrograms per liter) Malathion killed juvenile spot only after 14 days of continuous exposure. In the present experiment, the fish were continuously exposed for 26 wk. to 10.0 p.p.b. of Malathion in flowing sea water. The fish had no symptoms of distress during the chronic exposure and grew as much as the control fish. Since Malathion is a known inhibitor of the brain-enzyme cholinesterase, specimens were killed and examined every 2-wk. to determine the amount of enzyme activity. Brain cholinesterase was significantly lower in the experimental group than in controls throughout the experiment, and appeared to stabilize at about 70 percent of normal. This degree of inhibition appeared to have no adverse effect on the fish. One wk. after termination of the chronic exposure, the fish had regenerated near-normal levels of enzyme.

Fish that survived the chronic exposure to Malathion were subjected to a rapid drop in salinity to determine any change in their ability to adapt to this physiological stress. We observed no differences in reactions between control and experimental fish when the two groups were transferred directly from water with a salinity of 28 p.p.t. (parts per thousand) to water of 15 p.p.t. salinity. A gradual reduction to 9 p.p.t. caused distress behavior in both groups, but no fish died in 48 hr.

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

Longnose killifish, Fundulus similis, served as bioassay animals in another experiment to determine the effects on reproduction of long-term exposure to endrin, a chlorinated hydrocarbon insecticide. In a previous experiment we exposed spot to endrin but no observations could be made on reproduction because these fish spawn offshore.

The experimental killifish, after 6 months of continuous exposure to a sublethal concentration (0.10 p.p.b.) of endrin in flowing sea water, were divided into two groups and placed in large outdoor tanks and field pools. We divided a group of control fish in the same manner. Whole-body analyses (by gas chromatography) of a sample of the endrin-exposed fish revealed a residue of 92.0 p.p.b. endrin. The fish were lost from the field pools when high water inundated the area. The control and experimental fish in the outdoor tanks have not spawned, but the males have the characteristic breeding coloration. A small number of eggs were stripped from the endrin-exposed females and fertilized artifically with sperm from an endrin-exposed male. A few of the eggs hatched into apparently normal young.

We have exposed marine fish chronically to chlorinated hydrocarbon and organophosphorus insecticides, but only acute toxicity tests have been completed with the carbamate insecticides. A study is now in progress to determine how spot are affected by chronic exposure to the carbamate, Sevin® (carbaryl), a commonly used insecticide. The U.S. Department of Agriculture has recently approved its use in control of adult mosquitos. The toxicity of Sevin® to fish is relatively low compared to most of the chlorinated hydrocarbon insecticides. In the present experiment, juvenile spot have tolerated 100.0 p.p.b. of Sevin® in their environment for 3 months. Mortality has been about the same for control and experimental fish. This exposure will be continued at least another month, and then specimens of control and experimental fish will be examined for pathology and possible changes in enzyme levels. Surviving fish will be subjected to various physiological stresses.

Acute toxicity tests show that many of the organophosphorus insecticides are extremely toxic to marine crustaceans. To provide information on the effects of chronic exposure, we are exposing blue crabs, Callinectes sapidus, to two new phosphate insecticides, Abate and Dursban. Both of these compounds show such promise as mosquito larvicides that they may find their way into marine environments. Separate populations of crabs are being exposed to 1.0 p.p.b. Abate and Dursban in flowing sea water. Both insecticides are acutely toxic to crabs at a concentration of

10.0 p.p.b. and above. In addition to making observations on growth and frequency of molting, we hope to compare cholinesterase levels of experimental and control crabs.

CHEMICAL ASSAYS

Alfred J. Wilson, Jr., Project Leader

The laboratory analyzed 1,638 samples for residues of the commonly occurring chlorinated hydrocarbon insecticides (fig. 3)--aldrin, BHC, DDT, dieldrin, endrin, heptachlor, lindane, and methoxychlor. This twofold increase in samples over last year was due primarily to the nationwide monitoring program of the Laboratory at Gulf Breeze; 1,067 of the samples came from various stations along the Atlantic, Gulf, and Pacific coasts.

Because DDT and its metabolites have been found in most of the oyster samples from monitor stations, laboratory experiments were done to obtain information concerning its metabolism and uptake in oysters.

Eastern oysters (Crassostrea virginica) were exposed in separate running-water aquaria for 5 days to 0.5 p.p.b. of p,p' DDT; o,p' DDT; p,p' DDE; and p,p' DDD. Residue analysis of the tissues after exposure showed that the only significant conversion was the dehydrochlorination of p,p' DDT to DDE. Analyses were repeated on the above animals after flushing in an unpolluted environment for 6 days. Results were similar.

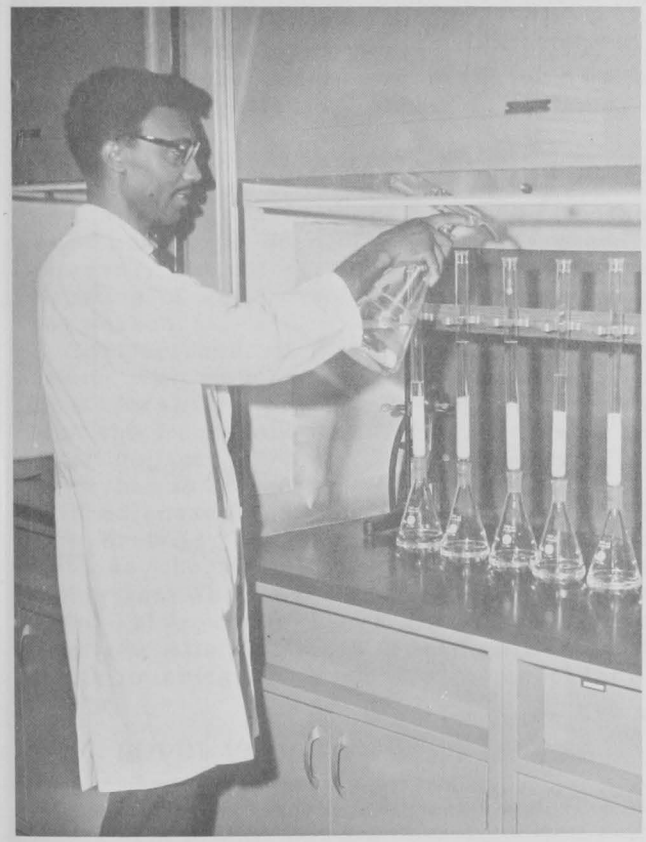

Figure 3.--The analysis of pesticide residues requires meticulous preliminary "cleanup" or removal of extraneous material from the samples.

Table 1.--DDT residues in oysters exposed for 20 days to DDT at two different concentrations, Gulf Breeze, Fla.

Exposure Concentration of DDT	RESIDUES IN OYSTERS		
	DDE	DDD	DDT
P.p.b.	P.p.m.	P.p.m.	P.p.m.
Control.....	0.017	0.020	<0.010
0.01.....	.044	.034	.11
0.001.....	.045	.022	.085

In a second series of experiments, groups of oysters were exposed to 0.01 and 0.001 p.p.b. of DDT for 20 days in running-water aquaria. Table 1 shows the residual DDT and metabolites in parts per million (milligrams per kilogram) that were detected in the tissue by electron capture gas-liquid chromatography after 20 days of exposure at the indicated concentrations. Each analysis was made on a pooled sample of eight animals. During the test, we analyzed the residues in the control group at 0-, 5-, and 10-day intervals to observe fluctuations in DDT levels due to any naturally occurring pollution in the water supply pumped from Santa Rosa Sound. Residue levels fluctuated less than 0.003 p.p.m. (parts per million) at the four sampling intervals.

The studies and experiments in progress will assist in the evaluation of the data being accumulated from our monitoring program.

We had the unusual opportunity during the year to sample two dead porpoises, which represent one of the final links in a marine food chain. Various tissues were analysed for pesticides. Considerable amounts were found, including more than 200 p.p.m. of DDT and its metabolites in the blubber of both animals and 0.05 to 2.0 p.p.m. of residues of dieldrin and endrin.

FAUNAL INVENTORY

Nelson R. Cooley, Project Leader

This study, which ended with the fiscal year, was a first attempt to systematically inventory the marine fauna in the Pensacola Estuary; to establish a checklist of species found there; and to assemble information on their seasonal occurrence, abundance, and habitats.

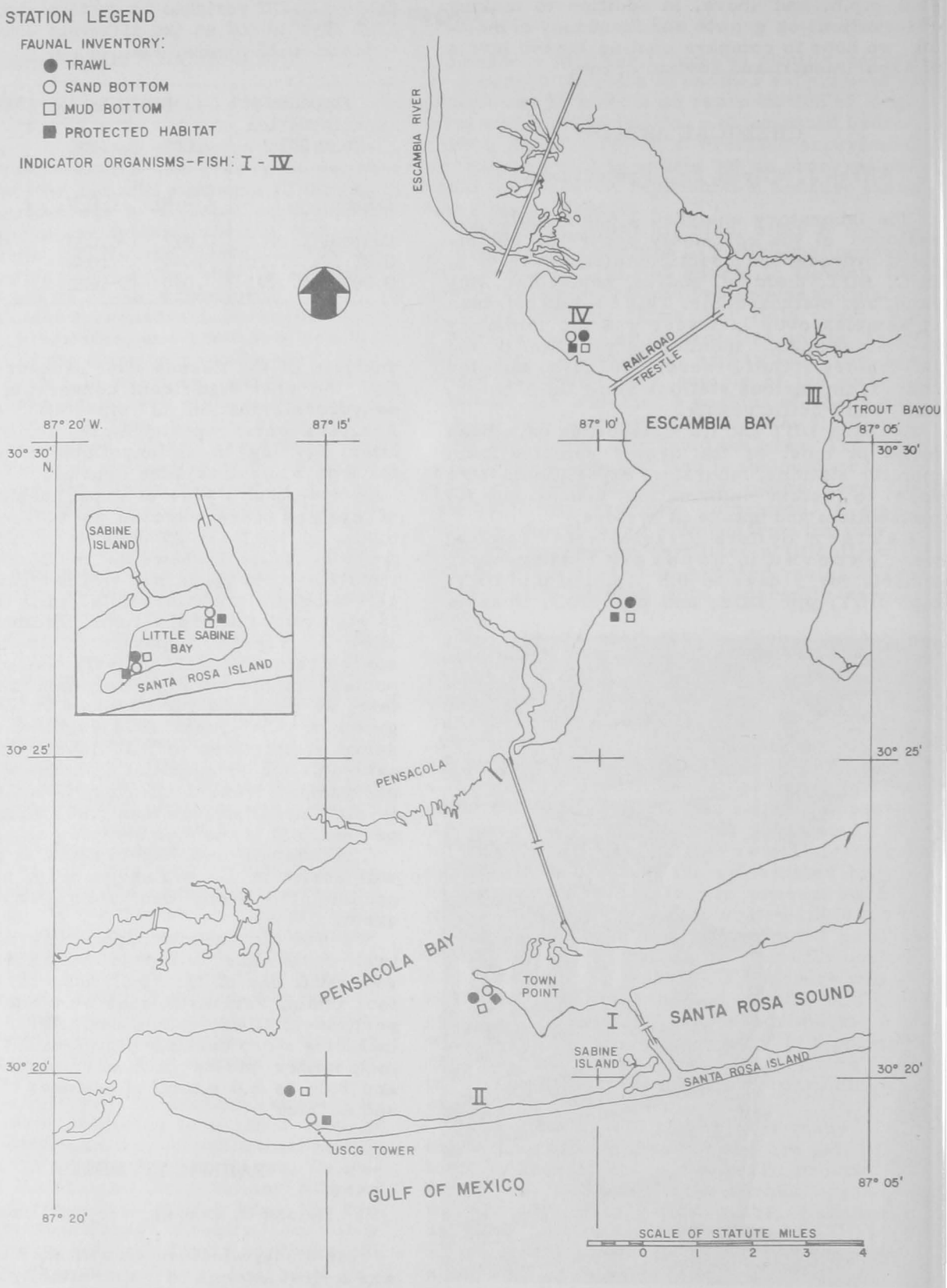

Figure 4.--Locations of sampling stations in the Pensacola Estuary for Faunal Inventory Study (symbols) and Population Dynamics of Fish (I-IV).

Standardized seasonal biological samples of pelagic and bottom animals were collected at stations established in low-, intermediate-, and high-salinity areas of the estuary. To permit better comparison with seasonal trends in salinity and water temperature, biological samples were collected when the seasonal hydrographic studies were made. A seasonal hydrographic study determined the hourly changes in surface and bottom water temperature and salinity during a single maximum-amplitude tidal cycle and was made simultaneously at all stations.

A checklist of Pensacola estuarine fauna compiled from identified specimens collected during this study and in other years includes 665 species in 469 genera and 269 families. Chief animals in the estuary are polychaete worms, gastropod and pelecypod mollusks, crustaceans, and fishes. 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.

Their preferences for certain substrates influenced the distribution of many bottom invertebrates. Most mollusks and many polychaetes occurred in sand and sandy mud. Grass beds harbored small shrimps and crabs. Submerged rocks provided attachment sites for barnacles, oysters, bryozoans, and calcareous tube worms and, in the most saline part of the estuary, sometimes sheltered octopi.

Spawning of 25 species of shrimps and crabs, 1 species of turbellarian, and 4 species of snails was observed and breeding of horseshoe crabs was seen. The sampling schedule, however, did not permit us to determine the duration of any individual breeding or spawning season.

Comparisons of the Pensacola estuarine fauna with faunas reported for other Gulf coast localities, principally in Florida, showed that the Pensacola fauna is more closely related to that of the Alligator Harbor, Fla., area than to faunas of other localities.

The Pensacola estuarine fauna, though mainly temperate in character, includes groups of species characteristic of the tropics (especially the West Indies); some species in the fauna also occur along the shores of the Western Atlantic Ocean from New England to South America, and some are cosmopolitan.

POPULATION DYNAMICS OF FISH

David J. Hansen, Project Leader

This project was initiated in the summer of 1963 to determine the status of two species of fish--pinfish, Lagodon rhomboides, and Atlantic croaker, Micropogon undulatus,--in the Pensacola estuary. Information gained will be used to determine whether changes caused by man have any noticeable effect on the abundance, growth, feeding, and migrations of these fish. The preliminary (prepollution) phase has been completed and a report is being prepared for publication.

Pinfish and croakers are extremely abundant in areas of high and low salinity, respectively. The two stations chosen to study the life history of each species are in the lower estuary, Santa Rosa Sound, for pinfish and in the upper estuary, Escambia Bay, for croakers (fig. 4).

Pinfish

Annual variations in the number of pinfish caught per trawl haul are large. Times of maximum and minimum abundance coincide from year to year. In all months of sampling in the summer and fall of 1965, except November, the number of fish caught per trawl haul was greater than in 1964 (fig. 5). Significantly more pinfish were caught in August and September of 1965 at station II than at station I.

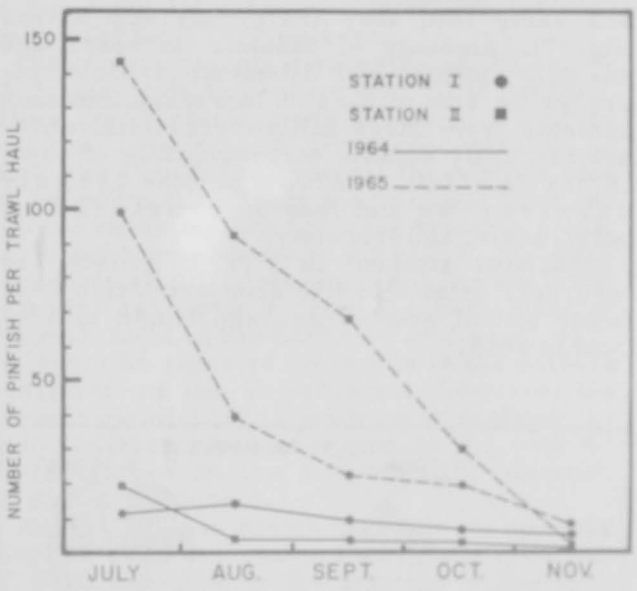

Figure 5.--Number of pinfish caught per trawl haul at Stations I and II in summer and fall, 1964 and 1965.

The type and amount of food in pinfish stomachs vary with time of year, location, and the size of fish. Monthly changes in foods ingested is not a function of time of day, as all fish were captured during the same time span. The amount of food in pinfish stomachs decreased in the fall of 1965 (fig. 6). This decrease may be correlated with decrease in available food or with the gulfward

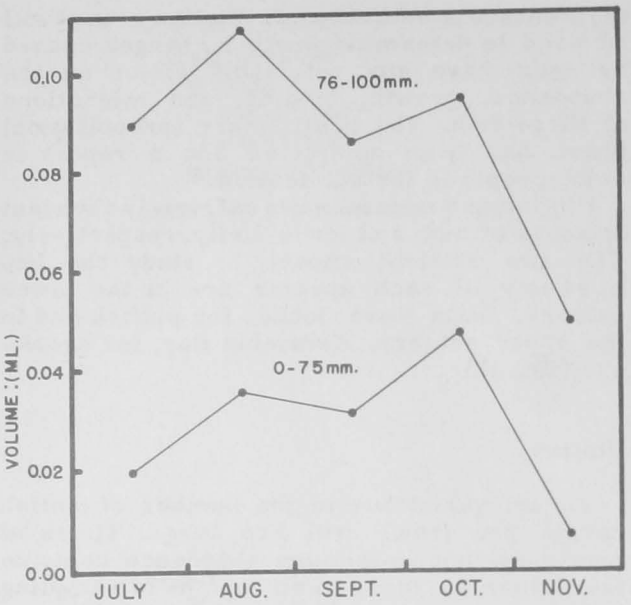

Figure 6.--Mean volume (milliliters) of material in 1,300 stomachs from large and small pinfish in the summer and fall, 1965.

migration of fish. Pinfish feed on a large variety of plants and animals. In the summer and early fall, they are mainly vegetarians (fig. 7). Amounts of diatoms decrease and vascular plants and filamentous algae increase as size of the fish increases. Stomach contents from large fall-caught pinfish characteristically contain large amounts of sand. In the late fall, pinfish become largely carnivorous and feed on crustaceans, polychaetes, and lancelets.

The size attained in 1 yr. of growth was inversely related to fish abundance; fish were fewer and of greater average length in 1964 than in 1965.

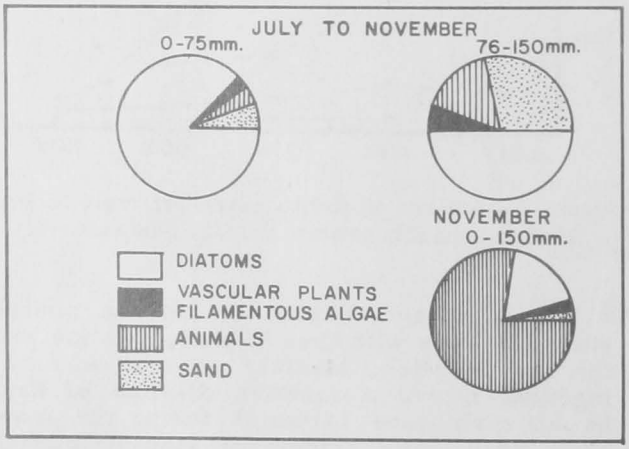

Figure 7.--Differences in the foods from stomachs of 1,200 large and small pinfish from July to November 1965 and from over 100 pinfish caught in November.

Pinfish can be aged by scales and by analysis of length-frequency distributions. Marginal increments on scales, percentage of scales with an annulus on different dates, and back calculations of length at annulus formation all indicate that annuli are formed mainly in March or April. The relation between standard length and scale radius is a straight line. Scales are formed at about 13 mm. (millimeters) (1/2-in.) standard length.

Most pinfish leave the estuary in the late summer and fall to spawn in the Gulf. At this time, pinfish over 80 mm. (3 in.) long have developing gonads. Since fish below 110 mm. (4 in.) are in their first year of life, it is possible that they spawn in that year. The gonads become fully developed during the spawning migration or after the fish reach the spawning site.

Atlantic Croaker

Average numbers of croakers caught per trawl haul varies annually, monthly, and between stations. Peak abundance is in the late spring or early summer. At one station, numbers caught in 1964 and in 1965 were not significantly different; at the other station, more fish were caught in all summer months in 1965 than in 1964.

Types and amounts of food in croaker stomachs vary with time of year, location, and size of the fish. Monthly changes in foods ingested is not a function of time of day, as all fish were captured during the same time span. Amount of food in the stomachs decreases in the late spring and summer, but an increase was observed in the summer of 1965 at one station. This increase, which presumably indicated an increase in available food, may explain why this area had more fish in 1965 than in 1963 or 1964. Croakers are carnivorous; polychaetes, arthropods, mollusks, and fish are the principal prey. At one station, polychaetes composed 82 to 96 percent of the food identified from all sizes of croakers caught in the summer and fall of 1965; fish and crustaceans made up most of the remainder. At the other station, stomach contents from croakers captured in the summer of 1965 showed that as the fish grew longer they ate fewer polychaetes and more crustaceans and fish. Polychaetes made up over 90 percent of the food in fish caught in the fall at this location.

Growth varied between years, averaging 0.06 mm. (0.002-in.) more per day for first year fish in 1965 than in 1964. On any sampling date, the average size of fish caught was greatest at the station with the highest salinity.

Croakers may spawn in the first year of life. First-year fish caught in the fall had developing gonads in both 1964 and 1965.

A variety of species of fish were routinely analyzed for residues of organochlorine pesticides. Residues vary with age; generally, 1-year-old fish have twice as much DDT in their tissues as fish in their first year of life. Different species of fish of the same year class from the same location have similar residues. Analyses of pesticide residues this year indicated that spraying for control of larvae of the dogfly, Stomoxys calcitrans, is responsible for the wide fluctuations and the high DDT content of fish from the lower estuary. Spraying for dogfly control in the upper estuary is limited; residues in fish collected there are lower and fluctuate less than in the lower estuary.

POPULATION DYNAMICS OF SEDENTARY FAUNA

Philip A. Butler, Project Leader (Acting)

Marine animal populations have seasonal and annual cycles of abundance as a result of many interacting factors, such as temperature and salinity, as well as disease. Knowledge of these natural cycles is indispensable to our understanding of how man affects the marine environment by dredge-and-fill operations and pollution.

During the past 15 years, we have continuously monitored changes in salinity, temperature, and the incidence of sedentary animals--primarily barnacles, oysters, and Bryozoa--at one station in Santa Rosa Sound. Appropriate meteorological data are available from a nearby weather station. These records show that drastic fluctuations in, for example, the number of oysters setting from year to year are not clearly associated with any one environmental factor. In 2 years when hydrographic features appeared similar, for example, the total oyster sets per unit area were 13 and 221.

All of these data are now being prepared for computer analysis; it is hoped that the interactions of the factors that cause population fluctuations will become more apparent. Because the local estuary is relatively unpolluted now, these data form a baseline for comparison with future changes.

FOOD CHAIN STUDIES

Robert F. Johnson, Project Leader

We started our food-chain studies with bacteria. Marine bacteria are used as food by several marine organisms, but more important is their direct influence on the environment through the regeneration of nutrients. We selected Pseudomonas piscicida as a representative species because it is common in shallow water in Florida.

Our experiments indicate that pesticides do not grossly affect this bacterium. No damage to growth rate or morphology was apparent when the bacterium was cultured in the presence of chlorinated hydrocarbon compounds (e.g., 10 p.p.m. of DDT) and organophosphorus compounds (100 p.p.m.). After extended periods of time small amounts of DDT actually stimulated growth.

Since DDT is normally dissolved in acetone for stock solutions, we tested P. piscicida's tolerance to acetone. The bacterium's growth is noticeably decreased by 2 ml. (milliliters) of acetone per liter of culture. Consequently, we added microliter quantities of acetone stock solutions to our cultures.

The uptake of DDT by this bacterium is fast and nearly complete: In culture media containing 1 p.p.b. of DDT, more than 90 percent was taken up in 24 hr. The only difference between a 24-hr. and a 48-hr. culture is in the metabolites of DDT in this bacterium; the total DDT remains the same. The uptake was measured by the removal of C^{14}-labeled DDT from solution and by electron capture gas chromatography. Adsorption is not an important part of this process; 10 washings of the cells did not remove a significant portion of the total DDT present.

Other experiments demonstrated the ability of P. piscicida to remove DDT from solution. When the bacteria were grown in a culture medium containing a given concentration of DDT and then fed to oysters, the oysters took up no more DDT from the bacteria plus the culture medium than from the bacteria alone.

To determine where in the cell P. piscicida concentrates DDT, we analyzed the bacterial cell wall separately from the remainder of the cell. Most of the DDT was in the cell wall, as would be expected since this is the surface presented to the environment; however, the percentage of DDT in the form of metabolites was considerably higher inside the cell (25 percent) than in the cell wall (13 percent), as shown in figure 8.

As the DDT is metabolized in its passage into the cell, the conversion favors a buildup of DDD and DDE in the interior of the cell. This tendency to convert DDT to DDD and DDE may play an important part in the estuaries as an initial step in the biological degradation or fixing of DDT. The data suggest that the remarkable rate of uptake of DDT is made possible by the immense surface area that the bacteria present to the environment and the lipidlike character of DDT that would allow it to penetrate the lipoprotein portion of the cell wall. This last characteristic would also be responsible for the high concentration of DDT in the cell wall.

The metabolite DDE is considerably less toxic than DDT. When we set up an artificial

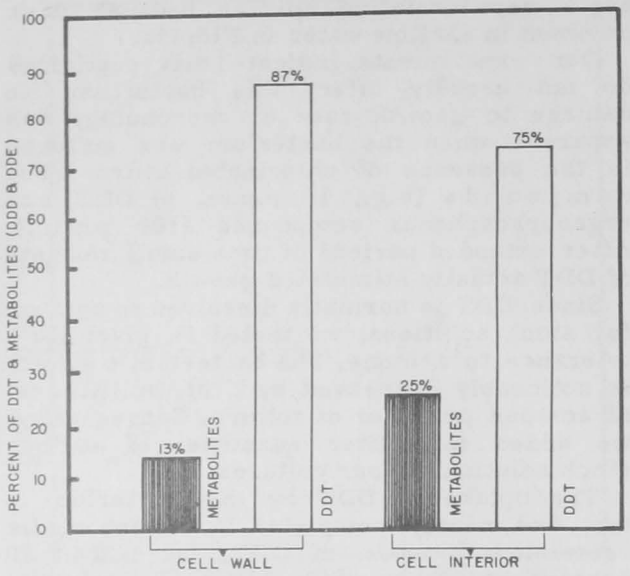

Figure 8.--Comparison of the distribution of DDT and its metabolites in the cell wall and cell interior of a marine bacterium (Pseudomonas piscicida).

food chain: marine bacteria→oysters→fish, the shift toward an increase in DDE is evident (fig. 9). This shift could be significant in nature and probably is responsible for the high residues of DDE in animals near the end of food chains (sea gulls, porpoises, ...). Most of the DDT residues in the animals that we analyzed during the year were in the form of its metabolite, DDE.

Since organophosphorus pesticides are toxic because of their capacity to inactivate the cholinesterases, one would expect them to be less toxic to bacteria than to higher organisms. Experiments have shown that these pesticides have little effect on P. piscicida; however,

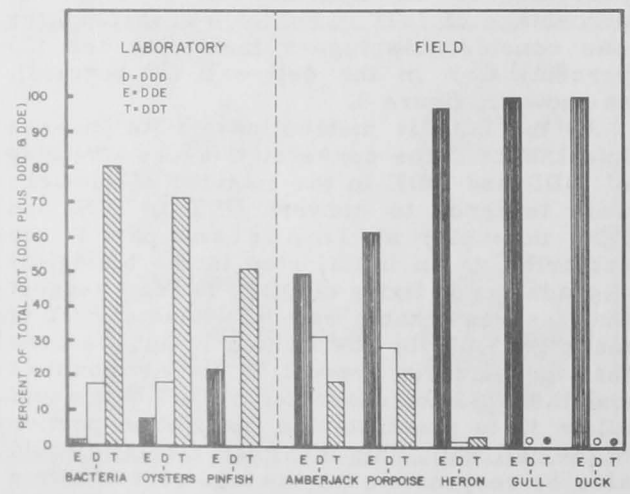

Figure 9.--Distribution of DDT and its metabolites, DDD and DDE, in representative organisms of a food chain.

this bacterium does diminish the toxic effect of Malathion (an organophosphorus compound). We have been unable to determine whether this detoxification is a direct or indirect process. Organophosphorus pesticides normally break down at pH's above 7.0. Therefore, the duration of toxicity in sea water, which is alkaline, is less than in fresh water. Bacteria create a microenvironment of high pH immediately adjacent to the cell; for P. piscicida, this pH ranges up to 9.5. The half-life of Malathion is 55 days at pH 6.0 and 4 to 5 days at pH 8.0. Therefore, any molecule of the pesticide that enters the microenvironment created by the bacteria will be quickly hydrolyzed in the high pH.

To check further the ability of P. piscicida to break down Malathion, we added the bacteria to phosphate-free sea water to which Malathion had been added. After 48 hr. the bacteria were removed and algal cells added (Chlorella sp.). After 5 days, the algal cells had produced 25 percent more cells in PO_3-free sea water containing Malathion alone (fig. 10).

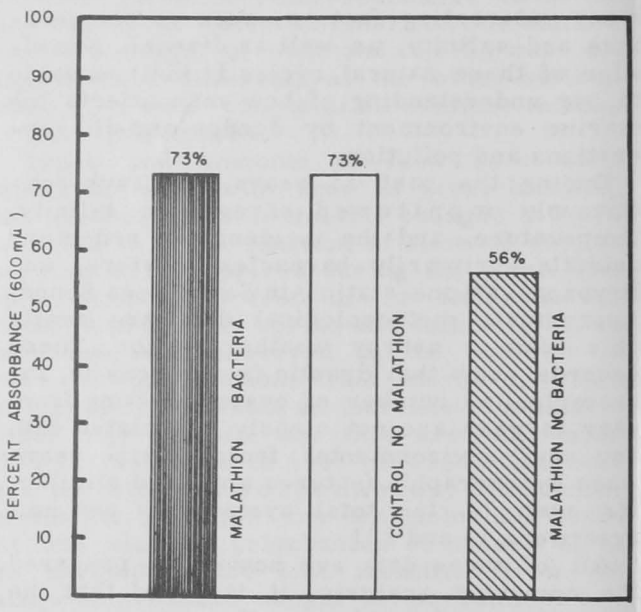

Figure 10.--Relative growth of algae, illustrating breakdown of malathion by a marine bacterium, Pseudomonas piscicida. Density of algal cells is determined by the amount of absorbance in a spectrophotometer.

The increased growth rate probably was due to the hydrolysis of the Malathion by the high pH created by the bacteria. This hydrolysis could provide a source of PO_4 for growth of algae. In the cultures in which no bacterial action had taken place, the algae grew until they had used all the available PO_4 contained within their cells; phosphate then became a limiting factor.

In further studies of the fate of pesticides in food chains, we found that both oysters and

P. piscicida are capable of metabolizing a very small fraction of the radioactively labeled DDT that enters them so completely that it is no longer recognizable. It becomes part of the metabolic pool and is utilized in the biochemistry of the cells.

This process was demonstrated by the following experiment: P. piscicida was cultured in media containing 1 p.p.m. DDT (C^{14}) and exhaustively washed and extracted. The cells were then acid-hydrolyzed under pressure; the hydrolysate was radioactive. To find out the nature of the substance that had been cross-labeled, aliquots of the hydrolysate were chromatographed, revealing the presence of radioactive amino acids. To make the C^{14} available for the formation of amino acids, the organism obviously had to break down the DDT molecule. The percentage of molecules so metabolized would be very small compared to the number of molecules that retained their original characteristics.

PHYSIOLOGICAL EFFECTS OF PESTICIDES

Hugh T. Holland III, Project Leader

Development and use of the organophosphorus pesticides have increased greatly in recent years, and these chemicals often are replacing the chlorinated hydrocarbons. Analytical techniques to detect residues of these compounds are unsatisfactory, however, with the exception of enzymatic methods based on the in vivo inhibition of acetylcholinesterase (AChE) in fish brains. The method used for assay of enzyme activity is as follows:

After the brain is removed and weighed (wet), the tissue is homogenized in a phosphate buffer. The homogenate is incubated with acetylcholine (ACh) as the substrate, and the residual ACh determined colorimetrically. Activity is reported as micromoles of ACh hydrolyzed per milligram (mg.) of brain tissue per hour at 25° C.

Much of our work to date has been directed toward determination of normal AChE activity for several common estuarine fishes of the Pensacola area. Our data show a decrease in activity per unit weight as the brain weight increases, but individual variation from the line of best fit for the data is generally less than 10 percent. Consequently, normal enzyme activity can be estimated from the brain weight, and deviations expressed as percentage of normal activity.

We studied the sensitivity of estuarine fishes to various organophosphorus pesticides by exposing them to sublethal concentrations in running sea water. Fish were killed and examined at 24-hr. intervals to determine their brain enzyme activity. One of the chemicals tested significantly inhibited AChE activity within 48 hr. at a concentration of 0.001 p.p.m. (table 2). We found that a particular pesticide may be species-specific. Guthion reduced the enzyme activity of sheepshead minnows (Cyprinodon variegatus) by 90 percent in 24 hr. at a concentration of 0.01 p.p.m., but did not affect spot under similar conditions. Recovery to normal enzyme levels after exposure varied with time, concentration, pesticide, and species, but in general required at least 1 wk.

During the year, we initiated a program for monitoring organophosphorus pesticides, in which fish brain AChE activity is used to determine if pollution by these chemicals is present. Eight laboratories along the Atlantic and Gulf coasts cooperated by periodically sending us frozen specimens of several selected fish species. We made 741 assays in conjunction with this study. Our data show that enzyme activity for a particular species is nearly constant throughout its range, so it is possible to use the same kind of fish to measure pollution in different areas. We detected several areas of minor pollution during the year.

We found high AChE activity in certain tissues of several species of shrimp (fig. 11) and crabs, and demonstrated that this

Table 2.--Effects of 48-hr. exposure to various organophosphorus pesticides on brain AChE activity of estuarine fish, Gulf Breeze, Fla.

Pesticide	Concentration	Species[1]	AChE activity
	P.p.m.		Percent
Malathion.....	0.1	S	76
Malathion.....	.1	C	39
Dibrom.....	.05	S	<10
Dibrom.....	.05	C	79
Dibrom.....	.001	M	76
Parathion.....	.01	S	<10
Parathion.....	.01	C	26
Guthion.....	.01	S	79
Guthion.....	.01	C	<10
Diazinon.....	.001	S	100
Diazinon.....	.001	M	74
Bayer 38156...	.001	S	76
Bayer 38156...	.001	C	82
Bayer 38156...	.001	M	58
Dursban.....	.001	S	38
Thimet.....	.0005	S	84
Thimet.....	.0005	C	68
Thimet.....	.0005	M	69

[1] S = spot, Leiostomus xanthurus.
C = sheepshead minnow, Cyprinodon variegatus.
M = striped mullet, Mugil cephalus.

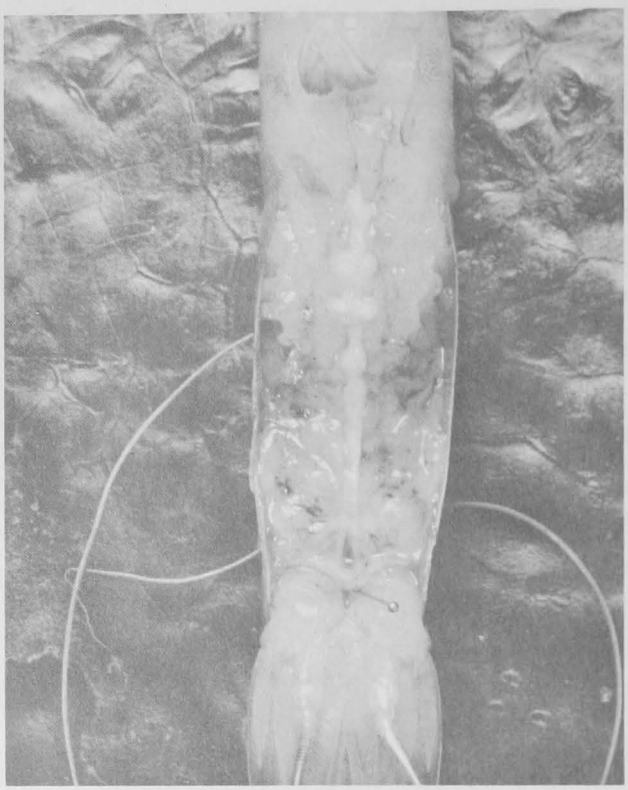

Figure 11.--Ventral nerve cord of a shrimp, which is assayed for cholinesterase activity to determine effects of pesticides on this commercially important species. Studies on invertebrates are in the developmental stages but will soon be incorporated into our program.

activity is sometimes inhibited by extremely low concentrations (less than 1.0 p.p.b.) of organophosphorus pesticides. We are currently perfecting AChE assay methods for invertebrates, and hope that they can be used to study the effects of sublethal concentrations of pesticides.

ARTIFICIAL SELECTION OF FISH

Hugh T. Holland III, Project Leader

The first report of pesticide resistance in insects was published more than 50 years ago; now more than 120 resistant species are known. The genetic, physiological, and biochemical foundations for insect resistance are well documented in the literature. On the other hand, vertebrates have been expectedly slow to develop resistance. Researchers in Canada developed a DDT-tolerant strain of laboratory mice, but it was not until 1963 that naturally occurring populations of fishes resistant to insecticides were found. These fishes populated areas known to be grossly contaminated by pesticides through direct application and agricultural runoff. The continuation of resistance for several generations when the fish are transferred to uncontaminated water suggests that they possess genotypes that enable them to survive concentrations of pesticides lethal to their nonresistant brethren. The genetic mechanism, selective agents, and number of generations required to produce a resistant population are unknown.

We initiated studies in 1964 to determine if genetic resistance to pesticides in sheepshead minnows could be demonstrated by selectively breeding survivors of DDT exposure tests. Tests were made in 20-l. (liter) (5.3-gallon) plastic aquaria to which an acetone stock solution of pesticide was added to give the desired concentration of toxicant. Control groups received acetone only. Tap water adjusted to a salinity of 4 p.p.t. with artificial sea salt was used. Water temperature was 21 ± 1° C. during the tests. Adult sheepshead minnows were seined from marsh ditches on nearby Santa Rosa Island and held in the laboratory 24 hr. to eliminate weak or injured individuals. Fish that survived these tests were transferred to artificial pools on Santa Rosa Island similar to the natural habitat of the species so that they could spawn. Their offspring were tested in various pesticides when they had attained suitable size, and the survivors of these tests were returned to the field pools to serve as parents of the next generation of fish.

In 1964 we found that first-generation offspring of sheepshead minnows that survived DDT exposure in July were more sensitive to both DDT and endrin than were control fish. Fish of the F_1 generation of brood stock selected in April and May of 1965 did not exhibit this increased susceptibility to pesticides; however, second and third generation offspring of these parents were significantly more sensitive than were control groups. In July of 1965 we repeated the previous summer's work with similar results--the F_1 fish were pesticide-sensitive.

To summarize, fish that survived pesticide exposures which killed most of the fish tested tended to have offspring more sensitive to pesticides than control fish. This sensitivity could be moderated in the F_1 generation by selecting brood stock earlier in the year, but we can offer no explanation for this phenomenon. We hope that continuing studies will help resolve questions as to the mechanism of resistance in fishes.

PESTICIDE MONITORING PROGRAMS

Philip A. Butler,
Pesticide Research Coordinator

Local

During the past 12 mo., we continued to monitor monthly plankton and oyster samples from the Pensacola estuary for levels of organochlorine pesticide residues. Eleven of 12 oyster samples and 30 of 37 plankton samples contained residues of DDT and its metabolites. The concentrations ranged from less than 5 to about 80 p.p.b. on a wet-weight basis. These levels are comparable to those in oysters in many other parts of the country and are not considered indicative of undue pollution.

As in earlier years, we found pesticide residues in plankton to be highest in mid-summer when local insect control programs were active. Highest levels in oysters, however, were in early spring and apparently reflect the appearance of DDT pollution in the rivers that drain into the estuary from the surrounding agricultural land.

National

We began surveillance of organochlorine pesticide pollution in estuaries on the Atlantic and Pacific coasts last year, using shellfish as bioassay animals. Mollusks, especially oysters, are able to store pesticides as body residues, even when the concentration of these compounds in the environment is very low. In addition, their habitat at the junction of river and ocean waters, their fixed position on the bottom, and their widespread occurrence combine to make them most useful in this monitoring program. A major factor in the success of the program has been the widespread understanding of the need for more knowledge of pesticide pollution and the willingness of many State and university scientists to participate in the accumulation of the data.

The bioassay animals must be collected at regular intervals. Stations must be selected on the basis of drainage basins and the permanence of the populations of mollusks. Samples must be prepared with precision if we are to have confidence in data on residues in the range of 10 to 100 p.p.b. Our success in developing a technique for dry processing and shipment of the samples without recourse to freezing has greatly facilitated the logistics of the program.

During the first year, cooperative agreements were initiated in six coastal States. The program is cooperative in the true sense of the word. The contracting agencies have the knowledge needed to place stations in critical locations. They provide the essential field supervision and assume full responsibility for the collection and integrity of the biological samples. Our laboratory's responsibility lies in the maintenance of accuracy in chemical analyses, in the reporting of data, and in keeping abreast of technological developments that may affect the program.

In most States, we have written contracts or agreements by which the Bureau of Commercial Fisheries provides the chief financial support for the work--but this is not always true. In New Jersey and Texas, for example, the Bureau furnishes only the laboratory equipment, and the agency provides the personnel and field equipment. In Florida, the Conservation Department provides most of the equipment and personnel. In Maryland, the Bureau's Biological Laboratory at Oxford provides the equipment and personnel.

The Eastern oyster is the primary bioassay animal on the Atlantic and Gulf coasts, but the soft clam (Mya arenaria) is used at seven stations in Maine, and the hard clam (Mercenaria mercenaria) and mussel (Mytilus edulis) are used in New York. On the West coast, the Pacific oyster (Crassostrea gigas) is the chief assay animal, but at some stations the native oyster (Ostrea lurida) and the Eastern oyster are used. Of 13 monitoring contracts now in force, 7 are with the Conservation Departments of the respective States and 6 are with universities or university-affiliated marine laboratories.

The program is still growing, but our coverage is nearly complete on Atlantic, Gulf, and Pacific coasts and includes more than 150 stations that are sampled monthly. Some States, such as Alabama and Louisiana, are not included in our program because the shellfish populations are being monitored by other agencies.

At present, all samples are analyzed at the Gulf Breeze laboratory--not because we think it has a priority on excellence but rather because (in view of the temperamental nature of chromatographic techniques and equipment) it seems logical to have all samples handled by the same laboratory. If the methods are faulty, the same errors will affect all data.

Electron capture gas chromatography involving two different columns is used in the analytical procedures. The equipment makes possible the scanning of each sample for 10 of the most widely used organochlorine pesticides. Sensitivity of the technique is in the range of 1 to 5 p.p.b., but only residues above 9 p.p.b. are measured and reported. Identified residues below 10 p.p.b. are recorded simply as a trace.

Analyses indicate a surprising number of shellfish areas are apparently free from

pesticide pollution. In the Gulf of Mexico, which receives the drainage from half the continent, however, it is difficult to find an oyster sample that does not have some residue of DDT or its metabolites. Other pesticides identified in routine samples include endrin and dieldrin, but these occur at only a few locations.

I stress the fact that at no time have the analyses indicated residues which would cause concern for human health.

This program is only one phase of our study of the pesticide pollution problem. Several areas are under surveillance for possible changes in fish populations due to organophosphorus pesticides (see earlier section on physiological effects of pesticides and fig. 12), and the pelagic fish populations of both the Atlantic and Pacific Oceans are being sampled for pesticide residues.

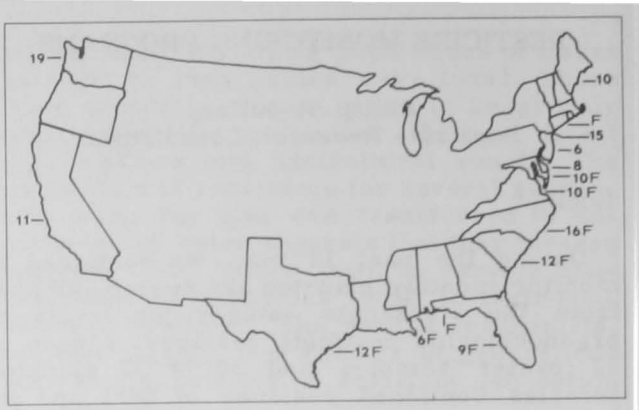

Figure 12.--The number and distribution by States of fixed monitor stations. Numbers indicate where mollusk populations are sampled for organochlorine pesticide residues. Locations designated by "F" indicate where estuarine fish populations are regularly sampled for organophosphorus pesticide residues.

RESEARCH CONTRACTS

TOXIC RESIDUES IN MARINE FOODS

Institute of Marine Resources of the University of California at Berkeley; Contract Period--January 1965 to March 1966

The investigation objectives were to determine the extent of residues of DDT and its metabolites in coastal and pelagic food fish and the degree of variation in residue levels in individuals of the same age and species.

In general, appreciable residues were found to exist but not to the extent that they would be hazardous to humans. In some species the residues appeared to accumulate with age, but in others the reverse was true. Some residue was usually present, even in fish collected in the mid-Pacific. A final report is being prepared for publication.

EFFECTS OF PESTICIDES ON OSMOREGULATORY ABILITY OF ESTUARINE FAUNA

University of South Florida; Contract Period--June 1966 to June 1967

The purpose of this project is to determine the effects of subacute levels of organophosphorus pesticides on osmoregulation in crabs and fish. This physiological process is important in estuarine animals, enabling them to adjust to marked changes in salinity. Change of this ability by pesticides could have a serious impact on migratory patterns of important commercial fishery species such as shrimp, crabs, and menhaden.

KINETICS OF DDT IN A MARINE FOOD CHAIN

California State College at Long Beach Foundation; Contract Period--May 1966 to April 1967.

The objective of this project is to introduce DDT into the food chain as a residue in algae and then to follow its metabolism and eventual detoxification as it moves progressively through worms and fish. This food chain can be observed under controlled laboratory conditions; it will be possible to measure with precision residue movements that now are only guessed at in nature.

ACCUMULATION AND LOSS OF 2,4-D BE RESIDUES IN OYSTERS AND CLAMS

Chesapeake Biological Laboratory, University of Maryland; Contract Period--June 1966 to June 1967.

The aims of this project are to determine whether, after herbicides are used to control watermilfoil, residues build up in clam and oyster populations that would affect their commercial use. The chemical analysis of shellfish tissues for 2,4-D is to be standardized and the possible effects of 2,4-D on shellfish biology will be studied.

EVALUATION OF CHEMICALS FOR NATIONAL FISHERY CENTER

Bureau of Sport Fisheries and Wildlife; Contract Period--April to September 1966.

This agreement made it possible for the Gulf Breeze laboratory to provide its facilities for testing the suitability of various

plastics and sealants proposed for use in the salt-water circulation system of the new aquarium. Most compounds tested gave off substances highly toxic to marine fauna during the first few days of exposure; in most instances, these substances dissipated on continued exposure. Some of the materials tested were clearly unsuited for the proposed use. Two of the materials had special qualities that may make them desirable for other purposes in marine research; they will be tested further.

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