A REPORT ON THE COOPERATIVE BLUE CRAB STUDY - SOUTH ATLANTIC STATES[1]

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ABSTRACT

The cooperative blue crab study was designed to determine the cause(s) of blue crab mortalities and to delineate significant factors affecting the relative abundance of marketable crabs. A multiphased approach provided background information relative to regional hydrological characteristics, diseases and parasites, and residual pesticides associated with blue crab populations.

Standardized routine monitoring at 20 South Atlantic sampling areas provided hydrological data that were illustrative of typical seasonal patterns.

A total of 195 blue crab samples (1,950 individual crabs) was collected from established stations and processed for pesticide analyses. Chlorinated hydrocarbon pesticides were detected in all samples.

Approximately 300 blue crabs were collected by each state from established stations for histopathological data. The organism most emphasized during this study, *Paramoeba perniciosa,* was detected along with several other potential pathogens, but at levels not sufficient to cause blue crab mortalities.

Laboratory studies were conducted to determine the effects of selected hydrological factors and pesticides on blue crab mortalities under controlled conditions. Upper and lower thermal tolerance limits for adult crabs were defined at various test salinities. Generally, test crabs were less tolerant at low salinities and high temperatures and at high salinities and low temperatures.

Delineative screening of DDT and Toxaphene showed these compounds to be more toxic at lowered salinities (8.6‰) with toxicities increasing as temperatures ranged above and below 15° C at all test salinities. Toxicity was slightly greater at the lower thermal extremities. Mirex, the technical compound, was relatively non-toxic to adult and sub-adult crabs. However, Mirex in a granulated bait showed delayed toxicity when ingested by juvenile blue crabs (< 3 inch carapace width) in test concentrations of 0.036 grams/liter. Crab survival time and metabolic rates were correlated with environmental combinations.

INTRODUCTION

Massive mortalities and a decline in abundance of the blue crab (*Callinectes sapidus,* Rathbun) occurred along the South Atlantic coast from 1966 through 1968 (figure 1). First mortalities were reported in June 1966 from Holden Beach, North Carolina to Ossabaw Sound, Georgia and continued sporadically through the summer. Further mortalities occurred in June 1967 beginning in Georgia and spread up the coast as far as the Santee River in South Carolina during the summer. Mortalities beginning in April of 1968 were confined to the Georgia coast. These declines affected the economic stability of the crab industry and commercial fishermen witnessed a drop in production from the high of 40.2 million pounds in 1964 to 24.4 million pounds in 1968.

A series of biological conferences were held between the four affected states and the Bureau of Commercial Fisheries. These sessions culminated in a cooperative research program between the South Atlantic states, with federal financing by the U. S. Bureau of Commercial Fisheries as authorized under Section 4-b (disaster funds) of PL 88-309.

Standardized methods and procedures were confirmed by committee members of the participating states, and a standard format for recording all field and laboratory data was designed. Prior to initiation of the program each state individually selected five locations to monitor monthly.

Since it is probable that a combination of factors contributed to the decline in blue crab populations, this study was undertaken in four phases: Hydrology, Occurence and Abundance of Pesticides, Blue Crab Diseases and Parasites, and Laboratory Studies of Factors Affecting Crab Mortalities.

Figure 1.—Scenes such as this were common in some areas of South Carolina, North Carolina, and Georgia during 1966, 1967, and 1968.

Study Areas

A preliminary survey was made in each state to locate five sampling stations. In choosing the stations, it was necessary that they 1) were representatives of the various saltwater ecosystems found in each state, 2) were located in a commercial crabbing zone, and 3) were, if possible, in an area where previous crab mortality(ies) had occurred. The map on the back cover depicts the general location of the 20 stations. Throughout the text the study areas will be referred to as station numbers corresponding to those on the map and in the appendix.

Phase 1. HYDROLOGY

Methods and Materials

The following parameters were monitored monthly at each station:

(1) *Bottom temperature* was recorded with a rapidly equilibrating mercury immersion thermometer.

(2) *Dissolved oxygen* was measured by the azide modification of the Winkler method (Standard Methods, APHA 1965).

(3) *pH* was determined with a LaMotte block comparator accurate to ± 0.25 pH units.

(4) *Salinity* was determined with a temperature compensating American optical refractometer calibrated in index of refraction.

(5) *Turbidity* was measured with a Secchi disc.

All data, except turbidity, were derived from bottom water samples collected in a non-contaminating three-bottle train sampler as described by Swingle and Johnson (1953).

Results and Discussion

A summary of the hydrological characteristics is presented in Tables 1 through 4.

South Atlantic Coast

Bottom water temperature for the ten month study period ranged from 3.3 to 31.0° C (Table 1). Dissolved oxygen readings ranged from 1.7 to 11.6 ppm (Table 2). Salinities ranged from 1.1 to 35.5‰, with an over all average of 24.6‰ (Table 3). Turbidity readings varied from a low of 10 cm to a high of 250 cm (Table 4). pH readings for all study areas varied only 1.5 pH units, from 7.0 to 8.5.

North Carolina

Temperatures appeared to follow usual winter to summer fluctuations with a low 3.3° C registered at station 5 in March and a high of 31.0° C at station 5 in August.

The highest dissolved oxygen readings were recorded in March and the lowest in September at all five stations.

Salinity was recorded at sea water concentrations at stations 1 and 2. The lowest level recorded (11.9‰) was at station 3.

pH changed little during the ten months of testing, but did tend to be more basic in the fall.

Turbidity increased in the summer, decreasing as the water temperature cooled.

South Carolina

Bottom water temperatures were distributed by seasonal ranges at each station. Minimum water temperatures ranged from 8.7° to 10.0° C, upper temperature levels varied from 29.0° to 31.0° C.

Salinities varied by station over a wide range. Lowest salinity readings (2.7 - 10.7‰) were preceded by heavy rainfalls with absolute minimum values recorded on ebb tides. These low salinities usually re-

covered rapidly with tidal flushing.

Turbidity and dissolved oxygen followed a general seasonal pattern with low readings during the warmer months and ebbing tides. The pH measurements were relatively constant at sea water levels of 8 units.

Georgia

Seasonal bottom temperatures ranged from 10° to 31° C. Variations between high and low temperatures were similar at every station. The sharpest rise in temperature (12.5° C) occurred between March and April at station 1. All other stations also showed a marked rise in bottom water temperature during this period. Temperatures reached a peak in July at all stations. The highest recorded bottom temperature (31° C) was at station 5. Temperatures dropped as much as 9° C between October and November.

Dissolved oxygen levels ranged from 3.0 to 9.4 ppm. Levels at station 2 stayed at 3.0 - 3.2 ppm during June, July, and August. Dissolved oxygen levels at the other four stations were somewhat higher during this period ranging from 3.2 to 5.7 ppm. The lower dissolved oxygen at station 2 did not appear to harm the local crab population.

Salinities ranged from 15.1‰ (November, station 5) to 32.3‰ (July, station 4). The greatest seasonal fluctuation (15.6‰) occurred at station 4.

pH ranged from 7.3 to 8.2 with the greatest variation of only 0.6 units occurring at station 1. Highest pH readings were recorded at station 4 which was located near a polluted area.

Turbidity readings ranged from 177 cm (March, station 1) to 22 cm (August, station 1). The most stable turbidity readings were recorded at station 3.

Florida

Bottom water temperatures ranged from a low of 13.5° C in December, at station 5, to a high of 31.0° C in August at station 3. The greatest temperature decline occurred from October to November, for all stations, and the greatest increase in April to May for stations 2, 3 and 5. Stations 1 and 4 showed the greatest increase during May and June. The narrowest seasonal fluctuations occurred during summer months, with station 2 showing the most variation.

Lowest dissolved oxygen for all stations was 1.7 ppm taken at station 3 during April. Station 3 also had the lowest overall seasonal average of 3.6 ppm, while station 2 showed the highest average with 5.5 ppm. Highest dissolved oxygen was 7.2 ppm found at station 5 during June.

Bottom salinities fluctuated considerably at all stations. Station 1 had the lowest salinity ranging from 1.1 to 26.9‰. Station 5 fluctuated least, ranging from 17.2 to 31.2‰. Salinity patterns at stations 2 and 4 (nearest the ocean) fluctuated from 13.5 to 34.5‰ and 23.2 to 35.5‰. Salinities were generally highest during the summer months, except for station 1 which peaked in May and declined rapidly during August, reaching a low of 1.1‰ in October and November.

The highest Secchi disc reading was 155 cm at station 1, July, but station averages (76 cm) were much lower. During September and October, stations 2 and 3 were less than 50 cm, but both were sampled during flood tide when there were strong turbid currents.

TABLE 1.—*Summary of observed bottom temperature data from each state. Temperature in °C*

	N. Carolina			S. Carolina			Georgia			Florida			South Atlantic		
Sta.	Min.	Max.	Avg.	Min.	Max.	Avg.	Min.	Max.	Avg.	Min.	Max.	Avg.	Min.	Max.	Avg.
													For All Stations		
1	10.0	28.0	20.5	8.7	30.8	20.9	10.0	30.0	21.3	15.5	30.0	24.9			
2	9.4	28.0	20.8	10.0	29.0	22.1	10.0	29.5	22.1	16.5	29.9	24.3			
3	7.8	28.5	20.2	10.0	31.0	22.0	10.0	29.5	21.6	15.5	31.0	24.8	3.3	31.0	22.2
4	6.7	27.8	20.1	8.7	30.3	21.5	10.0	29.5	22.4	15.0	30.0	24.6			
5	3.3	31.0	19.8	10.0	29.5	21.6	12.0	31.0	23.3	13.5	30.0	24.3			

TABLE 2.—*Summary of observed dissolved oxygen data from each state.*

Dissolved oxygen in ppm

	N. Carolina			S. Carolina			Georgia			Florida			South Atlantic		
Sta.	Min.	Max.	Avg.	Min.	Max.	Avg.	Min.	Max.	Avg.	Min.	Max.	Avg.	Min.	Max.	Avg.
													For All Stations		
1	4.1	8.6	7.2	3.0	8.0	6.3	4.5	9.2	6.2	3.5	7.0	5.5			
2	5.2	9.6	7.8	5.0	9.0	6.1	3.0	9.4	5.4	3.6	6.5	5.3			
3	5.4	9.8	7.0	5.0	8.0	6.8	3.7	9.4	6.2	1.7	5.8	3.6	1.7	11.6	6.2
4	6.8	10.8	8.9	4.0	8.0	6.2	5.0	9.3	6.5	2.9	6.8	4.5			
5	6.0	11.6	7.9	5.0	9.0	6.2	3.2	8.6	5.5	2.6	6.8	4.9			

TABLE 3.—*Summary of observed salinity data from each state.*

Salinities in ppt.

	N. Carolina			S. Carolina			Georgia			Florida			South Atlantic		
Sta.	Min.	Max.	Avg.	Min.	Max.	Avg.	Min.	Max.	Avg.	Min.	Max.	Avg.	Min.	Max.	Avg.
													For All Stations		
1	29.6	34.5	32.2	2.7	32.3	25.3	16.2	28.1	22.8	1.1	26.9	16.6			
2	29.6	35.3	32.9	10.7	22.0	16.2	18.9	27.5	23.8	13.5	34.5	27.7			
3	11.9	31.8	20.6	23.0	32.3	29.0	16.2	27.5	21.9	16.2	32.3	26.3	1.1	35.5	24.6
4	11.9	28.0	22.5	17.0	32.0	25.6	16.7	32.3	24.9	23.2	35.5	28.6			
5	18.9	31.2	25.2	14.0	26.9	21.0	15.1	29.1	23.0	17.2	31.2	26.8			

TABLE 4.—*Summary of observed turbidity data from each state.*

Turbidities recorded in cm

	N. Carolina			S. Carolina			Georgia			Florida			South Atlantic		
Sta.	Min.	Max.	Avg.	Min.	Max.	Avg.	Min.	Max.	Avg.	Min.	Max.	Avg.	Min.	Max.	Avg.
													For All Stations		
1	55	155	84	10	56	28	22	177	86	65	155	98			
2	55	89	75	10	145	42	25	122	61	43	120	78			
3	46	135	66	15	85	41	38	112	70	43	105	68	10	250	70
4	52	120	92	16	147	49	22	147	72	60	150	91			
5	56	250	104	15	120	36	43	167	75	60	135	90			

Phase II.—OCCURENCE AND ABUNDANCE OF PESTICIDES

Methods and Materials

Ten blue crabs were collected monthly from each of the 20 sample stations for pesticide residue analyses. Standard otter trawls were used for collections during most of the sampling period, crab pots being utilized only in the colder months.

Preanalyses preparation of the crab samples was accomplished by taking 30 ± 5 grams of soft tissue from the ten crabs comprising a composite sample. The soft tissue, consisting of pieces of gill, hepatopancreas, heart, intestine, testes, ovarian eggs, and seminal receptacles from each crab, was placed together in a pint jar and chilled. A desiccant mix, consisting of 10% QUSO (a microfine precipitated silica) and 90% anhydrous sodium sulfate was added to the sample. It was then put into a freezer for two hours after which it was ground with a blender into a free flowing powder. Processed samples, placed in aluminum foil, were folded and shaped into tight rolls and stored in plastic bags with their appropriate data sheets. This technique, recommended by the Bureau of Commercial Fisheries Biological Laboratory at Gulf Breeze, Florida, prevents spoilage of the sample and degradation of the pesticide residues for at least 30 days without refrigeration.

The South Carolina State Board of Health Laboratory in Columbia, South Carolina, was under contract to analyze the 195 samples for pesticide residues.

These monthly samples were analyzed by gas chromatography for the following chlorinated hydrocarbons: Aldrin, BHC, Dieldrin, DDT, DDE, DDD, Endrin, Heptachlor Expoxide, Methoxychlor, Mirex, Toxaphene, and Chlordane. The only pesticide residues found in recordable quantities were DDT, DDD, DDE, Dieldrin, and Mirex.

The procedure developed by Maunder (1964) was used in extracting pesticide residues from the crab samples. Cleanup was accomplished using the standard florisile technique for nonanionic pesticides. Samples were analyzed on an electron capture gas chromatograph. All completed pesticide data were returned to the participating states.

OCCURRENCE OF CHLORINATED HYDROCARBONS IN BLUE CRABS

A total of 50 blue crab samples (500 individual crabs) was collected from established stations, by biologists from each state, and processed for pesticide analyses. Chlorinated hydrocarbon pesticides were detected in all samples. DDT and its metabolites were found in 100% of the samples. The highest values for DDT, DDD, and DDE in the crab samples were .247 (South Carolina), .188 (North Carolina), and .231 ppm (Georgia). Mirex was found in 35% of the samples at a maximum level of .389 ppm (Georgia). Dieldrin occurred in only 19% of the samples with a maximum level of .072 ppm (Georgia) (Table 5).

Monthly variations in the occurrence of chlorinated pesticides are shown in figure 2. Peak levels of total DDT in crabs occurred during early spring and summer while Dieldrin reached maximum levels during May and October. Mirex was found at highest levels from April through July.

Although these data are not sufficient for predicting seasonal variations, some degree of interpretation was attempted. Monthly fluctuations of total DDT and Dieldrin probably indicate seasonal differences in agricultural activities that were magnified by maximum fresh water runoff during summer and fall. The presence of Mirex can be directly associated with fire ant control.

Since the parent compound DDT was generally present in higher quantities than its metabolites DDD and DDE, the crabs were probably exposed to pesticide runoff. There is also an indication of residue buildup from transmission through the food web, since metabolites of DDT are present in all samples (Keil and Priester, 1969).

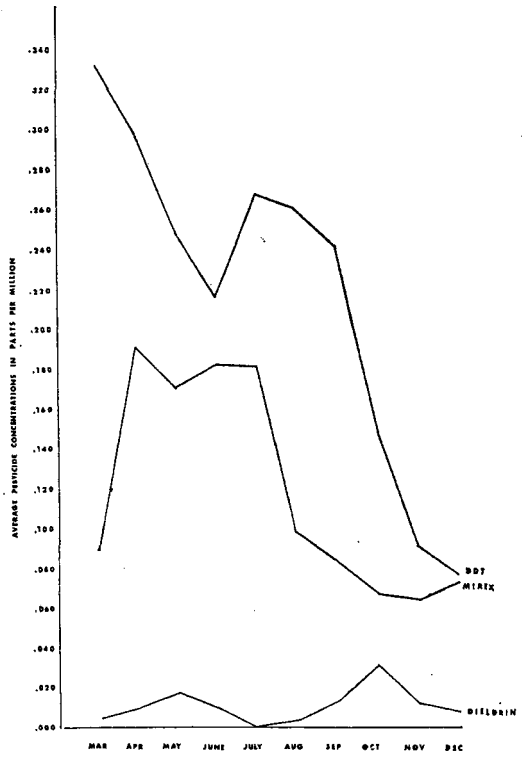

Figure 2—Average monthly occurence of DDT (total), Mirex, and Dieldrin in crab samples collected at 20 South Atlantic stations.

North Carolina

A summary of pesticide levels from the 50 crab samples collected in North Carolina are shown in Table 6. Of the 50 samples analyzed all were found to contain varying concentrations of DDE, DDD, and DDT. Only 10% of the total number showed any trace of Dieldrin and 16% contained Mirex. DDE, DDD, and DDT showed their highest concentrations in the spring with DDE, reaching a level of .122 ppm and DDD and DDT reaching highs of .188 and .213 ppm. Dieldrin was detected in the March and December samples but in very low concentrations, the highest being .005 ppm. Mirex was found in October, November, and December samples, the highest concentration being .045 ppm in December.

South Carolina

Chlorinated hydrocarbon pesticides were detected in all 50 of the blue crab samples collected in South Carolina. Table 7 shows that DDD, DDE, and DDT were found in all 50 samples ranging from .009 to .160 ppm, .011 to .180 ppm, and .012 to .247 ppm. Mirex was found in 44% of the samples at concentrations in the range of .005 to .209 ppm. Dieldrin was detected in 28% of the samples at a range of .002 to .019 ppm.

TABLE 5.—*Chlorinated Hydrocarbon Pesticide Residues in Blue Crab Samples from 20 South Atlantic Stations.*

Pesticide	No. of Samples Examined	No. of Samples % Positive	Residue in Parts Per Million Mean	Residue in Parts Per Million Low	Residue in Parts Per Million High
DDD	195	100	.073	.009	.188
DDE	195	100	.064	.010	.231
DDT	195	100	.086	.012	.247
Total DDT[1]	195	100	.219	.034	.517
Mirex	195	35	.076	.005	.389
Dieldrin	195	20	.012	.002	.072

[1] DDT and its Metabolites (DDD and DDE)

TABLE 6.—*Chlorinated Hydrocarbon Pesticide Residues in Blue Crab Samples from North Carolina*

Pesticide	No. of Samples Examined	No. of Samples % Positive	Residue in Parts Per Million Mean	Residue in Parts Per Million Low	Residue in Parts Per Million High
DDD	50	100	.051	.010	.188
DDE	50	100	.053	.013	.122
DDT	50	100	.077	.012	.213
Total DDT[1]	50	100	.181	.043	.491
Mirex	50	16	.023	.005	.045
Dieldrin	50	10	.003	.002	.005

[1] DDT and its Metabolites (DDD and DDE)

Georgia

DDT and its metabolites were present in all 50 of the samples collected in Georgia. These pesticides were found in varying amounts as follows: DDT, .018 to .176 ppm; DDE, .015 to .231 ppm; and DDD, .012 to .179 ppm (Table 8). These pesticides stayed at somewhat constant levels until the last three months of the study (October, November, and December) when concentrations declined sharply.

Dieldrin, present in 36% of the samples, ranged from .004 to .072 ppm. Mirex was found in 54% of the samples, ranging from .015 to .389 ppm. Mirex was found in samples collected at station 3 more frequently than at any other station. Most Mirex residues were found in samples from July through December.

Florida

The highest DDT residues in crabs were found during the spring months, especially April which showed an average of .136 ppm. Summer DDT averages varied least with slightly lower amounts than spring (.075 to .077 ppm). During fall and winter months a declining trend for DDT was accompanied by the appearance and increase of Mirex. DDT reached its lowest level (.018 ppm) during December. Mirex first appeared in September with an average level of .019 ppm, then increased to an average level of .061 ppm by December. Dieldrin appeared during October only, at a level of .008 ppm (Table 9).

TABLE 7.—*Chlorinated Hydrocarbon Pesticide Residues in Blue Crab Samples from South Carolina*

Pesticide	No. of Samples		Residue in Parts Per Million		
	Examined	% Positive	Mean	Low	High
DDD	50	100	.076	.009	.160
DDE	50	100	.081	.011	.180
DDT	50	100	.092	.012	.247
Total DDT[1]	50	100	.249	.034	.517
Mirex	50	44	.088	.005	.209
Dieldrin	50	28	.009	.002	.019

[1] DDT and its Metabolites (DDD and DDE)

TABLE 8.—*Chlorinated Hydrocarbon Pesticide Residues in Blue Crab Samples from Georgia*

Pesticide	No. of Samples		Residue in Parts Per Million		
	Examined	% Positive	Mean	Low	High
DDD	50	100	.068	.012	.179
DDE	50	100	.077	.015	.231
DDT	50	100	.092	.018	.176
Total DDT[1]	50	100	.237	.053	.425
Mirex	50	54	.162	.015	.389
Dieldrin	50	36	.017	.004	.072

[1] DDT and its Metabolites (DDD and DDE)

TABLE 9.—*Chlorinated Hydrocarbon Pesticide Residues in Blue Crab Samples from Florida*

Pesticide	No. of Samples		Residue in Parts Per Million		
	Examined	% Positive	Mean	Low	High
DDD	45	100	.066	.012	.180
DDE	45	100	.047	.010	.114
DDT	45	100	.082	.018	.196
Total DDT[1]	45	100	.195	.040	.468
Mirex	45	31	.031	.005	.164
Dieldrin	45	2	.008	.008	.008

[1] DDT and its Metabolites (DDD and DDE)

Phase III—BLUE CRAB DISEASES AND PARASITES

Methods and Materials

A disease and parasite sample of 30 crabs was collected monthly at selected stations in each state, along with data and samples for Phases 1 and 2. Crabs were collected as described previously.

Live crabs were processed at the laboratories within a few hours after collection. Each was examined externally and internally for signs of diseases or parasites. Hemolymph smears from each crab were obtained by cutting off the distal articulation of the fifth leg (dactylopodite or back fin) and allowing the hemolymph to drip freely and clot on a glass slide. Small pieces of the hepatopancreas, gill, gonad, muscle, heart, intestine, and eye stalks were taken from each crab. The tissue samples and hemolymph smears were each assigned a code number and preserved in a 10% neutral-buffered, formalsaline solution.

Observations, remarks, and other pertinent information (crab size, sex, and ecdysis state) were recorded on data sheets. After monthly samples were processed, they were forwarded to the U. S. Bureau of Commercial Fisheries Biological Laboratory at Oxford, Maryland, for diagnostic services.

Results and Discussion

Occurence of the "gray crab disease" (Sprague and Beckett, 1966) in wild populations of blue crabs was associated with massive crab mortalities in the South Atlantic states during 1966, 1967, and 1968. This condition, caused by *Paramoeba perniciosa* which infests itself in a crab's hemolymph and body tissues (figure 3), was the main target for histological investigations during this study.

As shown in Table 10 the frequency of crabs positive for diseases and parasites was not significant.

Paramoeba perniciosa was found in only 18 crabs (1.5 percent of all crabs sampled). North Carolina accounted for 14 of the infected crabs while Georgia had three and Florida had one. Two were taken in May and sixteen in June. The Oxford Laboratory will publish additional findings in the near future.

The occurence of *Urosporidium crescens* is directly associated with the trematode metacercaria *Microphallus* which it parasitizes. This hyperparasite may actually help crabs by destroying trematode parasites which are common in the crab's muscle tissue and hepatopancreas.

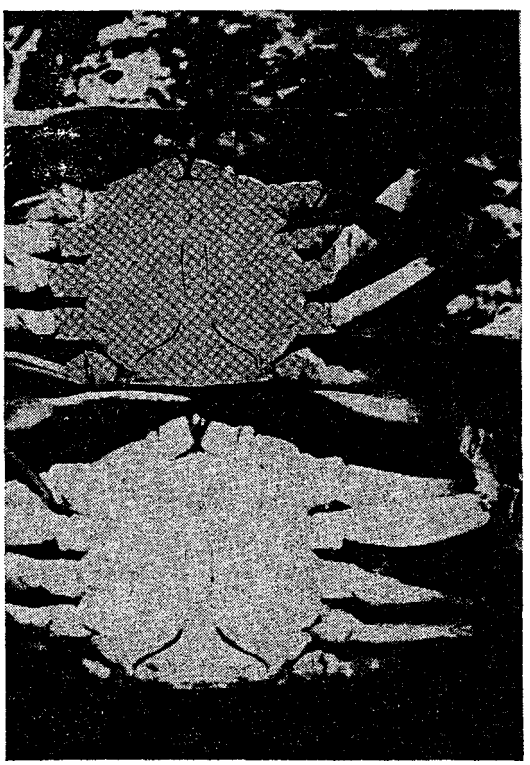

Figure 3--Blue crabs infected with *Paramoeba pernicosa* often exhiblt a gray coloration on their ventral side. This picture clearly shows the different coloration of an infected crab (top) and a normal crab (bottom).

TABLE 10.—*Summary of Diseases and Parasites found in* 1,200 *Blue Crabs Sampled from March through December* 1969. *By frequency of Positive Animals.*

	Parasites				Commensals		Other[1]
Month	*Paramoeba*	Metacercaria	*Urosporidium*[3]	Microsporida	*Balanus*	*Octolasmis*[2]	
Mar.	—	1	1	—	9	5	2
Apr.	—	—	—	5	5	3	5
May	2	—	—	—	6	5	5
June	16	—	—	1	9	—	2
July	—	—	—	—	8	12	—
Aug.	—	6	6	3	5	1	1
Sept.	—	3	3	—	7	4	—
Oct.	—	2	2	1	12	6	8
Nov.	—	1	1	1	2	2	3
Dec.	—	1	1	—	10	2	2

[1] Necrotic lesions associated with Chitinoclastic Bacteria

[2] Not recorded in North Carolina Samples

[3] Hyperparasite that parasitizes the trematode metacercaria, without the *Urosporidium* present the metacercaria are not readily detectable

Microsporidian infections occurred in crabs from North Carolina and Florida. Those found in North Carolina were associated with a small localized crab mortality. A special mortality sample of ten crabs, collected at Ocean Isle Beach near station 4, contained 5 crabs which appeared to have light to heavy microsporidian infections. Since no other casual agents were discovered, these infections could have caused this minor crab mortality.

Current information concerning the commensal *Octolasmis (mulleri) lowei* on the gills of blue crabs suggests that it is a potential pathogen. This organism occurred frequently but could not be related to crab mortalities. The organism *Balanus eburneus*, the common turtle barnacle, is not considered a potential pathogen. It was the most common organism found on blue crabs during this study. Both *Octolasmis* and *Balanus* were often found infecting the same crab, which was usually in its ultimate instar.

A total of 28 specimens exhibiting an unusual exoskeletal disease was collected at various stations in North and South Carolina. The nature of this disease was similar to that described by Rosen (1966). Routine examinations of these crabs, which were held alive in fiber glass tanks at relatively constant temperatures (20° C ± 2° C) and salinities (26‰), showed that the infection manifests from a superficial necrosis. Eight of the specimens showed signs of the syndrome on their sterna with numerous punctiform "corrosive" marks.

Necrotic lesions also occurred on the dorsal carapace of all specimens. One crab had an advanced case which eventually resulted in death; this animal's distal section of the lateral spine was detached to such an extent the gill filaments were visable. The causative agent involved was unknown, however, a review of the literature (Hess, 1937) implies that chitinoclastic bacteria are one possible agent. Zobell (1946) relates this bacteria as a common commensal on marine crustaceans. According to Rosen (1966) this disease or a similar syndrome was found more frequently in crabs living under crowded conditions for long periods. The specimens caught in South Carolina were all taken in deeper high salinity waters during the colder months.

Phase IV—LABORATORY STUDIES OF FACTORS AFFECTING CRAB MORTALITIES[1]

Methods and Materials

Since there was an absence of crab mortalities and the occurence of *Paramoeba* was so sparse, laboratory experiments were focused on the effects of certain residual pesticides on blue crabs. Pesticides were considered for bioassay in accordance with regional frequency distribution and in the final selection, DDT, Toxaphene, and Mirex were specified by the committee members as the problem compounds.

Experimental crabs

Bioassay specimens were collected from Wadmalaw Sound and stocked in reserve holding tanks prior to acclimation in the laboratory. Crabs that had just moulted or those designated as red-line peelers were segregated from the test groups. Only adult crabs with a carapace width of 5 inches or more were used in the preliminary screening tests. Juvenile crabs less than 3 inches in carapace width were used in certain delineative screening tests. All crabs were fed cut bait during the holding period prior to testing.

During the collection periods, water temperatures ranged from 24° to 33° C. Crabs to be used in low temperature experiments were collected during the colder months and conversely those for high temperature tests were taken during the summer. Salinitities in the collection area ranged from 20 to 26 parts per thousand.

Bioassay tanks and controls

Eight wooden tanks (48x24x12 inches) molded over with fiberglass were used as immersion baths. Each tank was equipped with coils of copper tubing (⅜ inch o. d.) for heat exchange and a corner airlift system to facilitate even temperature circula-

[1] This phase of the study was conducted at Bears Bluff Laboratories, Wadmalaw Island, South Carolina.

tion. Custom made glass aquaria (16x12x9 inches) were used as the test tanks for holding crabs. A total of three aquaria, each containing 20 liters of sea water, was immersed in each of the bath tanks. The 24 temperature controlled units were used simultaneously for delineative screening tests. The preliminary screening was conducted in a constant temperature room (ambient temp. 20° ± 3° C) with an additional 24 bioassay tanks.

An industrial air compressor designed for continuous duty at an operating range of 25 to 50 psi was utilized as a central unit for supplying oxygen to each bioassay tank. The aquaria were fitted individually with an adjustable air valve for controlling dissolved oxygen in each test.

Water temperatures were maintained relatively constant (± 1° C) throughout the experiments. A Blue M portable cooling unit [1] was used to cool water in a 200 gallon reserve tank. The water was then pumped continuously through the heat exchangers and returned to the reservoir for re-cooling. To prevent excessive evaporation and a loss of exchange efficiency, each immersion tank was covered with a sheet of 1-inch styrofoam insulation. A schematic diagram of the bioassay laboratory is shown in figure 4.

[1] Mention of tradename does not imply endorsement.

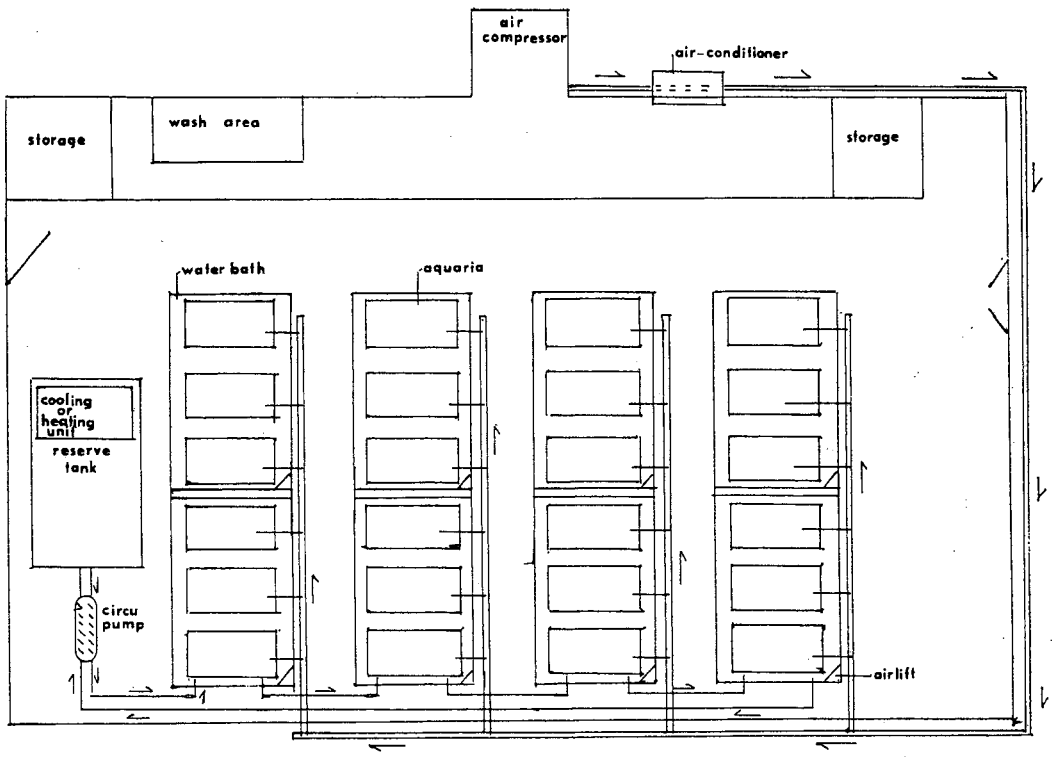

Figure 4—Lay-out of bioassay laboratory

For experiments requiring above ambient water temperatures, a Blue M immersion heater was used in the reservoir with heated water being pumped through the exchange system. Although the heater maintained reservoir temperatures to within ± 2.5° C, there was a loss of heat efficiency during circulation. To compensate for this loss, a thermostatically controlled pyrex glass immersion heater was used in each aquaria for finer accuracy.

Reduced salinities were obtained by mixing sea water with tap water in desired proportions. Increased salinities were obtained with Rila sea salts.

Bioassay Standards

Static bioassays were conducted over 96-hour exposure periods as described in Standard Methods, APHA (1965). Blue Crabs exposed to extremes of temperature and salinity were considered dead when no movement could be detected upon close observation. When exposed to lethal or near lethal concentrations of pesticides, test crabs would remain in a moribund condition for hours without recovery or reaction to mechanical stimulation. Therefore, a crab's death point was assumed upon its los of equilibrium or "overturning" in the pesticide solutions. All results were expressed as median tolerance limits (TLm) which is that concentration causing a 50% mortality or loss of equilibrium within 96 hours. Five concentrations in logarithmically increasing quantities (plus a control without pesticide additive) were tested in each set of bioassays. Six crabs were placed in each concentration and control. Prior to each test, experimental crabs were acclimated for 24 hours at constant temperatures and salinities which were gradually increased or decreased by 2 unit intervals per day until the desired testing conditions were established.

Since the pesticides were relatively insoluble in sea water, acetone was used as a solvent in preparing stock solutions of each compound. Stock solutions were titrated into the test medium to obtain desired concentrations. Acetone in volumes equivalent to the largest in a dosage series for any pesticide was always used in control tanks.

The delineative pesticide screening data were statistically analyzed according to the methods of Litchfield and Wilcoxon (1949) to determine TLm values, variations, slope functions, and 95% confidence intervals.

Lethal Levels of Temperature and Salinity

Sixty-six combinations of temperature and salinity were tested to determine the effects of abrupt changes of these environmental factors on blue crabs. Dissolved oxygen concentrations were recorded as a function of the two variables (Green and Carritt, 1967). Table 11 gives a summary of the results.

Cold induced blue crab mortalities occurred at all combinations of salinity at 0° C. At 5° C crab survival was higher at the lower salinity range of 8.6 to 13.4‰. As temperatures increased to the upper extremes (30° to 36° C) crab survival became higher at the maximum salinities. Generally, crabs were less tolerant at low salinities and high temperatures and at high salinities and low temperatures. The TLm values were estimated from the experimental data by straight-line graphical interpolation (Litchfield and Wilcoxon, 1949). Upper and lower tolerances at various salinities are listed in Table 12.

By plotting these data graphically on temperature and salinity coordinates as in figure 5, an almost linear relationship illustrating the zone of thermal tolerance is established for upper and lower values.

TABLE 11—*Reactions of adult Blue Crabs to Temperature and Salinity combinations with recorded dissolved oxygen.*

Expressed as Percent Survival over 96 hours

	Test Salinities ‰											
	8.6		13.4		19.3		24.2		30.1		36.0	
°C Temp.	D. O. ml/l	% Sur.	D. O. ml/l	% Sur.	D. O. ml/l	% Sur.	D. O. ml/l	% Sur.	D. O. ml/l	% Sur.	D. O. ml/l	% Sur.
0	9.6	0	9.4	0	9.0	0	8.7	0	8.4	0	8.0	0
5	8.6	80	8.3	60	7.9	40	7.6	20	7.3	0	7.1	0
10	7.5	100	7.3	100	7.0	100	6.8	100	6.5	40	6.3	20
15	6.7	100	6.5	100	6.3	100	6.1	100	5.9	100	5.7	40
18	6.3	100	6.1	100	5.9	100	5.7	100	5.5	100	5.3	40
21	5.9	80	5.7	80	5.6	100	5.4	100	5.2	100	5.0	80
24	5.6	20	5.4	80	5.2	100	5.1	100	5.0	100	4.8	100
27	5.3	0	5.1	40	4.9	100	4.8	100	4.7	100	4.5	100
30	5.0	0	4.8	40	4.7	80	4.6	100	4.4	100	4.3	80
33	4.7	0	4.6	0	4.4	0	4.3	100	4.2	100	4.0	100
36	4.5	0	4.4	0	4.3	0	4.1	0	4.0	40	3.9	80

TABLE 12—*Estimated 96-hour TLm for adult Blue Crabs at various salinities*

Sal. ‰	Acclimation temp °C ± 2	TLm Values °C Upper	Lower
8.6	20	22.0	3.2
13.4	20	26.1	3.8
19.3	20	30.6	5.6
24.2	20	34.2	6.5
30.1	20	35.2	10.7
36.0	20	35.2	18.5

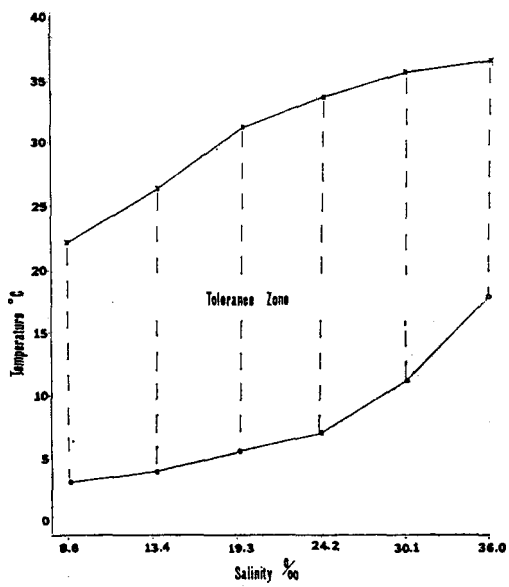

Figure 5—Upper and lower thermal tolerances for adult blue crabs exposed to abrupt changes in temperature and salinity.

On the lower end of the correlation, the minimum thermal tolerance limits decrease as salinities increase. The upper tolerance limits show a reverse pattern with maximum tolerance at 35.2° C and a downward trend corresponding with decreasing salinities. By connecting the upper and lower thermal tolerance limits, the range of all tolerable temperatures is shown at each salinity. Tagatz (1969) diagramed upper and lower thermal 48-hour TLm values against acclimation temperatures. His results showed that at both high and low salinities the upper and lower TLm values increased with increases in acclimation temperatures. His data generally agree with results of this study. Acclimation time and temperature was an obvious factor in the final results of the two studies. The percentage of adult crab survival apparently becomes greater as the difference between acclimation and test temperature decreases.

There appears to be an important physiological-ecological relationship among the tolerance limits at various temperatures and salinities. The metabolic rate of crustaceans is generally temperature oriented with higher rates of metabolism corresponding with temperature increases (Waterman, 1960). King (1964) also showed that lower salinities had a marked increase on blue crab metabolism. This indicates that at low salinities and high temperatures the strain of osmoregulation would add to the stress of metabolism and thus decrease the upper TLm values at such combinations. In a reverse situation, low temperatures may reduce metabolic activity to such a degree that it would be difficult

for crabs in high salinities to maintain a favorable gradient between external and internal salinities. Rees (1966) stated that blue crabs in full strength sea water (35‰) maintained a blood concentration slightly below that of the water regardless of temperatures between 10° and 30° C. However, Tan and Van Engel (1966) showed that blood osmoconcentrations of adult blue crabs were hypertonic to 10‰, 20‰, and 30‰ salinities at 20° C. Rees (1966) further emphasized that in most cases higher blood concentrations were maintained with temperature decreases. This would indicate that blue crabs can successfully regulate their internal environments near the lower end of their thermal tolerances. Rees (1966) also stated that adult female blue crabs showed less regulatory abilities than adult males in the lower salinities. This differential ability to regulate sodium in the blood may well explain why sexually mature females prefer higher salinity waters, especially during fall and winter months.

Effects of Chlorinated Pesticides on Blue Crabs

Preliminary Screening

Initial tests with DDT and Toxaphene indicated a rather high level of toxicity to adult blue crabs; technical mirex in solution was relatively non-toxic to adult and sub-adult crabs. However, if ingested, Mirex, in the form of granulated bait was toxic to juvenile crabs.

All test crabs died after 24 hours of continuous exposure to 1.0 ppm DDT. Toxaphene killed 100% of the test crabs after 72 hours exposure at 10 ppm. Technical Mirex in suspension had measurable effects only at the end of 72 hours exposure in concentrations exceeding 500 ppm. Mirex granulated bait (85% corncrib grit, 15% soybean oil, and 0.3% Mirex) ingested by juvenile crabs showed delayed toxicity when present at equivalent rates of 1.25 pounds per acre.

Test crabs responded to the more toxic polychlors within a few hours after exposure, displaying extreme sensitivity to external movement and sound. Reactions began with increased activity and erratic swimming, and ended with convulsions and a loss of equilibrium.

Delineative Screening

Results from testing combinations of temperature and salinity against varying pesticide concentrations are recorded as 96-hour TLm values in Table 13. DDT was more toxic than Toxaphene or technical Mirex at all combinations. A comparative relationship is illustrated in figure 6.

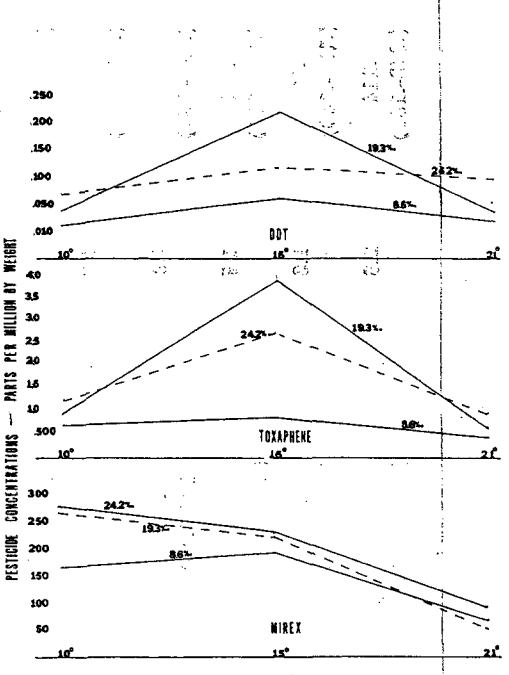

Figure 6—Comparative 96-hour TLm values of DDT, Toxaphene, and Mirex at various combinations of temperatures and salinity.

Table 13—*Toxicity of pesticides on adult blue crabs at various temperatures and salinities (96-hour TLm)*

TLm (PPM) with 95 percent Confidence Interval and Slope Functions							
Sal.	Temp.	DDT		Toxaphene		Mirex	
‰	°C	TLm	SF	TLm	SF	TLm	SF
	10	.019 (.009-.036)	1.5	.580 (.460-.920)	2.1	159 (83-302)	3.0
8.6	15	.054 (.030-.084)	2.5	.900 (.470-1.70)	3.0	180 (128-250)	1.8
	21	.035 (.021-.057)	1.9	.370 (.180-.700)	3.2	72 (48-108)	2.1
	10	.043 (.025-.078)	3.1	.960 (.590-1.50)	2.7	260 (173-390)	2.0
19.3	15	.213 (.160-.280)	1.7	3.80 (.270-5.20)	1.8	220 (137-352)	2.6
	21	.045 (.030-.067)	1.8	.770 (.570-1.00)	1.7	56 (40- 78)	1.7
	10	.080 (.070-.110)	1.5	1.20 (.910-1.50)	1.8	265 (188-371)	1.8
24.2	15	.120 (.100-.140)	1.3	2.70 (1.3- 5.9)	3.4	220 (152-275)	2.0
	21	.114 (.073-.180)	2.6	1.00 (.570-1.75)	3.2	105 (75-147)	1.3

All three compounds were more toxic with decreasing salinity. At each salinity, however, the defined tolerance limits were higher at 15° C. Above and below this mid-point, the TLm values decreased with toxicity being more pronounced at the lower extremes for DDT and Toxaphene. Mirex was more toxic at higher temperatures within each salinity bracket. This compound, however, cannot be classified with DDT and Toxaphene since its order of toxicity as a contact poison is comparatively low.

Lethal levels of DDT were established within relatively narrow confidence intervals, indicating consistent toxic effects with little range between concentrations causing survival and death. The calculated slope functions were low (ranging from 1.3 to 3.1) and the regression was steep, indicating that toxicity was accurately defined at low levels. In sharp contrast, Marking (1966) found the slope function p. p.' - DDT for goldfish to be 6.02. This was indicative of a flat curve with wide confidence intervals and, consequently the toxicity was difficult to define accurately.

The confidence limits for the TLm values of Toxaphene were somewhat wider than those of DDT and the calculated slope functions larger. This indicates that increased concentrations in the survival and mortality range produced less effect within the 96-hour bioassay.

The toxicity of Mirex in acetone solution was difficult to define accurately as evidenced by the high TLm values and wide confidence intervals. This compound remained in solution only a short time before precipitating out. Mirex granulated bait-4X was bioassayed with adult, sub-adult, and juvenile crabs. Adult (5 inches or more), and sub-adult (3 to 5 inches) crabs were not outwardly affected by the bait material even in equivalent doses of 10 times the standard application rate of 1.25 pounds per acre. However, juvenile crabs (< 3 inches) exhibited extreme sensitivity to the bait. At concentrations of .036 grams per 2.8 square feet of surface water area[1], juvenile crabs showed variable signs of delayed toxicity at temperature - salinity combinations. Table 14 presents a summary of replicate tests conducted with the bait.

There were delayed toxic effects at all combinations except 10° C. An increase in dosage from .036 g/liter to 1 g/liter did not substantially alter the survival times. Follow-up experiments with high concentrations indicated a threshold reaction time; a stage was reached when further increases in concentration did not shorten survival time. Extensive observation showed the juvenile test crabs ingesting particles of the bait. These crabs were transferred to non-contaminated aquaria and monitored. After 96 hours in pesticide free water, the crabs showed acute irritation which resulted in spasmotic muscle contractions, a loss of equilibrium and finally death at the end of 192 hours. These data suggest that Mirex bait acts as a stomach poison and, if ingested by juvenile crabs, is a definite mortality factor.

Figure 7 illustrates the relationship between temperature and relative toxicity of the bait.

[1] Equivalent in gram wt./water surface area to 1.25 lbs./acre as calculated from aquaria measuring 16.7 inches.

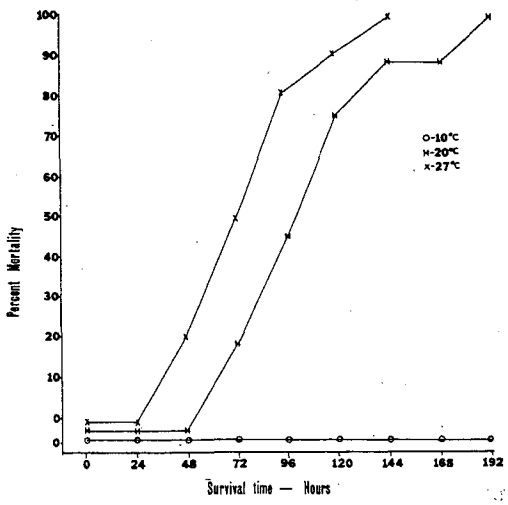

Figure 7—Survival curves for juvenile crabs exposed to equivalents of 1.25 lbs./acre Mirex bait at salinity of 22‰.

TABLE 14.—*Average survival data from replicate tests on juvenile blue crabs exposed to Mirex Granulated Bait - 4X[1]. .(The numbers under each concentration indicate percent survival after the given exposure time)*

	Bait Concentrations						
	.036 g[2]					0.5 g[3]	1.0 g[4]
	22‰			30‰	10‰	22‰	22‰
Exposure Time Hours	10°C	20°C	27°C	15°C	20°C	20°C	20°C
0	100	100	100	100	100	100	100
24	100	100	100	100	100	100	100
48	100	100	80	100	75	100	94
72	100	81	50	100	63	75	94
96	100	63	20	100	38	50	69
120	100	25	10	75	38	31	44
144	100	13	0	50	38	19	25
168	100	13	0	50	25	6	13
192	100	0	0	44	0	0	6

1 Controls averaged 90% survival or better in all tests.

2 Gram equivalent to standard application rate.

3 Gram equivalent to 13.8 times standard application rate.

4 Gram equivalent to 27.7 times standard application rate.

As depicted, the toxicity rate appears to be temperature dependent. At 10° C there was no mortality recorded but as temperatures increased from 20° to 27°C the survival times and rates decreased. This would indicate that toxicity might be defined as a function of metabolism. A partial relationship between salinity and toxicity of the bait material is evident. However, temperatures appeared to be the major factor of influence. Further experimentation showed that small crabs which had ingested Mirex bait at 27° C could survive for an extended time at 10°C. However, as temperatures were gradually increased there were concurrent mortalities. Interpretation of these data indicates that juvenile crabs ingesting the bait during winter months could possibly survive throughout the colder months, but as seasonal temperatures increased mortality would occur.

These data are purely suggestive but from preliminary evaluations, it would appear that acute toxicity of the Mirex bait depends on (1) availability of the bait to hungry crabs, (2) size and age of the exposed crabs, and (3) the season of exposure.

Related Discussion

The toxic effects of DDT and Toxaphene on blue crabs have been thoroughly documented. Butler (1963) presented data on the effective concentration (EC_{50}) of both these compounds on juvenile blue crabs. The 48-hour EC_{50} for DDT and Toxaphene was .01 and .33 ppm, respectively. Comparing these results with those obtained from this study, the adult crabs tested under similar conditions (24‰ and 21° C) are approximately 10 times more tolerant to DDT and 3.4 times more resistant to Toxaphene. This study gave evidence that decreased temperature was a definite factor affecting the toxicity of DDT and Toxaphene. Bridges et al. (1963) also found that toxicity of DDT increased with temperature decrease.

Butler (1963) listed Mirex in solution as a relatively non-toxic pesticide to juvenile crabs. The 48-hour EC_{50} was a high as 2 ppm. Later studies by McKenzie (1969) and Lowe (1969) suggested that the bait formulation was a stomach poison rather than a contact poison to juvenile blue crabs. The toxic effects, however, were delayed but once the bait material was ingested moribundity was evident af-

ter several days. Field studies conducted at Bears Bluff Laboratories indicated that standard applications of Mirex directly to the estuary had no observable effects on adult and juvenile crabs which were caged within the test zone. Further studies indicated that adult crabs could accumulate extremely high residual levels of Mirex (8,860 ppm) in the stomach and still maintain an apparent resistance to the compound.

Miscellaneous Studies

Diseased Crabs

North Carolina reported 14 blue crabs positive for *Paramoeba* from Brunswick County near the South Carolina line. Immediately, biologists obtained live specimens from the area for bioassay. The test crabs were delivered to Bears Bluff Laboratories and stocked in fiberglass tanks with water temperatures and salinities adjusted to equal those of the sampling area. Control crabs were collected from Wadmalaw Sound and stocked in a comparable environment. Hemolymph was withdrawn from the North Carolina crabs and injected into the hinge of the dactylus of 10 control crabs and into the cardiac sinus of 5 controls. All except one of the injected crabs survived without any gross morphological or physiological changes. Test crabs showed no signs of the "gray crab syndrome" and temperature-salinity variations failed to show correlation between mortalities and diseases. Later analyses of hemolymph smears were negative for *Paramoeba*.

Summary and Conclusions

The cooperative Blue Crab Project was initiated as a result of massive blue crab mortalities, which occurred in 1966, 1967, and 1968, along with a general decline in crab production on the South Atlantic coast. This study was a cooperative effort between the states of North and South Carolina, Georgia, and Florida.

The main purpose of the study was to investigate environmental and pathological factors possibly associated with blue crab mortalities. Water chemistry, pesticides, diseases, and parasites were monitored monthly, from March 1 to December 31, 1969, at selected stations along the South Atlantic coast. During this period no major mortalities occurred at these stations thus preventing a correlation of data and crab mortalities.

A total of 195 hydrological samples was taken at the 20 stations. Results of the routine monitoring phase for hydrology are useful in illustrating seasonal changes in the blue crab study areas. In general, seasonal variations were typical when compared with available hydrological data.

A total of 1,950 blue crabs was processed and analyzed for pesticide residue levels. All samples contained DDT and its metabolites, 35% contained Mirex and 20% Dieldrin. From these data it can be assumed that most, if not all, blue crabs along the South Atlantic coast contain varying amounts of DDT, DDE, and DDD.

Pesticide concentrations lethal to blue crabs in the natural environment are difficult to establish. Because of the tendency of organisms to concentrate these hydrocarbons through the food chain to levels well above that of the surrounding medium, a relatively low initial concentration may ultimately cause mortalities. In addition, there is undoubtedly a synergistic affect of pesticide levels and other environmental abnormalities such as industrial pollution (Lowe, 1965).

Pesticide data collected during this study indicates a definite contamination of estuarine waters, representing a potentially dangerous situation, which should be kept under surveillance. In future pesticide sampling it is suggested that more information be obtained by analysis of water and bottom samples in addition to tissue samples.

Blue crabs collected and processed for diseases and parasites totaled 1,170. Although apparently prevalent in two prior years, there were no major outbreaks of "gray crab sickness" during this study. Several potential pathogens were present but not abundant.

Data collected under the disease and parasite phase of the study will contribute to the overall body of knowledge being compiled by the Bureau of Commercial Fisheries Laboratory at Oxford, Maryland. This data is expected to be of value in the event of future mass mortalities.

Laboratory tests showed crabs to be less tolerant to pesticides at low salinities and high temperatures and at high salinities and low temperatures. Chlorinated hydrocarbon pesticides were most toxic at low salinity levels. Mirex, the technical compound, was relatively non-toxic to adult and sub-adult crabs. However, Mirex in a granulated bait was toxic to juvenile blue crabs if ingested.

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APPENDIX

Appendix Table A—*Hydrological data collected at the five North Carolina sampling stations.*

Month	Bottom Temp. (°C)	DO (ppm.)	Salinity (‰)	pH	Turbidity (cm.)
			Station 1		
Mar.	10.0	8.6	31.00	7.4	77
Apr.	21.1	7.7	32.90	7.4	61
May	20.0	5.8	32.85	7.5	64
June	26.4	8.4	34.47	7.5	76
July	28.0	7.3	33.93	7.5	80
Aug.	27.2	7.2	29.62	7.8	75
Sep.	25.0	4.1	29.62	7.8	55
Oct.	23.3	6.5	33.93	8.3	95
Nov.	14.4	7.7	30.69	8.3	100
Dec.	10.0	8.2	32.85	8.1	155
			Station 2		
Mar.	9.4	9.6	35.30	7.4	70
Apr.	21.1	8.3	33.40	7.2	79
May	20.0	7.3	33.39	7.5	80
June	26.4	8.3	31.23	7.8	89
July	28.0	8.3	29.62	7.5	85
Aug.	27.8	8.6	32.85	7.8	68
Sep.	26.7	5.2	31.77	7.8	77
Oct.	22.8	6.4	34.47	7.8	55
Nov.	16.7	7.7	32.85	8.2	65
Dec.	9.4	7.9	33.93	8.0	85
			Station 3		
Mar.	7.8	9.8	12.00	7.6	46
Apr.	18.9	7.4	21.50	7.0	67
May	20.0	8.5	31.77	7.8	54
June	26.5	6.0	11.85	7.5	68
July	28.5	7.2	12.38	7.5	70
Aug.	27.8	6.0	15.62	7.4	56
Sep.	25.6	5.4	28.54	7.8	58
Oct.	21.7	5.6	26.38	7.8	47
Nov.	15.6	6.8	21.00	7.8	63
Dec.	9.4	7.4	25.31	7.8	135
			Station 4		
Mar.	6.7	10.8	22.00	7.5	107
Apr.	18.3	9.5	24.60	7.3	113
May	20.6	9.4	25.31	7.5	105
June	27.0	9.2	24.60	7.5	86
July	27.5	9.2	23.69	7.5	85
Aug.	27.8	8.8	11.85	7.5	52
Sep.	27.8	6.8	21.00	8.2	62
Oct.	22.2	7.7	24.23	8.2	85
Nov.	14.4	8.5	26.50	7.2	104
Dec.	8.3	8.9	19.92	8.3	120
			Station 5		
Mar.	3.3	11.6	22.00	7.5	70
Apr.	18.3	8.9	26.50	7.2	104
May	22.2	6.2	31.23	7.8	73
June	26.4	6.2	26.92	7.5	85
July	28.0	7.2	25.31	7.7	80
Aug.	31.1	7.8	28.54	7.8	56
Sep.	26.7	6.0	25.31	8.2	86
Oct.	19.4	7.6	21.00	8.2	67
Nov.	13.3	8.4	18.85	7.8	166
Dec.	8.9	9.5	25.85	7.9	250

Appendix Table B—*Hydrological data collected at the five South Carolina sampling stations*

Month	Bottom Temp. (°C)	DO (ppm.)	Salinity (‰)	pH	Turbidity (cm.)
			Station 1		
Mar.	8.7	8.0	31.8	8.5	32
Apr.	19.5	3.0	31.9	8.5	56
May	24.1	8.0	28.0	8.0	10
June	26.8	6.0	28.0	8.0	16
July	30.8	6.0	32.3	8.0	25
Aug.	26.5	8.0	2.7	7.5	24
Sep.	25.0	5.0	24.0	7.5	25
Oct.	24.0	6.5	25.0	8.0	28
Nov.	13.0	6.0	22.6	8.0	27
Dec.	11.0	7.0	27.0	8.0	38
			Station 2		
Mar.	10.0	9.0	13.0	8.5	145
Apr.	19.0	7.0	20.2	8.5	83
May	26.0	6.0	12.3	8.0	40
June	28.0	7.0	16.0	8.0	25
July	29.0	7.0	14.0	8.0	20
Aug.	28.0	7.5	10.7	8.0	25
Sep.	27.5	3.0	22.0	8.0	35
Oct.	24.0	5.0	18.0	8.0	10
Nov.	18.0	5.0	18.3	8.0	20
Dec.	12.0	5.0	18.0	8.0	20
			Station 3		
Mar.	12.0	8.0	30.8	8.5	85
Apr.	19.1	8.0	30.0	8.5	85
May	24.0	7.0	32.3	8.0	45
June	26.0	5.0	25.0	8.0	15
July	31.0	6.0	30.0	8.0	35
Aug.	27.0	7.0	31.0	8.0	30
Sep.	27.5	7.0	32.0	8.0	30
Oct.	26.0	7.0	32.0	8.0	30
Nov.	18.0	6.0	24.0	8.0	20
Dec.	10.0	7.0	23.0	8.0	33
			Station 4		
Mar.	8.7	8.0	29.0	8.5	147
Apr.	18.2	7.2	28.7	8.5	85
May	24.0	8.0	29.0	8.0	35
June	27.5	6.0	29.0	8.0	30
July	30.3	5.0	32.0	8.0	25
Aug.	29.0	6.0	27.4	8.0	20
Sep.	27.0	4.0	17.0	8.0	66
Oct.	24.0	6.0	18.0	8.0	16
Nov.	15.0	5.0	29.0	8.0	35
Dec.	12.0	7.0	17.0	7.5	30
			Station 5		
Mar.	13.5	9.0	23.9	8.5	120
Apr.	23.0	7.0	84.2	8.5	60
May	26.0	5.0	23.1	8.0	25
June	29.5	5.0	25.8	8.0	35
July	29.0	6.0	26.9	8.0	20
Aug.	27.0	6.0	19.0	7.0	16
Sep.	27.0	6.0	19.0	8.0	16
Oct.	19.0	6.0	15.0	8.0	15
Nov.	12.0	7.0	14.0	8.0	25
Dec.	10.0	5.0	20.0	8.0	25

Appendix Table C—*Hydrological data collected at the five Georgia Sampling Stations*

Month	Bottom Temp. (°C)	DO (ppm.)	Salinity (‰)	pH	Turbidity (cm.)
			Station 1		
Mar.	10.0	7.8	24.77	7.7	177
Apr.	22.5	6.6	23.69	7.8	81
May	23.0	5.5	16.15	7.8	86
June	28.5	5.1	19.38	7.7	56
July	30.0	4.8	24.77	7.7	61
Aug.	27.0	4.5	22.08	7.7	22
Sep.	26.0	4.9	19.92	7.3	81
Oct.	19.0	6.4	23.16	7.8	59
Nov.	16.5	7.4	25.85	7.8	125
Dec.	10.0	9.2	28.08	7.7	111
			Station 2		
Mar.	11.5	8.2	26.92	7.6	122
Apr.	20.5	6.8	27.46	7.7	112
May	25.0	4.4	24.77	7.6	76
June	28.0	3.0	22.62	7.6	61
July	29.5	3.0	21.00	7.6	36
Aug.	29.0	3.2	25.85	7.7	55
Sep.	26.0	4.3	18.85	7.7	25
Oct.	25.0	4.1	22.08	7.6	33
Nov.	16.5	7.2	23.69	7.9	48
Dec.	10.0	9.4	24.23	7.9	42
			Station 3		
Mar.	12.0	8.6	24.23	7.9	112
Apr.	20.0	7.8	27.46	7.9	81
May	24.0	5.4	21.54	7.7	86
June	28.5	3.9	22.62	7.8	84
July	29.5	4.3	24.23	7.7	50
Aug.	28.5	3.7	17.77	7.6	50
Sep.	28.0	4.4	16.15	7.3	38
Oct.	21.0	6.4	22.66	7.7	58
Nov.	14.0	8.5	23.16	7.9	55
Dec.	10.0	9.4	18.85	7.8	81
			Station 4		
Mar.	17.0	7.0	23.69	7.8	61
Apr.	19.0	7.1	25.85	7.7	132
May	22.5	5.8	23.69	7.9	86
June	26.0	5.2	20.46	7.9	112
July	29.5	5.7	32.31	8.1	147
Aug.	29.0	5.7	31.23	7.9	61
Sep.	25.5	5.0	16.69	7.6	25
Oct.	27.0	6.3	22.08	7.7	36
Nov.	18.0	7.4	28.00	8.2	22
Dec.	10.0	9.3	25.31	8.0	38
			Station 5		
Mar.	18.0	6.8	24.23	7.4	167
Apr.	21.0	6.5	26.38	7.9	112
May	24.5	5.4	26.92	7.8	64
June	28.5	4.2	26.38	7.8	58
July	31.0	4.6	29.08	7.9	61
Aug.	28.5	3.2	27.46	7.6	61
Sep.	25.5	5.2	18.85	7.6	43
Oct.	25.5	4.5	17.23	7.5	61
Nov.	18.5	6.2	15.08	7.7	60
Dec.	12.0	8.6	17.77	7.8	60

Appendix Table D—*Hydrological data collected at the five Florida Sampling Stations*

Month	Bottom Temp. (°C)	DO (ppm.)	Salinity (‰)	pH	Turbidity (cm.)
			Station 1		
Mar.	-	-	-	-	-
Apr.	21.5	4.7	19.92	7.4	70
May	25.0	5.7	26.92	7.9	105
June	29.5	6.9	24.77	8.0	120
July	30.0	6.3	24.23	7.8	155
Aug.	29.0	5.2	15.62	7.5	140
Sep.	28.5	5.3	12.38	7.5	85
Oct.	25.5	3.5	1.08	7.2	70
Nov.	20.0	5.0	1.08	7.5	70
Dec.	15.5	7.0	23.69	8.0	65
			Station 2		
Mar.	-	-	-	-	-
Apr.	20.5	3.6	32.31	7.7	90
May	26.0	5.3	33.93	8.0	120
June	27.0	6.5	33.93	8.0	95
July	29.9	5.6	33.39	7.6	90
Aug.	27.9	6.2	34.47	8.0	90
Sep.	28.0	4.8	24.85	7.6	45
Oct.	25.0	4.8	15.62	7.5	43
Nov.	18.0	5.1	13.46	7.5	75
Dec.	16.5	6.1	26.91	7.5	55
			Station 3		
Mar.	-	-	-	-	-
Apr.	20.8	1.7	30.15	7.0	70
May	25.5	2.5	31.23	7.5	60
June	29.5	1.9	29.62	7.4	75
July	29.5	4.0	32.31	7.5	105
Aug.	31.0	5.3	31.23	7.5	60
Sep.	29.0	4.7	21.54	7.2	45
Oct.	24.5	2.3	16.15	7.0	43
Nov.	17.5	4.1	19.92	7.5	85
Dec.	15.5	5.8	24.23	7.5	65
			Station 4		
Mar.	-	-	-	-	-
Apr.	20.3	2.9	30.15	7.4	70
May	25.0	3.4	30.69	7.5	75
June	30.0	4.9	30.15	7.6	95
July	29.5	5.2	30.69	7.6	145
Aug.	30.0	5.3	35.54	8.0	150
Sep.	29.0	3.1	23.16	7.5	60
Oct.	24.5	4.4	23.69	7.6	75
Nov.	18.5	5.4	29.62	8.0	80
Dec.	15.0	6.8	26.92	7.6	70
			Station 5		
Mar.	-	-	-	-	-
Apr.	18.6	2.6	29.08	8.0	65
May	25.5	3.6	29.62	7.5	90
June	29.5	7.2	25.31	7.5	90
July	30.0	6.7	29.08	7.7	135
Aug.	30.0	5.4	30.69	7.5	90
Sep.	27.5	4.0	26.92	7.5	75
Oct.	24.5	3.1	17.23	7.2	60
Nov.	18.5	4.8	23.16	7.7	85
Dec.	14.5	6.8	29.62	8.0	127

Appendix Table E—*Pesticide residue levels found in the soft tissue of blue crabs collected in North Carolina. Pesticide levels are expressed in ppm.*

Month	Sta.	Lab.No.	DDE	DDD	DDT	Dieldrin	Mirex
March	1	C-36	.063	.090	.170	-	-
	2	C-17	.058	.084	.195	-	-
	3	C-18	.086	.072	.134	.005	-
	4	C-19	.067	.072	.111	-	-
	5	C-21	.122	.087	.099	-	-
April	1	C-40	.065	.057	.078	-	-
	2	C-37	.060	.028	.058	-	-
	3	C-34	.120	.067	.134	-	-
	4	C-50	.076	.048	.089	-	-
	5	C-20	.063	0.47	.099	-	-
May	1	C-47	.043	.026	.055	-	-
	2	C-33	.099	.188	.213	-	-
	3	C-41	.076	.052	.080	-	-
	4	C-22	.53	.090	.173	-	-
	5	C-42	.051	.024	.051	-	-
June	1	C-44	.048	.031	.065	-	-
	2	C-43	.017	.020	.031	-	-
	3	C-49	.100	.063	.085	-	-
	4	C-45	.014	.016	.027	-	-
	5	C-46	.024	.013	.037	-	-
July	1	C-51	.076	.048	.089	-	-
	2	C-38	.035	.040	.083	-	-
	3	C-32	.108	.075	.127	-	-
	4	C-48	.053	.021	.059	-	-
	5	C-39	.047	.055	.109	-	-
August	1	C-107	.080	.068	.108	-	-
	2	C-109	.040	.051	.070	-	-
	3	C-158	.060	.090	.095	-	-
	4	C-113	.045	.062	.080	-	-
	5	C-111	.042	.060	.082	-	-
September	1	C-103	.075	.080	.095	-	-
	2	C-115	.060	.060	.085	-	-
	3	-	.057	.073	.092	-	-
	4	C-104	.060	.080	.085	-	-
	5	C-110	.051	.070	.082	-	-
October	1	C-163	.022	.035	.030	-	-
	2	C-161	.023	.035	.036	-	.012
	3	C-114	.092	.081	.130	-	-
	4	C-153	.042	.032	.036	-	.020
	5	C-154	.042	.034	.051	-	-
November	1	C-162	.023	.035	.025	-	-
	2	C-160	.038	.039	.036	-	.005
	3	C-156	.068	.026	.035	-	.008
	4	C-155	.018	.014	.020	-	-
	5	C-157	.026	.030	.035	-	.020
December	1	C-181	.013	.012	.027	.003	.045
	2	C-183	.020	.011	.012	.003	-
	3	C-179	.020	.014	.023	.004	.045
	4	C-182	.020	.010	.025	.002	.030
	5	-	.013	.012	.027	-	-

Appendix Table F—*Pesticide residue levels found in the soft tissue of blue crabs collected in South Carolina. Pesticide levels are expressed in ppm.*

Month	Sta.	Lab.No.	DDE	DDD	DDT	Dieldrin	Mirex
March	1	C-16	.095	.107	.247	-	.112
	2	C-15	.134	.085	.110	-	.089
	3	C-13	.096	.149	.172	-	.074
	4	C-14	.086	.126	.162	-	-
	5	C-12	.153	.117	.247	.005	-
April	1	C-85	.113	.134	.120	-	.140
	2	C-87	.115	.114	126	-	.100
	3	C-88	.120	.072	.063	-	.120
	4	C-94	.083	.072	.088	.014	-
	5	C-86	.134	.095	.095	-	-
May	1	C-91	.059	.048	.074	.020	.125
	2	C-93	.165	.101	.087	.020	.209
	3	C-94	.083	.072	.088	.014	-
	4	C-88	.120	.072	.063	-	.179
	5	C-90	.050	.031	.029	-	-
June	1	C-97	.082	.107	.128	.014	-
	2	C-96	.050	.129	.106	-	-
	3	C-92	.096	.025	.071	.019	-
	4	C-99	.043	.076	.104	-	-
	5	C-98	.057	.107	.126	-	-
July	1	C-101	.072	.114	.151	-	-
	2	C-102	.108	.160	.166	-	-
	3	C-117	.085	.102	.130	-	.086
	4	C-62	.123	.058	.103	-	-
	5	C-100	.061	.126	.130	-	-
August	1	C-63	.092	.051	.065	.004	.150
	2	C-66	.108	.072	.083	-	.075
	3	C-65	.180	.116	.099	-	.085
	4	C-64	.163	.076	.123	-	-
	5	C-61	.117	.087	.106	-	-
September	1	C-121	.126	.114	.120	-	-
	2	C-119	.140	.104	.130	-	.080
	3	C-118	.095	.110	.105	-	.106
	4	C-120	.104	.101	.135	-	-
	5	C-116	.115	.130	.162	-	-
October	1	C-141	.020	.029	.033	-	-
	2	C-138	.048	.096	.108	-	-
	3	C-139	.030	.040	.051	-	-
	4	C-140	.050	.064	.068	-	-
	5	C-142	.068	.060	.046	-	.030
November	1	C-193	.022	.015	.015	.002	.038
	2	192	.024	.016	.027	.002	-
	3	190	.017	0.10	.015	-	-
	4	191	.011	.009	.018	-	-
	5	185	.030	.022	.016	.004	.010
December	1	187	.012	.010	.012	.005	.010
	2	188	.028	.017	.018	-	.052
	3	186	.030	.010	.014	.002	-
	4	189	.015	.011	.019	.005	.005
	5	194	.030	.025	.023	-	.067

Appendix Table G—*Pesticide residue levels found in the soft tissue of blue crabs collected in Georgia.*
Pesticide levels are expressed in ppm.

Month	Sta.	Lab.No.	DDE	DDD	DDT	Dieldrin	Mirex
March	1	C-22	.101	.081	.108	-	-
	2	C-23	.047	.067	.097	-	-
	3	C-24	.101	.179	.145	-	-
	4	C-25	.064	.072	.083	-	-
	5	C-26	.094	.104	.106	-	-
April	1	C-6	.082	.101	.134	-	-
	2	C-7	.073	.036	.106	.006	-
	3	C-9	.143	.060	.108	.011	.294
	4	C-10	.231	.060	.176	-	.308
	5	C-8	.075	.075	.165	.010	-
May	1	C-2	.055	.068	.109	-	-
	2	C-1	.045	.052	.073	-	-
	3	C-4	.088	.085	.142	-	-
	4	C-3	.077	.098	.126	-	-
	5	C-5	.075	.090	.114	-	-
June	1	C-125	.092	.097	.115	-	.165
	2	C-126	.085	.090	.120	.004	-
	3	C-127	.120	.090	.160	.008	.201
	4	C-84	.063	.048	.067	-	-
	5	C-83	.072	.047	.058	.005	-
July	1	C-82	.072	.043	.071	-	.298
	2	C-124	.102	.120	.136	-	.120
	3	C-73	.120	.102	.160	-	.225
	4	C-123	.082	.098	.112	-	-
	5	C-122	.075	.068	.089	-	-
August	1	C-131	.081	.103	.109	-	.106
	2	C-128	.090	.101	.098	-	-
	3	C-132	.102	.075	.120	-	.103
	4	C-129	.120	.108	.125	-	.108
	5	C-130	.065	.090	.108	-	.070
September	1	C-136	.080	.092	.130	.015	.195
	2	C-133	.090	.089	.095	-	.080
	3	C-135	.030	.034	.039	-	.070
	4	C-134	.070	.115	.105	-	.095
	5	C-137	.072	.085	.120	-	.105
October	1	C-172	.069	.038	.044	.072	.330
	2	C-173	.139	.039	.054	.018	.190
	3	C-171	.139	.079	.043	-	.038
	4	C-169	.015	.057	.048	-	-
	5	C-170	.038	.025	.029	-	.015
November	1	C-197	.040	.023	.037	.025	.389
	2	C-196	.038	.016	.018	.014	.127
	3	C-199	.078	.060	.063	.012	.140
	4	C-198	.024	.023	.060	.030	-
	5	C-195	.022	.012	.024	.005	-
December	1	C-204	.019	.016	.018	.025	.143
	2	C-201	.026	.015	.046	-	.209
	3	C-203	.072	.030	.062	.005	.150
	4	C-202	.043	.030	.093	.030	.112
	5	C-200	.040	.020	.035	.008	-

Appendix Table H—*Pesticide residue levels found in the soft tissue of blue crabs collected in Florida.*

Pesticide levels are expressed in ppm.

Month	Sta.	Lab.No.	DDE	DDD	DDT	Dieldrin	Mirex
April	1		.064	.155	.196	-	-
	2		.072	.155	.166	-	-
	3		.046	.060	.114	-	-
	4		.060	.068	.079	-	-
	5		.108	.180	.180	-	-
May	1		.055	.068	.090	-	-
	2		.070	.084	.102	-	-
	3		.075	.098	.120	-	-
	4		.036	.043	.065	-	-
	5		.114	.096	.117	-	-
June	1		.036	.120	.108	-	-
	2		.048	.060	.076	-	-
	3		.065	.080	.165	-	-
	4		.064	.078	.095	-	-
	5		.029	.040	.055	-	-
July	1		.032	.048	.065	-	-
	2		.049	.051	.089	-	-
	3		.068	.095	.128	-	-
	4		.075	.085	.096	-	-
	5		.080	.095	.130	-	-
August	1		.048	.072	.099	-	-
	2		.043	.080	.045	-	-
	3		.065	.090	.110	-	-
	4		.055	.082	.098	-	-
	5		.066	.095	.101	-	-
September	1		.012	.021	.049	-	-
	2		.023	.039	.068	-	.009
	3		.026	.064	.042	-	.030
	4		.044	.096	.117	-	-
	5		.070	.060	.090	-	-
October	1		.024	.035	.078	.008	.038
	2		.042	.042	.036	-	.025
	3		.021	.058	.038	-	.028
	4		.018	.046	.040	-	-
	5		.030	.029	.029	-	.015
November	1		.065	.080	.097	-	.015
	2		.028	.025	.036	-	.010
	3		.020	.025	.045	-	.005
	4		.019	.018	.022	-	.020
	5		.019	.039	.024	-	-
December	1		.044	.037	.101	-	.164
	2		.012	.017	.028	-	.031
	3		.010	.012	.018	-	.010
	4		.026	.015	.026	-	-
	5		.027	.032	.036	-	.040

Descriptions of Stations

North Carolina

Station 1. Eastern Channel is located between the inland waterway and Ocean Isle Beach and extends 3.0 miles from Tubbs Inlet to the Ocean Isle Beach Bridge. Mean annual salinity is about 32‰ and the sampling depths were from 1 to 5 meters.

Station 2. Located where Shallotte Creek and Saucepan Creek join the Shallotte River at the Shallotte Inlet. Mean annual salinity is about 33‰ and the sampling depths were from 1 to 3 meters.

Station 3. The Elizabeth River is a tidal river about 2 miles long surrounded by extensive marsh. It extends from the Intracoastal Waterway to the Cape Fear River. Mean annual salinity is about 21‰ and sampling depths were from 2 to 5 meters.

Station 4. Located on Stones Bay about 5.0 miles in from the New River Inlet. Mean annual salinity is about 23‰ and the sampling depths were from 1 to 3 meters.

Station 5. Located adjacent to Core Sound in Jarrett Bay and between Williston and Wade Creeks. Mean annual salinity is about 25‰ and sampling depths were from 1 to 3 meters.

South Carolina

Station 1. Harbor River, a narrow channel winding through marshland, is located 4.5 miles south of McClellanville and is tributary to the Intracoastal Waterway opening into Bulls Bay. This system is a lower distributary of the Santee River Delta with a mean annual salinity of approximately 25‰. Sampling depths ranged from 1 to 10 meters.

Station 2. Wando River is a main branch of Charleston Harbor and runs northeasterly from its mouth at the Cooper River for about 17.5 miles. The mean annual salinity is around 16‰ and sampling depths ranged from 5 to 10 meters.

Station 3. Point of Pines is located along the western shores of North Edisto River about 3 miles from the ocean. This estuary is an important nursery area for immature blue crabs. The mean annual salinity is around 32‰ and sampling depths were 5 to 8 meters.

Station 4. Folly River is an estuary intersecting Charleston Harbor at the northern end and Stono River at the south. The river proper is about 3.5 miles in length and terminates in a narrow sound filled with marsh islands and a network of winding channels. Mean annual salinities run around 26‰ and sampling depths were 1 to 5 meters.

Station 5. Whale Branch is a natural connection or cut-off between Coosaw and Broad Rivers near Beaufort. It is approximately 1/3 of a mile wide and 2 to 7 meters deep at low water. The tidal amplitude, runs nearly 2.5 meters and exceeds 3 meters on spring tides. This station is 17 miles from the ocean and salinities are around 20‰.

Georgia

Station 1. Located where Grimball Creek joins the Skidaway River. Grimball Creek drains a large marsh area. Skidaway River is part of the Intracoastal Waterway. This station was strongly influenced by rainfall and tidal flow. Sampling depths varied greatly in a small area ranging from 1 to 15 meters.

Station 2. Located where Ashley Creek empties into St. Catherines Sound. Ashley Creek originates in an extensive marsh. Sampling depths range from 2 to 6 meters.

Station 3. Sapelo River is a large body of water that runs directly into Sapelo Sound. This station was chosen for collecting disease and parasite samples because of the massive mortalities that had occurred there the two previous years. Sampling depths varied from 2 to 8 meters.

Station 4. Located at the southeast tip of St. Simons Island in St. Simons Sound; the area is fed by rivers polluted by industries located in Brunswick. Sampling depths varied from 1 to 12 meters.

Station 5. Crooked River is the southern-most station in Georgia. The sample area was near Crooked River State Park. Sampling depths ranged from 2 to 5 meters.

Florida

Station 1. Florida's southernmost station site, located 8 miles west of St. Johns Inlet between two small tributaries, Clapboard Creek and Browns Creek; depth 2 to 6 meters.

Station 2. In Nassau Sound, near the southern tip of Amelia Island; bottom type sandy mud, continually scoured by rapid flow of water during tidal changes. Two large rivers drain into this basin, the Nassau and the South Amelia; depth 5 to 6 meters.

Station 3. Approximately 4 miles west of Fernandina Beach near the head of Lanceford Creek, abundant in mud flats and poorly drained swales; depth 3 to 8 meters.

Station 4. Near the ocean in Cumberland Sound between Tiger Creek and Amelia River; bottom type sandy mud; three main rivers flow into this area, the Jolly, the St. Marys, and the Amelia, creating strong cross currents and extensive mixing of waters; depth 1 to 5 meters.

Station 5. In St. Marys River near the outflow of Jolly River; Florida's northernmost station joining boundaries with Georgia; depth 3 to 8 meters.