COLOGICAL EFFECTS BRANCH DATA EVALUATION REPORT

1. Chemical: Bolero 8EC 108401

2. Test Material: Bolero 8EC is 84% ai

3. Study Type: Three-year field study with biological and residue monitoring.

4. Study ID: Impact of Bolero Runoff on a Brackish Water Ecosystem. Performed by Biospherics, Inc. Project No. 382-1983. Date of study report: January, 1985. Sponsored by Chevron Chemical Company. Data Accession Nos. 256967, 252022.

5. Reviewed by: Daniel Rieder

Wildlife Biologist

EEB/HED

Daniel Kiale

Date: AUG 6 1985

6. Approved by: Norm Cook, Section Head

Section 2 EEB/HED - ruman cui

Date: _____ 8/4.85

7. Conclusions:

This study is categorized as supplemental. It partially fulfills the data requirement to determine the environmental effects of using Bolero 8EC on rice. The study showed adverse effects of Bolero on some fish species Gambusia affinis, Dormitator maculatus, and Poecilia latipenna when Bolero was used. No effects were seen on overall fish production. A fish kill was reported; dead Brevoortia patronus were found near sample station 4. This kill is attributed to Bolero. The study also showed some adverse effects to several invertebrate populations. There were substantial declines in numbers of gravid shrimp at two sample stations during the years Bolero was used compared to the baseline year.

The study does not show that Bolero can be used on rice without adverse effects to freshwater and estuarine environments. It does not rebut EEB's presumption that the use of Bolero on rice will have severe adverse effects on aquatic ecosystems.

The following information pertaining to this study must be provided by Chevron:

1. Page 106 of the baseline report.

2. The additional 1984 gravid shrimp data mentioned on page 11, para. 3 of the February, 1985 Fish and Wildlife Safety Study (EPA ACC# 256957).

- 3. Quantification of the fish kill observed in 1984 near sample station 4.
- 4. The reference for the literature cited in the statistics methods section.

Weber 1973 William and Dorris 1968

5. Possible sources of Bolero residues found at the treatment site during the baseline year and at the control station (9) during the two treatment years.

8. Background:

This field study was requested because EEB considered that the use of Bolero 8EC on rice would pose an unreasonable adverse acute and chronic effect on aquatic/estuarine organisms.

Bolero is moderately toxic to highly toxic to fish. It is very highly toxic to aquatic invertebrates, highly toxic to very highly toxic to marine invertebrates.

Species		Results (LC50)	T.M.
Sheepshead minnow Sheepshead minnow Sheepshead minnow Bluegill sunfish Rainbow trout Channel catfish	iuvenile	900 ppb 659 ppb >150 ppb 1.6 ppm 1.15 ppm	95.1% 95.1% 95.1% Bolero 8EC 95.5%
Daphnia magna Daphnia magna Daphnia magna	21-day LC5	> 1 ppb	95.5% 94.4% 95.2%
Grass shrimp Grass shrimp Panaeid shrimp	56-day LC ₅₍ pink white	570 ppb 264 ppb	95.2% Bolero 8EC Bolero 8EC Bolero 8EC Tech.
Ghost Shrimp Mysid Shrimp Mysid Shrimp Eastern Oyster	MATC >	470 ppb 1.08 ppm 288 ppb 19 < 30 ppb 320 ppb	Bolero 8EC Bolero 8EC 95.1% Tech Bolero 8EC

A previous field monitoring study was performed in Texas, Halls Bayou, 1979. This resulted in residues from 28 and 53 ppb up to 0.40 and 0.41 ppm in water adjacent to treated rice fields.

Based on this information a full-scale, multi-yeared field study was requested and made a requirement of the requirements for a conditional registration.

The purpose of the study was to demonstrate that the actual use of Bolero would not result in the expected adverse effects, or demonstrate the extent of those effects.

Objectives

This field study was designed to measure:

 Residues of thiobencarb in water, sediment, fish and invertebrates;

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- invertebrate populations;
- fish populations; and
- aspects of shrimp fecundity.

The data from the treatment years/sites were to be compared with a baseline (untreated) year and a control site. The contention was that if Bolero was going to affect the invertebrate or fish populations, the effects would be detected by comparison of the measurements listed above.

10. Discussion of Individual Test or Studies: N/A

11. Methods and Materials:

Study Area

EEB believed a study site which incorporated a rice field draining into a tidal estuary would answer the question of both aquatic and estuarine hazard while addressing the specific concern for shrimp reproduction.

According to the report, a preliminary survey from February 16, to February 18, 1982, resulted in the choice of a site two miles north of Matagorda, Texas, on the west side of Texas Highway 60. Figure 1 is a diagram showing the study site and its approximate location in South Central United States. Water from several hundred acres of "intermittent" rice fields drain into the ditch which borders the west side of the Mike Ottis Farm. (See figure 2.) "Intermittent" means not all the rice fields are planted in rice each year, however, sufficient acreage was planted and treated in each of the two treatment years to provide maximum typical exposure. The ditch is a permenant man-made bayou that flows into the Colorado River (in Texas). It was formed many years ago when a dike was built around Matagorda to protect the town from hurricane floods. The test site ditch is 120 to 140 feet wide, 1 to 1.5 feet deep. There are 2 to 3 causeways that allow vehicles to cross the ditch. Waterflow (current and tide) is permitted through drain pipes under the causeways.

A reference site was chosen approximately 8 miles upstream in an oxbowlike estuary. It was similar to the study area in terms of substrate, water depth and emergent vegetation. Cattle grazing was the only agricultural practice in the immediate area of the oxbow.

Sample Sites

Four sample sites or stations (I, II, III, IV) were established within the test drainage ditch. See Figure I. The reference or control sample station (IX) was located at the reference site 8 miles upstream along the Colorado River. Additional stations were established along the Colorado River (VI and VIII).

Location and Description of Sampling Stations

Station Number	Location and Description
I	Northern end of study ditch. Approximately 135 feet wide and 1 to 1.5 feet deep. Substrate mud and detritus.
II	Approximately 1/4 mile south of Station I. Approximately 120 feet wide and 1 to 1.5 feet deep. Substrate mud and detritus.
III	Downstream of lowermost flap gate. Approximately 140 feet wide and 0.5 to 1.0 foot deep. Substrate mud and detritus.
IV	South of station III. At this point the main ditch flows west through a dredged channel to the Colorado River. Approximately 20 feet wide, 4 to 6 feet deep. Substrate soft sediments, gumbo clay, sand detritus.
V	Southern end of the drainage ditch approximately 1/2 mile south of station IV. Approximately 130 feet wide and 0.5 to 1.0 feet deep. Substrate mud and detritus. It would not receive runoff from the treated rice fields.
VI	Colorado River downstream of the confluence of drainage ditch. Width of river approximately 100 yards. Water depth dropped sharply to 15 to 20 feet. Only water and sediment residue samples were collected from this station.

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VIII

Colorado River upstream of the confluence of drainage ditch. No biological sampling at this station. Physical measurements (i.e., salinity, conductivity, temperature) and water/sediment residue samples only.

IX

Reference or Control Station, Colorado River oxbow approximately 8 miles upstream of the study site. Oxbow on the east side of the Colorado River just upstream of the Texas Route 521 bridge. Approximately 70 feet wide. Substrate, soft sediments, detritus.

Farming Practice

Several rice fields drain into the study area. Not only the Mike Ottis field immediately adjacent, but the fields further north and those across Highway 60. See figures 2 and 3. During the baseline year, no thiobencarb was used in the study area, however, the herbicides ordram, propanil and machette were applied.

During the first treatment year Bolero was applied at 4 lbs ai per acre to 502 acres (see figure 2) which drained into the test ditch. (see Table 1.) Ordram and propanil were also applied.

During the second treatment year Bolero was applied at 4 lbs ai per acre to 549 acres (See Figure 3) which drained into the test ditch. (See Table 2.) Ordram, basegran, and propanil were also applied.

Physical and Chemical Parameters

The physical and chemical parameters were measured at each test station and at the reference station throughout the 3-year period. This included measurement of salinity, temperature, conductivity, pH and dissolved oxygen. The tables do not show these data for the reference station until August 1982.

Salinity

Based on the salinity data this test site falls into the mesohaline salinity classification based on Hedgepath, 1953 as found in Gosner, K.L., 1971. As would be expected, the reference site had consistently lower salinity values and significant rainfall lowered salinity values at the test site.

Farm Practices for Rice Fields in the Vicinity of the BOLERO® Study Area

Farm	Field Number	Farmer	Acreage Treated	Date-Rice Planted	Date-BOLERO Applied	Date-1st Post- Application Flush	Amount of BOLERD® Applied	Amt. Additional Herbicide Applied
9-0	-	S. Armatta	72.0	3/28/83	4/10/83	4/15/83	2 qts./acre	Ordrame: 2 pints/ acre; Propanil:
A-275	~	M. Ottis	118.0	4/12/83	4/20/83	4/23/83	2 qts./acre	Propanil: 3 qts./
0-79	ო	M. Ottis	80.0	3/15/83	3/20/83	3/23/83	2 qts./acre	Propanil: 1 gal./ acre
0-88 0-88	ı	F. Harrison	75.0	4/01/83	4/15/83	4/20/83	2 qts./acre	Propanil and Basagran
1-275	*A-275 6,7,9	M. Ottis	100.0	5/15/83	6/01/83	6/06/83	2 qts./acre	
A-275	©	B. Ottis	57.5	4/12/83	4/20/83	4/28/83	2 qts./acre	Propanil: 3 qts./ acre; Ordram: 0.66 pints/acre

* - BOLERO® Test Fields ** - BOLERO® BEC-2 quarts/acre is equivalent to 4 lbs. ai./acre

Table 2.

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Farm Practices for Rice Fields in the Vicinity of the BOLERD® Study Area

153	153 162
3/22/84	3/22/84
4/5/84	4/5/84

Total acres with BOLERO® - 549

*80LERO® BEC-2 quarts/acre is equivalent to 4 lbs. ai./acre

The salinity level also tended to decline from station I.

Water Temperature

The temperature ranged from 22 °C to 30 °C in the spring and fall of all study years. It was 26 °C to 35 °C during the summer. Winter temperatures ranged from from 13 ° to 20 °C, although temperatures dropped to as low as 2 °C during January 1984.

Dissolved Oxygen

The dissolved oxygen rarely dropped below 6 mg/l, and it was normally much higher. One major exception took place during May of 1982 when the DO was between 2 and 6 mg/l.

Conductivity and pH

These values may be viewed by looking at the attached microfiche of the final reports.

Metrological Data

Barometric pressure, wind information and humidity were recorded in the vicinity of the study. Significant rainfall for each of the 2 treatment years is presented below:

DATE	RAINFALL (IN)
7/22/82	0.94
10/8/82	1.84
6/5/83	1.30
6/23/83	2.00
6/25/83	1.58
7/12/83	1.04
7/13/83	3.30
7/14/83	1.00
7/16/83	6.26
5/1/84	0.03
5/8/84	0.16
5/16/84	0.51
5/17/84	0.31
5/18/84	1.59
5/19/84	0.16
5/20/84	0.10
6/6/84	0.04
7/3/84	0.40
7/24/84	1.75
,	1.13

Statistics Used by Author:

The following extract describes tha authors statistics performed on the invertebrate and fish sampling data during the study.

"Estimates of diversity (information per individual) as defined by Shannon's formula were made:

$$\overline{D} = \frac{s}{\sum_{i=1}^{s}} n_1/N \log_2 n_i/N$$

where D=information per individual; N=total number of individuals; n;=total number of individuals in the ith species; s=the number of species in the sample for a given station. This index summarizes information concerning the types of organisms occurring. The distribution of large numbers of individuals per taxon result in a low information per individual (D) value. High D values result from a large number of taxa and even distribution of numbers of individuals per taxon (Weber 1973).

"Redundancy was calculated using the theoretical diversity extremes:

Dmax - Dmin

The redundancy expression is a measure of the dominance of one or more species and is inversely proportional to the wealth or variety of species (William and Dorris 1968).

"An index of percent similarity was computed to evaluate the similarity of species composition between stations. This index is expressed as:

$$PS_{C} = 100 - 0.5 \ge |a-b|$$

where PS_C is the percent similarity and a and b are the percentages of ith species in samples A and B. The PS_C is only an empirical measure and is not an estimate of a statistical parameter from which the samples are collected. PS_C values range from 0 to 100. A value of 0 indicates that the species composition in two samples is entirely different and a value of 100 indicates complete

"A series of one-way Analysis of Variance (ANOVA) tests were used to statistically examine the counts of gravid Palaemonetes pugio and the total number of Palaemonetes pugio occurring at stations in the study area during April 1983 through March 1984. A Duncan's New Multiple Range Test was performed on each weekly series of counts, either gravid shrimp or total numbers, to identify stations where these differences occurred. A log (y+1) transformation as suggested by Elliot (1971) was made on the counts to normalize

The author also performed ANOVA tests and the Duncan's test on the 1984 gravid shrimp data.

Residue Analysis

Residues of thiobencarb were measured in samples of water, sediment, fish and shrimp in each of the 3 study years. The baseline year analysis was to document residue levels from previous and current pesticide use. The analysis performed on samples taken during the 2 treatment years was to determine the residue levels due to test use of thiobencarb.

Baseline Residues

During the baseline year, sediment, water and biota samples were collected from each station every other week. Fish specimens, either Brevoortia patronus (Gulf menhadden) or Mugil cephalus (stripped mullet) and Palaemonetes pugio (grass shrimp) made up the biota samples. Additional sediment and water were collected following any significant rainfall and on day 1 and 3 postrain. A significant rain was defined as one in which 0.5" or more of rain fell in a 24-hour period.

The thiobencarb residues measured during the baseline year are presented in tables 3, 4, 5a, and 6a. Tables 5b and 6b show the residues of 4-Chlorobenzyl Methyl Sulfone (degradate of thiobencarb) in fish and shrimp.

Table 3 is the results of water sample analysis. The highest residue detected was 9 ppb on May 29, 1982, at Station 2. Other levels detected were later in the year.

Table 4 presents the sediment residue analysis during the baseline year. No residues were detected (all less than 0.03 ppm).

Table 5a shows the measured fish residues. Here the highest level was 11.9 ppm on June 10, 1982. Table 5b shows the degradate residues in fish. The highest level was 0.05 ppm on 9/2/82.

Table 6a shows the measured shrimp residues. There is a series of measured levels on June 10, 1982, including one at 7.9 parts per million. Table 6b shows degradate residues in shrimp. The highest level was 0.04 ppm on 8/19 and 9/2, 1982.

In studying the 1982 tables showing the fish and shrimp residues, it appears that one group of samples may have been contaminated. Note that it is replicate B on June 10, 1982, for both fish and shrimp that show the extraordinarily high residues. The measurements do not correspond with any rainfall. It is also likely that some Bolero was being used close enough so that it transported into the study area, possibly through drift. That would account for both the thiobencarb and degradate residues.

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Treatment Year 1 Residues

Sediment, water and biological samples were collected from April 19, 1983, to March 8, 1984, and analyzed. The results are presented in Tables 7, 8, 9, and 10.

Table 7 presents the water residue data. The first residues are detected on May 2, 1983. Substantially higher residues (21.8 and 18.2) occurred at station 2 about 1 month later (June 1, 1983) following application to the rice fields 6, 7, and 9 immediately adjacent to the ditch. There was no water discharge then so it was attributed to drift. These residues are detected until June 12, 1983, or for about 11 days.

Table 8 presents the sediment residue data. The first detectable residues occurred on May 5, 1983, (10 ppb). On June 5, 1983, the residues were as high as 40 ppb at station 2. Sediment residues are detected at low levels (10 ppb) throughout 1983.

Table 9 presents the fish residues. The highest fish residue, 2.4 ppm, was measured on June 2, 1983, station 2. Residues in fish were detected from April 23, 1983, (0.42 ppm) to June 28, 1983, (0.03 ppm).

Table 10 presents the shrimp residue data. The highest level detected was 0.97 ppm on June 2, 1983, station 2. Residues were detected from May 5, 1983, to June 12, 1983.

Bolero was detected several times in the control samples of fish and shrimp suggesting these organisms were exposed to Bolero either in the control estuary or moved in following exposure in the Colorado River.

Treatment year 1 residue results show that drift was the major route of contamination. The primary residues in the sediment, fish and shrimp correspond with the June 1, 1983, application and apparent drift event according to the study author. This is likely because there was no scheduled flushes or rain just before the residue were detected. This suggests that drift contributed substantially to residues in water compared to runoff.

The water residues detected on May 2, 1983 followed closely after early treatment of several fields (fields 1,2,3 and 4; see figure 2) which flow into the study bayou. However, the high residues due to drift on June 1, 1983, probably masked any subsequent residues due to runoff from these fields.

Degradate analysis was apparently not performed during 1983, no values were reported.

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Treatment Year 2 Residues

The results of residue analysis for 1984 are presented in Tables 11, 12, 13a & 13b, and 14a & 14b. Sampling was discontinued August 22, 1984.

Table 11 shows the water residues. The highest residue (25.1 ppb) was at station 1 on May 2, 1984. This corresponds directly with the first postapplication flush (May 2, 1984) from fields 1, 2, 3 farm A-275 which was treated on April 21, 1984, and drains directly into the ditch at station 1. Residue detection began on April 28, 1984, and ceased by May 21, 1984. Residues up to 2.3 ppb were detected at the control site. No sample data are reported for the time period 5/9 to 5/14, 1984 for stations 1, 2, and 3.

Table 12 shows the sediment residues. The highest residues were on May 7, 1984 (70 ppb) and May 18, 1984, (60 ppb). Sediment residues were detected from April 28, 1984, until sampling was discontinued in August. Here again substantial residues (50 ppb) were detected in control station sediment.

Table 13a shows the fish residues. On sample day May 4, 1984, at station 4, dead fish were collected and analyzed for residues. The levels were 3.5 and 3.6 ppm. The highest levels in live fish samples were 1.6 ppm on May 2, 1984, at station 1. Residues in fish were not detected after June 27, 1984. The high residues in the dead fish may reflect a "slug" of Bolero coming from field 24 Farm A-275 that was not detected in the water samples. Residues up to 0.22 ppm were detected in control fish (station 9).

Residues of 4-chlorobenzyl methyl sulfure (degradate) were barely (10 ppb) detected on April 29, 1984, and May 2, 1984, in fish. See table 13b.

Shrimp residues are presented in Table 14a. The highest residues (210 ppb) occurred at station 1 on May 4, 1984. Relatively high residues (130 ppb) occurred at station 9 (control) on the same date. No residues were detected after June 27, 1984. Degradate residues in shrimp are shown on table 14b. The highest level was 120 ppb on 5/2/84.

Most of the residues correspond well with treatments. The high fish and shrimp residues occurred at the same stations and shortly after the highest water residues.

They suggest either sample contamination, faulty analysis or an unknown or unreported source of thiobencarb at the control site. In any case, it eliminates the use of station 9 as a control or reference site.

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The residue analysis data show that thiobencarb will transport from a rice field at high enough levels to result in residues in fish and shrimp. Furthermore, there is a strong suggestion that thiobencarb actually caused fish mortality at least once during the study.

In all cases residues in captured fish and shrimp declined to below detection levels by the end of the season.

Biological Effects

Tables 21 and 22 show respectively the invertebrate and fish taxa collected during the study.

The purpose of sampling estuarine populations was to determine if the use of Bolero on rice fields would have an adverse effect on these populations.

Both fish and estuarine macroinvertebrate populations were sampled at sample stations 1, 2, 3, 4, and 9 (control) during 1982 (baseline), 1983 (treatment year 1), and 1984 (treatment year 2).

Fish

Fish were sampled at 2 week intervals by seine during the rice growing season. Three seine hauls were made at each of stations 1, 2, 3, (occasionally 4) and 9 on each sampling. The samples from each station were compared by date, baseline to treatment year 1 and then to treatment year 2.

Gambusia affinis and Dormitator maculatus showed a marked decline, decreasing at all stations each year. Poecilia latipenna increased slightly from baseline to treatment year 1 then declined substantially the second treatment year.

CDECTO	Number	Caught per	Sample Day
SPECIES	1982	1983	1984
Gambusia affinis	61.1	54.2	12.7
Poecillia latipenna	16.7	25.1	3.7
Dormitator maculatus	18.8	2.3	0 ,

The most abundant fish species at the treatment site were:

CDUCTES	Year	No.*(Per	cent)**
SPECIES Brevoortia patronus	1982 1239(75)	1983 2184(56)	1984
Anchoa mitchilli	128(8)	695(19)	6 997(88) 670(8)
Sample days	17	18	7

* Number per sample day
** Percent of entire catch that year.

The time period for sampling fish is shown graphically, underlined letters represent months when sampling was being conducted.

1982	<u>A</u>	M	J	J	A	s	0	N	D	J	F	<u>M</u>
1983	<u>A</u>	М	J	J	_A_	s	0	N	D	J	F	M
1984	<u>A</u>	М	J	J	_ <u>A</u>	s	0	N	D	. ј	F	м

Summary of Effects to Fish

Based on samples taken by seine during the 3-year study, Bolero caused a reduction in numbers of the Gambusia affinis, Dormitator maculatus, and Poecillia latipenna. Bolero also caused a decrease species richness (total number of species collected) during the second treatment year.

Based on sample sizes, Bolero did not cause a decline in most fish species sampled, nor did it adversely affect overall fish production.

Information from the control site was analyzed but is not considered in this discussion. The control site is disqualified because of contamination with Bolero (residues found in fish and shrimp in 1983 and water, sediment, fish and shrimp in 1984).

INVERTEBRATES

Sweep Nets

Sweep nets were used to collect aquatic invertebrates at stations 1, 2, 3, 4, and 9 (control) during the baseline year (1982), treatment year 1 (1983) and treatment year 2 (1984).

Fifty-two taxa were represented. The results from the baseline year were compared against 1983 and 1984 results. Based on this comparison, the following species/taxa showed a substantial decline.

Lymnaeidae
Physa. sp
Cambarinae
Callinectes sapidus
Belostomatidae
Ranatra sp.
Noteridae
Dytiscidae

The total number of collected species or taxanomic classification* are reported:

Richness $\frac{1982}{44}$ $\frac{1983}{45}$ $\frac{1984}{34}$

*Some invertebrates were not identified to species but were only classified to genus, subfamily or family.

Summary of Sweep Net Results

Collection numbers of several species declined during the treatment years and a noticeable drop in species richness occurred during treatment year 2. These effects were attributed to Bolero.

The results from control site samples were analyzed but not included because of contamination of the site with Bolero.

MULTIPLATE SAMPLERS

Multiplate samplers were used during the baseline year and the first treatment year.

The results of the samplers showed that isopods declined at stations 1, 2, and 3 during the treatment year. The other species/taxa did not show a decline.

Richness (no. of taxa represented in samples) did not change substantially between baseline year and treatment year.

The decline in isopods is attributed to the presence of $\ensuremath{\mathsf{Bolero}}$.

EKMAN GRAB SAMPLES

Benthic organisms were sampled during the baseline year and the first treatment year using the Ekman grab. The samples were taken approximately monthly or every other month so that eight samples were collected from each sample station (1, 2, 3, 4, and 9) between April 29, 1982, and March 12, 1983.

The results were compared by matching numbers of organisms caught during the baseline year to those caught during the treatment year for all stations including control. The control data are not included because of contamination by thiobencarb.

A total of 46 species or taxa were used in the comparison of Benthic organisms. Each treatment sample day result for each station was compared with the corresponding sample day during the baseline year. When the overall trend was a decrease in numbers caught, the species or group was considered to have declined because of treatment.

Of the 46 taxa, 22 or 47.8 percent decreased during the treatment year. The following species/taxa tended to decrease at the listed stations:

Species/Taxa	Station	Showing	Decrease
Nematoda		•	
Dero*		3	
Nais*		1,2	
Paranais litoralis		1,2	
Stephensoniana trirandrana	•	1	
Wapsa		2	
Aulodrilus piqueti*		2,3	
Limnodrilus cervix*		1,2,3	
Limnodrilus maumeensis		1,2,3	
Immature tubificidae*		< 2	
Hexagenia		1,2,3,	4
Chaoboridae		2	
Ceratopogonidae		4	
Clinotanypus*		3,4 1	
Procladius*		_	
Tanypus*		2,3	
Chironomus		1,2	
Cryptochironomus		3	
Demeijera		4	
Dicrotendipes		3	
Parachironomus		3 3 3	
Polypedilum*			
4.5		1,2,3	

^{*}Supported by the following analysis where totals "by station" are compared.

In another comparative analysis, the total numbers of each species or taxa caught in each year were compared, baseline to treatment by station. The following shows the taxa and the numbers captured by station. Underlined sets of numbers represent a decrease from 1982 to 1983.

(Baseline/Treatment)
Station (Number / Number)

Taxa	_1	2	3	4
Polychaeta* Enchytraeidae	265/175**	336/219	307/264	403/579
Dero*	0/0 6/0	$\frac{1/0}{6/0}$	$\frac{12/0}{2/2}$	0/2
Nais* Paranais litoralis	3/0	<u>6/0</u>	2/2 2/0	$\frac{2/0}{1/1}$
Specaria josinae	$\frac{6/0}{1/0}$	$\frac{35/25}{0/0}$	$\frac{15711}{25711}$	1/4
Stephensoniana tanydi Aulodrilus piqueti*	170	1/0	0/0 0/0	$\frac{1/0}{0/0}$
Limnodrilus cervix*	<u>20/2</u> 152/0	$\frac{8171}{9070}$	131/0	4/0
Immature tubificidae*	1194/123	$\frac{90/0}{1133/280}$	$\frac{38/0}{304/122}$	0/1 76/16
Clinotanypus* Procladius*	$\frac{437/285}{11/8}$	347/153	99/60	119/62
Tanypus*	$\frac{11/6}{102/27}$	$\frac{22/7}{51/21}$	$\frac{15/3}{104/42}$	0/3 6/6
Chironomini Dicrotendipes	0/0	2/0	$\frac{204/42}{2/0}$	0/0
Parachironomous	0/0 2/0	$\frac{1/0}{3/3}$	$\frac{7/0}{3/0}$	0/0
Polypedilum* Stratiomyidae	10/0	33/0	<u>570</u> 1	$\frac{2/1}{94/82}$
Detactomy tode	0/0	0/0	2/0	1/0

Summary

Ten taxa or 21.7 percent of the taxa represented by capture (46) showed substantial decline during the treatment year. Eight other taxa showed a less distinct decline. This effect is assumed to be due to treatment.

^{*} These taxa were considered to have experienced substantial declines during the treatment year compared to the baseline year. This conclusion based on this comparison is strongly supported by the previous data where each sample date result is analyzed year to year.

GRAVID SHRIMP

of the <u>Palaemonetes</u> <u>pugio</u> captured with sweep nets during each of the <u>3 years</u>, gravid females were counted and the results reported as a percent of total adult shrimp collected. The percents reported for the baseline year were compared to those reported during the first treatment year by station and sample date. That is, the percent of gravid shrimp captured at station 1 on August 16, 1982, was compared to the gravid shrimp at station 1 on August 17, 1983. See table 15. The problem with using the second treatment year is that sampling did not begin until August 3, 1982, and essentially ended on June 25, 1984, except for one sample on July 17, and one on August 19. Therefore, there is only one sample date in 1984 which corresponds to a sampling date in 1982. Changes were noted when the percents from August 3, 1982, to November 1, 1982, were compared to those from August 5, 1983, to October 25, 1983, at each station. The average percents for all the sample dates at each station are presented.

		Year	PERCENT
Station	82	83	CHANGE
1 2 3	7.8 8.7 11.0 23.3	4.2 4.0 13.0	46% decrease 54% decrease 15% increase
*	23.3	21.7 AVERAGE	7% decrease 23% decrease

There were decreases in percent of gravid shrimp during the treatment years compared to the baseline at stations 1, 2 and 4. The average percent decrease for stations 1 and 2 is 50%; the decrease at station 4 was less.

The decrease at station 2 was significant (14<14; alpha=5%) based on the Wilcoxon signed rank test as described in Statistical Methods by George W. Snedecor and William G. Cochran, Sixth Edition, 1974, The Iowa State University Press, pp 128-129.

The control site data was not compared since the site was contaminated with Bolero.

Summary

Bolero caused substantial declines in percent of gravid shrimp at two of the 4 sample stations. The water residue data support this finding, as the shrimp MATC is > 19 < 30 parts per billion and the water residues reach this level in both treatment years.

Table 15. Comparison of gravid shrimp from baseline to treatment year 1 to treatment year 2.

Dime	PERCENT	GRAVID	SHRIMP BY	STATION
DATES	I	II	III	
82 83	82 83	82 83	82 83	82 83
8/3 8/5	: 23.6 2.5	2.8 2.9	9.7 2.5	8.3 4.3
8/10 8/12	: 2.6 1.3	11.5 1.2	10.5 11.4	31.8 10.7
8/16 8/17	: 10.2 8.5	23.5 0	7.1 7.4	26.8 49.9
8/24 8/27	: 12.5 8.2	15.8 19.6	18.5 16.7	26.4 25.0
8/31 9/2	: 2.3 6.9	14.1 8.3	15.7 12.1	29.2 33.7
9/8 9/8	: 10.9 7.0	12.8 6.9	5.3 9.7	28.1 46.9
9/14 9/15	: 1.7 3.9	15.0 4.7	22.0 13.9	31.9 24.3
9/20 9/21	: 23 8.9	5.9 0	18.6 33.3	10.0 0
9/28 9/30	: 0 0.5	0 3.2	22.2 24.9	29.2 31.3
10/5 10/5	: 1.7 3.4	1.2 1.2	2.3 18.2	41.8 22.0
10/13 10/12	: 2.3 0	0 0	0 6.9	13.0 7.9
11/1 10/25	: 2.6 0	1.5 0	0 9.5	3.2 4.2
\overline{X} PERCENT CHANGE	7.8 4.2 46% (decrea	8.7 4.0 use) 54% (decrea	11.0 13.0 ase) 15%(increase)	22 2 21 7

AVERAGE CHANGE: 23% (decrease)

12. Reported Results:

The reported data are too voluminous to include in the DER.

13. Study Authors' Conclusions:

The study author concluded no adverse effects that he could attribute to Bolero. His rationale is based on the statistics he performed and his analysis.

Invertebrate:

1. The author noted that the total number of Peneaus shrimp collected during the 2nd treatment year was higher than the other two years. The values for all three years were:

Year : <u>1982</u> <u>1983</u> <u>1984</u> No. Peneaus: 2771 1504 2785

2. The author noted greater populations of macroinvertebrates during the second year, 1983, than the first year, but made no mention of this figure for 1984. The values for total numbers caught by sweep net were:

Year : 1982 1983 1984
Total :100869 112625 72751

- 3. The total number of grass shrimp collected during the three years did not decline from baseline to treatment year 1 to treatment year 2.
- 4. The author noted that the grass shrimp (Palaemonetes pugio) life cycles were similar in 1983 and 1984 to those in 1982. This means that the gravid females (when found) were collected during the same time period in each year.
- 5. Based on ANOVA testing, there was no statistical difference in the percent of gravid shrimp collected when thiobencarb residues were present than when they were not present within the same year. The author did not compare the results from 1982 to the two treatment years.
- 6. Some of the author's confusions were based on a comparison to station 9, which was intended to be a reference station. For example, it was noted that on only one occassion when thiobencarb was present was the average number of gravid shrimp females significantly greater at station 9 than at the other stations.

- 7. The study reported that the number of water boatman, Trichocorixa verticalis, collected at each treatment station increased each year. This was attributed to increased salinity.
- 8. The diversity index (D values) were calculated for each sampling day at each station. According to the author, the distributions were similar to those observed during the baseline years. See tables 16, 17, and 18.

Fish

9. The author reported no reductions in fish taxa or populations after Bolero applications. See figure 4. It was also stated that no new major taxa were identified in the second treatment year as compared to the first treatment year and the baseline year. Tables 19 and 20 show the seine D values for fish collected in 1983 and 1984. This information was not provided for 1982.

A fish kill was reported on May 3, 1984. Dead <u>Brevoortia</u> patronus were found near station 4. This corresponds to a May 1 and May 2, 1984 post application flush. No quantification of this kill was provided.

14. Reviewer's Discussion and Interpretation of Study Results:

A. <u>Test Procedure</u>

The test procedure was marginally acceptable. The biological sampling should have continued regularly throughout the summer of 1984. The gravid shrimp should have been collected earlier in 1982. That would have allowed a full 3-year comparison.

The use of other chemicals that are also toxic to aquatic organisms, i.e. ordram, basegran and propanil were considered acceptable because that is typical of how Bolero would be used. These chemicals were used in the baseline year and before and as such are considered part of the control; the effects observed during 1983 and 1984 are attributed to Bolero. Machete was used in 1982 only.

The contaminated samples during the baseline year mean that thiobencarb transported from some unknown source to the study site. The registrant should determine what this source was and quantify it. The thiobencarb residues were substantially lower during the baseline year than during the treatment years so biological comparisons between 1982 and 1983 and 1984 may be made.

A statement on page 11, paragraph 3, of the Fish and Wildlife Safety Study dated February 8, 1985, (Acc #256957) suggests that shrimp were collected until late October 1984. That may have been a typographical error otherwise the results of that sampling must be provided.

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	The product confidential statement of formula.
	Information about a pending registration action.
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The study itself was marginally adequate and there were several problems with the report.

- 1. Page 106 of the baseline report is missing, it is part of the aquatic invertebrate data table.
- 2. The results of sampling all organisms were not presented in a manner which allowed year-to-year comparison by station. The yearly data for each station should have been presented on one page, with the sample dates across the top and species/taxa down the side.
- 3. The species/taxa were not consistent from year to year. Sometimes the organisms were reported by species and genus then in subsequent years they were clumped into families and subfamilies
- 4. Summary (whole year) data were not presented for each year and each different sampling method.
 - The fish kill was not quantified.
- 6. Two citations were included in the text but not listed in the literature cited list.

Weber 1973 William and Dorris 1968

These problems will be overlooked as the reviewer reworked the data into useful format and the company was not given specific instructions on how the data should have been presented.

More information on the fish kill must be provided. The missing literature citations must be provided.

B. Statistical Analysis

The reviewer analyzed the data visually, using averages where applicable. Gravid shrimp data were analyzed using an ANOVA and Duncan's multiple range test.

C. <u>Discussion of Results</u>

The residue monitoring data from water, sediment, fish and shrimp samples show that Bolero will move from an aerially treated rice field via drift and runoff and occur in water at 20 to 30 ppb and sediment at 40 to 70 ppb. This Bolero may then accumulate in fish at up to 2.4 ppm and shrimp to 1 ppm. The residues in water declined to nondetectable levels within 1 week. The residues in sediment remained at detection levels 10 ppb throughout the summer in both 1983 and 1984.

Biological Monitoring

1. Fish

Two species of fish, Gambusia affinis and Dormitator maculatus showed a marked decline, with number per sample decreasing at each station based on a comparison of baseline to treatment years. Poecillia latipenna showed a marked decline in 1984.

Overall fish production and numbers of most fish species were not adversely affected by the use of Bolero based on sample sizes and composition.

The fish kill (Brevoortia patronus) means that there were probably "slugs" of thiobencarb substantially higher than those measured in water samples as the fish LC50 is higher than those residues reported. These slugs would occur during release of water shortly after an application. The high residues of thiobencarb implicate Bolero in the kills.

2. Aquatic Invertebrates

Sweep net sampling of aquatic invertebrates showed a marked decline in numbers of several species/taxa from baseline to treatment years 1 and 2. There was a substantial decrease in richness the last study year (44 in 1982, 45 in 1983, but 34 in 1984). The decline in numbers of certain taxa and reduced richness are attributed to the use of Bolero.

Multiplate sampling showed a marked decline in numbers of isopods from baseline to treatment year 1. There was no difference richness (number of species or taxa).

The Ekman grab was used to sample benthic organisms. Ten taxa or 21.7 percent of the taxa represented by capture (46) showed a marked decline from baseline to treatment year l. Species or taxa richness decreased from 45 taxa to 34 taxa. The decline in isopods and decline in numbers of taxa is attributed to the use of Bolero.

3. Shrimp Fecundity

Bolero caused significant (5% level) declines in the percent of gravid shrimp at station 2. This decline was 50 percent from the baseline year to treatment year 1 at stations 1 and 2. There was a slight increase at station 3. The average decrease for the 4 stations was 23%.

4. Response to Author's Conclusions

The reviewer disagrees with the authors conclusions that there were no changes in the results of biological sampling that could be attributed to Bolero.

The points will be addressed by number: (See section 13)

1. Study Authors' Point: The author noted that the total number of Peneaus shrimp collected during the 2nd treatment year was higher than the other two years.

Response: The increase of Peneaus sp. in 1984 was negligible over the numbers caught in 1982. Furthermore, the concern was for chronic effects to shrimp and these effects would not show up in commercial shrimp in this study because of their life history. Peneaid shrimp move out of estuaries to open water annually to spawn. The young "post-larval" shrimp move back into the estuaries to mature. It is unlikely that the actual numbers of commercial shrimp would decrease in a small test area such as this due to adverse chronic effects. The young Peneaid shrimp would redistribute to estuaries other than where their parents matured. It would only be over several years and extensive usage that the effects of Bolero on Penaeids would be detected.

2. Study Authors' Point: The author noted greater populations of macroinvertebrates during the second year 1983 than the first year, but made no mention of this figure for 1984.

Response: The overall numbers of macroinvertebrates collected by sweep nets in 1984 were less than in both 1983 and 1982. This is partly attributed to a smaller sampling effort in 1984. Overall populations of macroinvertebrates would not be expected to decline sharply, as losses would be replaced by immigration from unaffected areas.

3. Study Authors' Point: The total number of grass shrimp collected during the three years did not decline from baseline to treatment year 1 to treatment year 2.

Response: Since grass shrimp larvae are planktonic, they would redistribute to estuaries other than where they originated. Thus this study area would be repopulated from other undisturbed areas even if there was no reproduction in the study area at all. Indeed, the fact that the study area adult grass shrimp sample sizes remained high in spite of reproduction declines suggests that the study area was repopulated. This also suggests that the reduction in percent gravid shrimp noted at the study site did not occur along the rest of the Colorado River estuary system.

4. Study Authors' Point: The author noted that the grass shrimp (Palaemonetes pugio) life cycles were similar in 1983 and 1984 to those in 1982. This means that the gravid females (when found) were collected during the same time period in each year.

Response: It is probably true that thiobencarb residues do not totally change the life cycles of grass shrimp. However, the presence of these residues in 1983 and 1984 corresponded to a drop in the percent of gravid shrimp collected when compared to the baseline year.

5. Study Author's Point: Based on ANOVA testing, there was no statistical difference in the percent of gravid shrimp collected when thiobencarb residues were present than when they were not present within the same year. The author did not compare the results from 1982 to the two treatment years.

Response: It is unlikely that the percent of gravid shrimp would fluctuate immediately due to the presence of thiobencarb. Since grass shrimp females carry their eggs for 2 to 3 weeks, adverse effects would not be noted for several weeks after the residues appeared.

6. Study Authors' Point: Some of the author's conlusions were based on a comparison to station 9, which was intended to be a reference station.

Response: Station 9 is not a valid reference station because of the numerous residues of thiobencarb detected in fish and shrimp in 1983 and fish, shrimp, water and sediment samples in 1984.

7. Study Authors' Point: The study reported that the number of water boatman, <u>Trichocorixa verticalis</u>, collected at each treatment station increased each year. This was attributed to increased salinity.

Response: No comment as some less sensitive species would not be affected by thiobencarb. However, it suggests that the author has confidence that the sampling methods used will show population changes. Thus the species/taxa decreases noted from sampling results would also reflect population changes.

8. Study Authors' Point: The diversity index (D values) were calculated for each sampling day at each station. According to the author, the distributions were similar to those observed during the baseline years. See tables 16, 17, and 18.

Response: The diversity indices reported tended to fluctuate from station to station and sample day to sample day. This would make any biological interpretation of these values virtually impossible.

9. Study Authors' Point: The author reported no reductions in fish taxa or populations after Bolero applications.

Response: There were reductions in the number of species collected:

Year : $\frac{1982}{26}$ $\frac{1983}{31}$ $\frac{1984}{20}$

There were also substantial reductions from baseline to treatment years in the numbers of some species collected (i.e. Gambusia affinis and Dormitator maculatus).

The fish kill is attributed to the presence of thiobencarb in the water. This conclusion is supported by residue measurements.

D. Adequacy of Study

Category: Supplemental

This study showed that the use of Bolero on rice fields and measured residues in water and sediment corresponded to reductions on some fish and invertebrate species sampled during the 3-year field study. It does not rebut EEB's presumption that the use of Bolero will cause unreasonable adverse effects to freshwater and estuarine ecosystems.

The problems with this study are presented in Section 14, (Reviewers Discussion and Interpretation of the Study). use of other chemicals that are also toxic to aquatic organisms in the study area during the study is scientifically questionable, but it is acceptable from the viewpoint that this is a typical end use for Bolero on rice. EEB is aware that the use of these chemicals in the water casts doubt on the validity of attributing the observed results of the field study to Bolero. However, the adverse effects noted in the study are based on samples taken during the years when Bolero was used compared to the baseline year when Bolero was not used. Since the registrant was aware of EEB's reservations for the use of these other chemicals when the study was designed, EEB will accept the study as it If the registrant feels that the use of these other chemicals makes the results of this study questionable as to their applicability to the use of Bolero, EEB will agree to reject the study.

- 15. Completion of One-Liner: Completed.
- 16. CBI Appendix: N/A

Table 21 Invertebrate Taxa Collected During the BOLERO: Texas Biological Monitoring Study

PHYLUM: Nemertinea PHYLUM: Nematoda PHYLUM: Annelida CLASS: Polychaeta

ORDER: Sabellida Sabellidae

CLASS: Clitellata

SUBCLASS: Oligochaeta ORDER: Haplotaxida Enchytraeidae Naididae

Bratislavia unidentata (Harman, 1973) Chaetogaster sp.

Dero sp.

Haemonais waldvogeli (Bretscher, 1900)

Nais spp.

Paranais litoralis (Muller, 1784)

Pristina longiseta longiseta (Ehrenberg, 1828)

Pristina logisoma (Harman, 1977) Specaria josinae (Vejdovsky, 1883) Stephensoniana tandyi (Harman, 1975) Stephensoniana trivandrana (Aiyer, 1926) Wapsa c.f. mobilis (Liang, 1958)

Tubificidae

<u>Aulodrilus limnobios</u> (Bretscher, 1899) <u>Aulodrilus piguetti</u> (Kowalewski, 1914) <u>Limnodrilus cervix</u> (Brinkhurst, 1963) L. hoffmeisteri (Claparede, 1861) L. maumeenis (Brinkhurst and Cook, 1966) L. udekemianus (Claparede, 1862) Monopylephorus helobies (Loden, 1980) Peloscolex heterochaetus (Michaelson, 1926) Quistadrilus multisetosus (Smith, 1900)

CLASS: Hirudinea

ORDER: Piscicolidae

PHYLUM: Mollusca

CLASS: Gastropoda

ORDER: Basommatophora

Physidae **Physa** Lymnaeidae

Table 21 antid

PHYLUM: Arthropoda CLASS: Crustacea ORDER: Mysidacea Mysidae Taphromysis louisianae (Banner, 1953) ORDER: Decapoda Penaeidae Penaeus aztecus (Ives, 1891) Penaeus duorarum (Burkenroad, 1939) Penaeus setiferus (Linnaeus, 1767) Palaemonidae Palaemonetes pugio (Holthius, 1949) Macrobrachium acanthurus (Wiegmann, 1836) Macrobrachium ohione (Smith, 1874) Astacidae Cambarinae Portunidae Callinectes sapidus (Rathbun, 1896) Grapsidae Sesarma (Holometopus) cinereum (Bose, 1918) CLASS: Malacostraca ORDER: Isopoda ORDER: Amphipoda SUBORDER: Gammaroidea CLASS: Insecta ORDER: Ephemeroptera Baetidae Callibaetis sp. Potamanthidae Tortopus sp. Ephemeridae Hexagenia sp. Caenidae Caenis sp. ORDER: Odonata SUBORDER: Anisoptera Gomphidae

Aphylla protracta (Selys, 1859)

Dromogomphus

Aeschnidae

Anax junius (Drury, 1770)

Libellulidae

Cannacria gravida (Calbert, 1890) Celithemis eponina (Drury, 1773) Erythemis simplicicollis (Say, 1839) <u>Libellula luctuosa</u> (Brumeisteri, 1839) Libellula c.f. needhami (Westfall, 1943) Miathyria marcella (Selys, 1857) Orthemis ferruginea (Fabricius, 1775) Pachydiplax longipennis (Burmeister, 1839)
Tramea c.f. onusta (Hagen, 1861)

SUBORDER: Zygoptera

Lestidae Lestes

Coenagrionidae

Ischnura

Enallagma durum (Hagen, 1861) E. exsulans (Hagen, 1861)

ORDER: Orthoptera

Tettigonidae

ORDER: Hemiptera

Veliidae **Gaerridae** Pleidae

Belostomatidae

Nepidae

Ranatra sp.

Naucoridae

Pelocris

Corixidae

Corixinae

Notonectidae

Buenoa

ORDER: Trichoptera

Hydrophilidae

Leptoceridae **Nectopsyche**

ORDER: Coleoptera

Gyrinidae <u>Dineutus</u>

Haliplidae **Haliplus**

Dytiscidae

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Noteridae
        Hydrocanthus
     Hydrophilidae
        Enochrus
        Berosus
     Dryopidae
        <u>Helichus</u>
ORDER: Diptera
     Culicidae
     Chaoboredae
     Ceratopoganidae
     Chironomidae
        Tanypodinae
           Coelotanypus
           Clinotanypus
           Labrundinia
           Procladius
           Tanypus stellatus
           Tanypus sp. A.
           Pentaneuria
    Chironominae
     Chironomini
       Cryptochironomus
        Demeijera
        Dicrotendipes
       Endochironomus
       Glyptotendipes
        Parachironomus
       <u>Phoenopsectra</u>
       <u>Polypedilum</u>
       Tribelos
       Pseudochironomus
    Ephydridae
     Tanytarsini
       Cladotanytarsus
       Rheotonytarsus
       Lauterborniella
       Tanytarsus
    Orthoc ladinae
       Nanocladius
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Table 22 Fish Taxa Collected During the BOLERO: Texas Biological Monitoring Study

ORDER: Lepisosteiformes Lepisosteidae Lepisosteus spatula (Lacepede, 1803) "Alligator Gar" ORDER: Elopifomres Elopidae Elops saurus (Linnaeus, 1766) "Ladyfish" Megalops atlantica Valenciennes, 1846 "Tarpon" ORDER: Clupeiformes Clupeidae _ Brevoortia patronus Goode "Gulf menhaden" Dorosoma petenense (Gunther, 1866) "Threadfin shad" Dorosoma cepedianum (Lesueur, 1818)
"Gizzard shad" Engraulidae Anchoa mitchilli (Valenciennes, 1848) "Bay Anchovy" ORDER: Cypriniformes Cyprinidae Catostomidae Ictiobus bubalus (Rafinesque, 1818)
"Smallmouth Buffalo" ORDER: Atheriniformes Cyprinodontidae Cyprinodon variegatus (Lacepede, 1803) "Sheepshead minnow" Fundulus confluentus (Goode and Bean, 1880)
"Marsh Killifish" Poeciliidae Gambusia affinis (Baird and Girard, 1853) "Mosquitofish" Poecilia latipenna (Lesuer, 1821)
"Sailfin Molly" Atherinidae Menidia beryllina (Cope, 1869)

"Tidewater silverside"

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ORDER: Perciformes
      Centrarchidae
            Lepomis spp.
                  "Sunfish"
            Lepomis macrochirus Rafinesque, 1819
                  "Bluegill"
            Micropterus salmoides (Lacepede, 1802)
"Largemouth Bass"
            Pomoxis nigromaculatus (LeSeur, 1829)
                  "Black Crappie"
      Sciaenidae
            Cynoscion arenarius Ginsberg
"Sand Seatrout"
            Micropogonias undulatus (Linnaeus, 1766)
"Atlantic Croaker"
                   Red Fish
      Gerreidae
            Eucinostomus argenteus (Baird 1854)
                  "Spotfin mojarra"
      Carangidae
          <u>Caranx hippos</u> (Linneaus, 1766)
                 "Crevalle jack"
      Sparidae
           Lagodon rhomboides (Linnaeus, 1766)
      Muqilidae
           Mugil cephalus (Linnaeus, 1758)
"Stripped Mullet"
      Eleotridae
           Dormitator maculatus (Bloch, 1792)
                 "Fat Sleeper"
      Gobiidae
           Gobionellus spp.
ORDER: Plectognathi
     Tetraodontidae
           Sphoeroides parvus (Shipp and Yerger)
"Least Puffer"
ORDER: Pleuronectiformes
     Bothidae
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"Flounder"

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Weber

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