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Toxicity of Permethrin to Procambarus Clarkii, and the Effects of Permethrin-Induced Density Reduction and Supplemental Feeding on Stunted Crawfish Populations.

Herman Henry Jarboe
Louisiana State University and Agricultural & Mechanical College

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Jarboe, Herman Henry, Ph.D.

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300 N. Zeeb Rd.
Ann Arbor, MI 48106

TOXICITY OF PERMETHRIN TO PROCAMBARUS CLARKII, AND THE EFFECTS OF PERMETHRIN-INDUCED DENSITY REDUCTION AND SUPPLEMENTAL FEEDING ON STUNTED CRAWFISH POPULATIONS

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by
Herman H. Jarboe
B. S., University of Louisville, 1981
M. S., Mississippi State University, 1984
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ABSTRACT

The acute toxicity of permethrin to red swamp crawfish (Procambarus clarkii) was evaluated in laboratory studies. The mean static acute 96-h LC50 of permethrin to red swamp crawfish 8 - 12 mm (0.017 g), 25 - 35 mm (0.64 g), 45 - 55 mm (2.45 g), and 65 - 75 mm total length (TL) (8.98 g) was 0.44, 0.85, 1.30, and 0.81 µg/L, respectively. Permethrin toxicity did not differ among immature male, mature male and female P. clarkii . Crawfish surviving permethrin exposure exhibited no differences in post-exposure growth, survival, maturity attainment, or the production of viable young when compared to non-exposed controls ($P > 0.2$). At the third-instar stage of development, no evidence of teratogenesis was observed in crawfish produced from permethrin-exposed parents.

The 24-h LC50 of permethrin to red swamp crawfish (31 to 70 mm TL) in pond water ranged from 1.0 to 3.1 µg/L. Permethrin was applied to shallow earthen ponds at concentrations of 1.0 to 3.0 µg/L and population estimates conducted 12 - 13 days post-treatment indicated that pond crawfish populations were reduced by 54.4 to 83.1 % for P. clarkii and 100.0 % for P. acutus acutus. Crawfish less than 40 mm TL had higher mortality (range of 55 to 84 %) than larger crawfish (range of 42 to 79 %). Among crawfish greater than 40 mm TL, mature males had higher mortality (33 to 100 %) than immature males (range of 43 to 86 %) and females (range of 30 to 72 %). No permethrin residue could be detected in crawfish abdominal muscle (detection limit 6.7 ng/g) or hepatopancreas (detection limit 400 ng/g) by gas chromatography.

The use of permethrin to effect density reduction in combination with supplemental feeding was evaluated as a management technique to minimize stunting of crawfish at sub-marketable size. Crawfish populations which were fed formulated feed at a rate of 28 kg/ha three days per week had more harvestable crawfish (greater than 75

mm TL) (mean = 29 %, P = 0.01), larger mature males (mean = 70 mm TL, P = 0.01), greater total weight of crawfish harvested (mean = 300 kg/ha, P = 0.18), greater number of crawfish harvested (mean = 35,470 crawfish/ha, P = 0.16), and higher CPUE (mean = 0.16 kg/trap-set, P = 0.001) than crawfish populations receiving no formulated feed from April to June.

INTRODUCTION

Management of a commerical procambarid crawfish facility may necessitate a period when it is advantageous to drain ponds early in the production season to minimize financial loss. In Louisiana this most often occurs in April or May and coincides with peak harvest of "wild" crawfish from the Atchafalaya Basin (Dellenbarger and Luzar 1988). The presence of large crawfish in the market from the Basin reduces the price paid to producers of pond-raised crawfish that are often smaller in size. Premature draining of crawfish ponds in April or May stimulates crawfish to burrow when crawfish standing crop is high. The mature females that survive the summer in burrows, produce young that are recruited into the population when the pond is filled in the fall. A healthy female red swamp crawfish (Procambarus clarkii), 85 - 90 mm total length (TL), can produce 350 - 400 young (Huner and Barr 1984). Good pond management, such as maintaining adequate forage biomass, satisfactory water quality and the presence of fish exclusion devices, enhances survival of newly-hatched and holdover juveniles. The recruitment of young-of-the-year (YOY) crawfish from a large spawning population of adult females, and high survival of YOY from holdover juveniles that mature and spawn in mid-winter and spring create a situation where forage biomass is not sufficient to sustain growth of the crawfish population for an entire production season, and carrying capacity of the pond is exceeded.

Stunting of Crawfish

Stunted crawfish populations are characterized by slow growth of individuals and crawfish that mature at sizes often less than 75 mm TL (Avault et al. 1974; Huner and Romaire 1978; Romaire et al. 1978, and Huner and Barr 1984). Stunted crawfish are not desirable to seafood processors because they are smaller than the preferred market-size crawfish of 85 to 105 mm TL (Avault et al. 1974, de la Bretonne and Fowler 1976, Huner and Barr 1984).

In the 1985-86 and 1986-87 crawfish production seasons, 7.5 % of 47,000 ha of crawfish ponds surveyed experienced severe stunting.[1] Crawfish were so small that producers ceased harvesting in April and May, significantly reducing catch. These estimates of crawfish stunting were for the most severe cases, and double this area probably goes unreported.[1] Several methods to minimize stunting of crawfish in ponds are recommended, but they produce inconsistent results. The management procedures include: (1) drain the pond quickly over several days to strand and kill many crawfish before they burrow; (2) increase harvest intensity to reduce population density; and (3) add agricultural vegetative by-products (such as hay or rice straw) to ponds as a supplemental feed for crawfish.[2]

Density reduction of crawfish populations may assist efforts to minimize stunting in commercial crawfish ponds. In metal pools, Romaire et al. (1978) demonstrated that a combination of reducing the density of stunted crawfish from 12 to 6 crawfish/m^2 in combination with adding aquatic vegetation (Polygonum sp. and Jussiaea sp.) as feed induced stunted crawfish to grow. The relationship between crawfish density, food supply, and incidence of crawfish populations stunting, is poorly understood. The purpose of this study was to determine if density reduction and a program of supplemental feeding of self-sustaining crawfish populations in earthen ponds would be an effective management technique to increase the growth and

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[1] Results of an informal survey of Louisiana Cooperative Extension Service agents during the 1985 - 86 and 1986 - 87 crawfish production seasons used to determine the total area of crawfish ponds in Louisiana that experienced stunting.

[2] Personal communication from Larry de la Bretonne, Aquaculture Specialist Louisiana Cooperative Extension Service, Baton Rouge, Louisiana 70803.

subsequent yield of market-size crawfish from ponds that may otherwise produce stunted crawfish.

In this study, permethrin was chosen to reduce crawfish population densities. This compound was selected because both laboratory (Jolly et al. 1977 and Coulon 1982) and field studies (Coulon 1982) determined that low concentrations of permethrin were toxic to P. clarkii, and permethrin residue bioconcentration by crawfish was minimal. To selectively reduce crawfish population density it was necessary to gather more information on the short and long-term effects of permethrin on several size classes of crawfish.

Acute Toxicity of Permethrin to Red Swamp Crawfish

Synthetic pyrethroids have become one of the most effective group of insecticides to enter the market in recent years (Matsamura 1985). Permethrin [(3-phenoxybenzyl (+) cis, trans, 3-(2,2-dichlorovinyl)-2, 2-dimethylcyclopropane carboxylate)] is a broad spectrum synthetic pyrethroid insecticide that has low toxicity to non-target avian and mammalian species (Nishizawa 1971; Abernathy and Casida 1973), but the compound is toxic to crawfish at concentrations in the low parts per billion (Jolly et al. 1977; Coulon 1982).

Research on the effects of permethrin on P. clarkii assessed the acute toxicity of the compound on one (Coulon 1982) or two (Jolly et al. 1977) size classes of juveniles. To understand the susceptibility of a population of P. clarkii to permethrin requires that the acute toxicity of the compound be determined on several size classes that represent mature and immature individuals of both sexes. Exposure of the organisms to sublethal levels of the compound and subsequent observation for response, aids in understanding how this xenobiotic may influence the growth, survival, and reproduction of crawfish populations.

The Exposure of Red Swamp Crawfish to Pesticides in the Field

Field trials and simulated rice-crawfish pond studies have examined effects of several rice insecticides, herbicides and fungicides, or combinations of the three on crawfish production (Hendrick and Everett 1965; Hendrick et al. 1966; Change and Lange 1967; Hyde et al. 1972; Ekanem 1981; Coulon 1982; and Romaire 1983) .

The effects of aldrin and dieldrin on P. clarkii survival, growth, and reproduction were examined by Hendrick et al. (1966). Chang and Lange (1967) conducted laboratory and field evaluations of nine insecticides for control of red swamp crawfish in California rice fields. Mirex, an organochlorine insecticide used to control the imported fire ant, Solenopsis invicta , was tested alone, and in combination with malathion and carbofuran, in experimental earthen plots, to determine their effects on the survival and production of crawfish grown in the rice fields of southern Louisiana (Hyde et al. 1972). Ekanem (1981) compared growth, survival, yield, and reproduction of P. clarkii in simulated rice fields treated with combinations of the pesticides captafol, propanil, molinate, and carbofuran. Field toxicity of permethrin on P. clarkii and persistence of permethrin residues in P. clarkii were determined by Coulon (1982). Romaire (1983) evaluated the effects of the rice insecticide, carbofuran, on crawfish yield in 2.0 ha rice plots managed for rice and crawfish production.

To determine which pesticides will provide adequate crop protection and still be compatible with crawfish production in rice fields it is necessary to examine specific impacts of a wide range of pesticides on resident crawfish populations in field exposures. In addition to expanding the existing information on the effects of xenobiotics on P. clarkii, there is a need to develop simple experimental methodologies which mimic impacts of pollutants on resident populations of aquatic organisms.

ACUTE TOXICITY OF PERMETHRIN TO FOUR SIZE CLASSES OF RED SWAMP CRAWFISH (PROCAMBARUS CLARKII) AND OBSERVATIONS OF POST-EXPOSURE EFFECTS

OBJECTIVES

The objectives of this study were: (1) to determine the 96-h LC50 of permethrin to the following four size classes of red swamp crawfish (Procambarus clarkii); 8 - 12 mm (0.017 g), 25 - 35 mm (0.64 g), 45 - 55 mm (2.45 g), and 65 - 75 mm total length (TL) (8.98 g); and (2) determine the effects of sublethal permethrin exposure on growth, survival, and reproduction in red swamp crawfish that survived 96-h permethrin exposure.

MATERIALS AND METHODS

Acute Toxicity

A commercial formulation of permethrin (AMBUSH^{TM}, ICI Americas, Inc., Goldsboro, North Carolina) was used in all assays. The formulation was an emulsifiable concentrate that was 25.6 % active ingredient (a.i.).

The 96-h LC50 of permethrin to four size classes of P. clarkii was determined in static acute toxicity tests generally following procedures in "Methods for Acute Toxicity Tests with Fish, Macroinvertebrates, and Amphibians" (USEPA 1975), and "Standard Methods for Examination of Water and Wastewater" (APHA et al. 1985). The four size classes of Procambarus used were 8 - 12 mm (mean weight of 0.017 g), 25 - 35 mm (mean weight of 0.64 g), 45 - 55 mm (mean weight of 2.45 g), and 65 - 75 mm total length (TL, tip of the rostrum to the end of the telson) (mean weight of 8.98 g). A 24-h range-finding test was conducted prior to the 96-h definitive test to define the range of permethrin dilutions to be used in the definitive tests.

Procambarus clarkii were collected from ponds at Ben Hur Research Farm, Louisiana Agricultural Experiment Station, Baton Rouge, Louisiana, and transported to a laboratory of the Forestry, Wildlife, and Fisheries Building where they were acclimated at 21 - 23 C in 40, 50-L capacity polyethylene tanks (45-cm L x 30-cm W x 30-cm D) for 10 days. Crawfish were initially maintained in 10 L of water obtained from the ponds from which the crawfish were collected. About 25 % of the pond water was replaced daily with dechlorinated tap water adjusted to a water hardness of 100 mg/L as $CaCO_3$, so that after 4 days all the pond water had been replaced. Dissolved oxygen concentration in the acclimation tanks was maintained at 60 % of air saturation or greater. Crawfish were exposed to a 24-h light cycle. Crawfish were fed to satiation daily with formulated trout ration (40 % crude protein, Number 4, 0.5-mm dia) during acclimation, and feeding was suspended 48 h prior to tests. Less than 15 % mortality occurred among crawfish during acclimation, and most of the mortality resulted from cannibalism of recently molted crawfish.

Baton Rouge city water from subsurface aquifers was used as acclimation and dilution water. Water was passed through an activated charcoal filter to remove chlorine and organic matter, and then adjusted to a total hardness of 100 mg/L as $CaCO_3$ with anhydrous calcium chloride. Water was aerated to near total oxygen saturation before use in tests. The longest crawfish in each size class used in the toxicity tests was no longer than twice the length of the shortest crawfish, and the biomass per test container did not exceed 0.8 g/L. In toxicity tests with the 25 - 35 and 45 - 55 mm TL size classes the male to female ratio in each test aquarium was 1:1, and in the 65 - 75 mm TL size class one mature male, one female, and two immature males were tested per aquarium. Anatomical characteristics as illustrated by Culley et al. (1985) were used to identify mature from immature crawfish. Mature female P. clarkii can not be accurately

identified by external means from immature females, and no effort was made to separate them.

Intermolt crawfish (molt stage C, Huner and Barr 1984), randomly selected from the acclimation tanks, were placed into polyvinyl chloride (PVC) containers (one crawfish per container). The PVC cages (20.3-cm L x 5.1-cm dia) were used to separate crawfish and prevent cannibalism. The PVC cages were placed in exposure containers 30 min after permethrin had been added in dilution water. Aquaria of glass and silicone construction were lined with polyethylene bags for use as exposure containers. Water temperature and crawfish mortality were recorded every 6 h over the 24-h range-finding tests. In the 96-h tests, water temperature and mortality were recorded at 6-h intervals for the initial 24 h and at 24-h intervals thereafter. Crawfish were considered dead if they failed to respond to antennae or leg stimuli, and dead crawfish were removed as soon as they were observed. Dissolved oxygen (polarographic oxygen meter, Model 57, Yellow Springs Instrument Co., Yellow Springs, Ohio), pH (glass electrode) and ammonia (selective ion electrode) were measured initially and at the termination of both the range-finding and 96-h tests. Total hardness (titration with 0.01 M EDTA) and total alkalinity (titration with 0.02 N H_2SO_4) was measured at initiation of each test using analytical methods in APHA et al. (1985).

A 1 % stock solution (1,000 mg a. i./L) of permethrin was prepared using dilution (exposure) water as the solvent. Four test concentrations (10.000, 1.000, 0.010, and 0.001 mg/L a. i.) of permethrin and a 0 mg/L control were randomly assigned to duplicate exposure containers in the range-finding tests. In range-finding tests, 10 crawfish per test concentration (5 crawfish/exposure container) were used. Permethrin concentrations selected in the definitive tests were determined using a geometric series between those concentrations in the range-finding test at which 0 and 100 % crawfish mortality occurred (USEPA 1975). Six permethrin concentrations and

a 0 mg/L control were randomly assigned among 21 aquaria (three replicates per permethrin concentration) for each acute toxicity test. The acute toxicity test was repeated three times for each of the four size classes of crawfish. The number of crawfish used in each toxicity test varied between 12 and 30 crawfish per test concentration to maintain biomass at 0.8 g/L or less (Appendix Tables 1 - 12).

Post-Exposure Growth and Survival

A 28-day post-exposure growth and survival study was conducted using crawfish from each size class that survived permethrin exposure in the 96-h acute toxicity tests. Crawfish in each size class were separated into groups corresponding to the respective permethrin exposure concentration. The total number of crawfish used for the post-exposure tests in the 8 - 12, 25 - 35, 45 - 55 and 65 - 75 mm TL size classes were 60, 62, 57 and 36 crawfish, respectivly. Crawfish of each size class and each permethrin exposure group were transferred to 19-L glass aquaria containing 5 L of aerated, pesticide-free exposure water and maintained for 28 days. Each crawfish was placed in a separate PVC cage (10.2-cm L x 12.7-cm dia) and fed to satiation daily with trout ration. Water was exchanged every 3 days to maintain satisfactory water quality. Water temperature was measured daily, and the pH, dissolved oxygen, total hardness, total alkalinity and ammonia (un-ionized) were determined weekly. Crawfish were checked daily for molts and mortality. On day 0, 14 and 28, crawfish were measured to the nearest mm, blotted dry with tissue paper and weighed to the nearest 0.01 g on a top-loading balance.

Post-Exposure Reproduction

Form I males and females (with well-developed cement glands) that survived permethrin exposure were mated to each other, and those from the controls were treated likewise. The total number of permethrin-exposed and control female crawfish used in reproductive studies was 19 and 16, respectively. Single mature male and female

crawfish of similar size were placed together in a 50-L polyethylene container containing 5 L of water, and mating activity was observed for 24 h. After 24 h, males were removed and the containers containing females were covered to provide continuous darkness (Huner and Barr 1984). Water was replaced every 3 days. Females were fed daily, and observed for oviposition and hatching of oocytes. One week after hatching, young were separated from females, counted, and 15 young crawfish per female were examined with a stereoscopic microscope (10 - 100 X magnification) for gross deformities. The number of females that spawned and number of young that hatched were compared between permethrin-exposed and control crawfish 75 days after mating.

Statistical Analysis

Mortality data from each of the four static acute toxicity tests were analyzed with probit analysis to determine the 96-h LC50 ± 95 % confidence limits for permethrin exposure in each crawfish size class (Finney 1971). In the probit analysis, the LC50 was determined using the highest permethrin concentration at which 0 % mortality occurred and the lowest permethrin concentration at which 100 % mortality occurred. The unpaired t-test was used to determine if differences existed in (1) mortality between immature male and female crawfish in the 96-h toxicity tests, (2) percent of females experiencing oviposition, and (3) number of young hatched in the post-exposure reproduction study.

Differences in mortality between the mature and immature forms in a 96-h acute toxicity test with the 65 - 75 mm TL size class were determined with analysis of variance (Steel and Torrie 1980). Differences in total weight gain, total length increase, molting frequency, and mortality of crawfish among the permethrin exposure concentrations in each size class from the post-exposure study were determined with analysis of variance. Polynomial orthogonal contrasts (Hicks 1973) were used to

describe the relationship of the response variables to exposure concentrations (Steel and Torrie 1980). Statistical differences were declared significant at $\alpha \leq 0.05$.

RESULTS

Acute Toxicity

P. clarkii in the 8 - 12 mm TL size class were the most sensitive to permethrin exposure, and 45 - 55 mm TL P. clarkii were the least sensitive. The mean 96-h LC50 for 8 - 12 mm TL P. clarkii was 0.438 µg/L which was about one-third the 96-h LC50 for P. clarkii 45 - 55 mm TL (mean of 1.298 µg/L). Permethrin tolerance of crawfish 25 - 35 mm TL and 65 - 75 mm TL was similar as indicated by 96-h LC50s of 0.854 and 0.813 µg/L, respectively (Table 1). Permethrin toxicity was manifested most rapidly (by 6 h) in the smallest size class, and mortality among all size classes of crawfish was maximized within 24 to 48 h of exposure. The quality of exposure water during the toxicity tests was maintained within ranges acceptable for optimum crawfish survival (Hymel 1985, Table 2). DO was maintained at 6.4 mg/L or greater and un-ionized ammonia (NH_3-N) remained less than 0.04 mg/L during all toxicity tests.

Clinical signs of permethrin toxicity in insects (Gammon 1981) were evident in crawfish of all size classes exposed to this compound. Initially, crawfish displayed hyperactive movement that ceased after 30 min. Within 2 h, crawfish exposed to permethrin were hyperactive again. Within several minutes following the second manifestation of hyperactivity, Procambarus displayed ataxia. Ataxia was followed by paralysis and then death. Clinical signs of permethrin toxicity were displayed in less than 1 h by the 8 - 12 mm TL size class of P. clarkii and mortality was observed within 3 h of permethrin exposure.

The tolerance of immature male and female P. clarkii to permethrin exposure did not differ. Total survival of immature male and immature female crawfish in the 25 - 35 and 45 - 55 mm TL size classes after 96 h of exposure was 54 and 46 %, respectively. Mortality among female (mature and immature), mature males and immature males in the

Table 1. Concentration of permethrin that is lethal to 50 % of Procambarus clarkii of 8 - 12, 25 - 35, 45 - 55, and 65 - 75 mm TL size classes in a 96-h exposure.

Size class (mm)	96-h LC50 (µg / L) Replicate 1	2	3	Mean
8 - 12	0.499 (0.451, 0.554)[1]	0.282 (0.231, 0.346)	0.532 (0.464, 0.620)	0.438 (0.382, 0.507)
25 - 35	1.047 (0.902, 1.235)	0.695 (0.595, 0.831)	0.819 (0.677, 1.023)	0.854 (0.725, 1.030)
45 - 55	1.368 (1.232, 1.536)	1.266 (1.129, 1.436)	1.266 (1.129, 1.436)	1.298 (1.163, 1.469)
65 - 75	0.803 (0.729, 0.893)	0.645 (- 0.082, 0.818)	0.992 (0.899, 1.104)	0.813 (0.515, 0.938)

[1] The lower and upper 95 % confidence limits are listed in parentheses beneath the LC50 value.

Table 2. Mean (± 1 SD) dissolved oxygen, pH, temperature, total alkalinity, total hardness, and conductivity of exposure water.

Dissolved Oxygen (mg/L)		pH		Temperature	Total Alkalinity (mg/L)	Total Hardness (mg/L)	Conductivity
0	96 h	0	96 h	(C)	as $CaCO_3$	as $CaCO_3$	(µohms/cm)
				8 -12 mm TL Size Class			
8.1 ± 0.4	6.8 ± 0.4	8.5 ± 0.0	7.9 ± 0.1	21.8 ± 0.5	172.7 ± 19.1	99.4 ± 1.0	486 ± 9.4
				25 - 35 mm TL Size Class			
8.8 ± 0.4	7.4 ± 0.2	8.5 ± 0.0	8.1 ± 0.1	21.2 ± 0.4	170.3 ± 7.0	98.3 ± 0.7	506 ± 4.5
				45 - 55 mm TL Size Class			
8.3 ± 0.6	6.9 ± 0.7	8.8 ± 0.6	8.3 ± 0.5	22.7 ± 0.6	162.3 ± 11.6	98.0 ± 1.3	493 ± 4.2
				65 - 75 mm TL Size Class			
8.2 ± 0.2	6.4 ± 0.1	8.6 ± 0.0	8.4 ± 0.6	23.1 ± 0.2	161.2 ± 10.2	98.4 ± 0.9	490 ± 0.0

Un-ionized ammonia was not detected in exposure water of any test.

65 - 75 mm TL size class did not differ. Mean survival of P. clarkii in the 65 - 75 mm TL size class in the toxicity tests was 56 % for immature males, 54 % for females (mature and immature), and 37 % for mature males. Mortality among maturity stages of P. clarkii at the various concentrations of permethrin exposure is illustrated in Figure 1.

Post-Exposure Growth, Survival and Reproduction

P. clarkii in the 8 - 12 mm TL size class that survived permethrin exposure in the acute toxicity test exhibited a significant increase in weight, length, and molting frequency in the 28-day growth study ($P < 0.05$); however, increase in size and molting frequency was not correlated with prior permethrin exposure (Table 3). Mean weight gain at all permethrin concentrations ranged from 0.01 to 0.02 g/week and total length increase averaged from 1 to 2 mm per week. Molting frequency ranged from 1 to 3 molts per crawfish in 28 days. Post-exposure mortality of crawfish in the 8 - 12 mm TL size class ranged from 0 to 40 % and post-exposure mortality decreased with an increase in prior permethrin-exposure concentration ($P < 0.05$).

Mean total weight and length increase for P. clarkii in the 25 - 35 mm TL size class ranged from 0.2 to 0.4 g/week and 2 to 4 mm/week, respectively. There was a significant increase in growth with increase in prior level of permethrin exposure ($P < 0.05$). Mortality of P. clarkii 25 - 35 mm TL size class in the 28-day growth study ranged from 0 to 20 % among exposure, concentrations and crawfish averaged 2 molts (Table 4).

P. clarkii in the 45 - 55 mm TL size class that survived permethrin exposure in the acute toxicity test displayed no differences in weight gain, total length increase, and average number of molts in the 28-day growth study (Table 5). Mean total weight gain and length increase of P. clarkii in the 45 - 55 mm TL size class was 1.0 g/week and 3 mm/week, respectively. Crawfish in the 45 - 55 mm TL size class averaged 2 molts and

Figure 1. Toxicity of permethrin to mature male (Form I), immature male (Form II) and female red swamp crawfish (Procambarus clarkii) 65 - 75 mm TL.

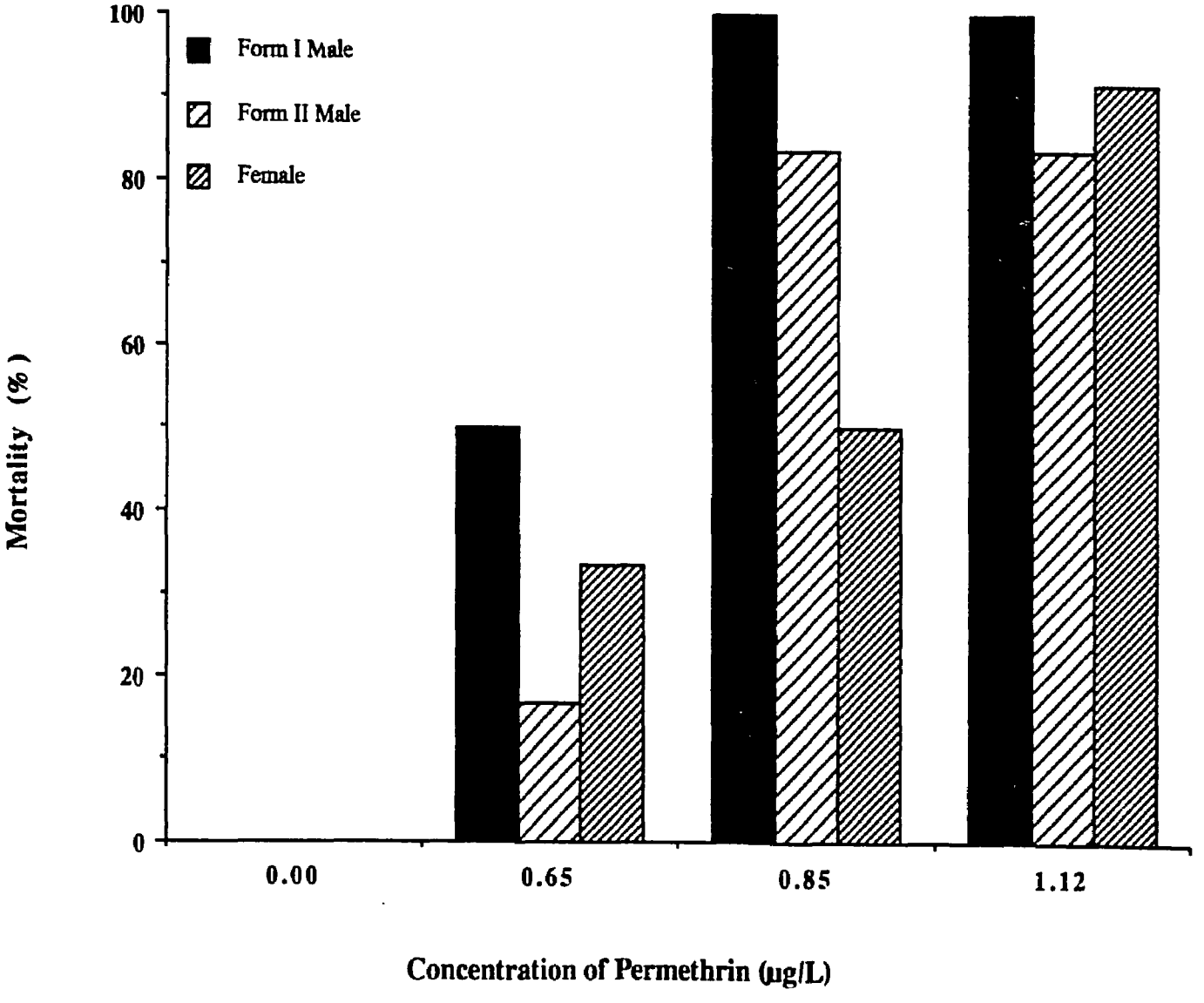

100
80
60
40
20
0
Form I Male
Form II Male
Female
Mortality (%)
0.00
0.65
0.85
1.12
Concentration of Permethrin (µg/L)

Table 3. Mean (± 1 SD) weight gain, length increase, molts, and survival of red swamp crawfish (Procambarus clarkii) 8 - 12 mm TL at 22.0 C after 28 days following a 96-h static exposure to permethrin.

Exposure Concentration (µg / L)	N	Initial Total Weight (g)	Initial Total Length (mm)	Weight Gain (g)	Length Increase (mm)	Average Number of Molts	Mortality (%)
0	10	0.016 ± 0.004	9 ± 0.6	0.09 ± 0.04	7 ± 3.1	1.1 ± 0.9	40
0.099	10	0.015 ± 0.003	9 ± 0.3	0.05 ± 0.02	4 ± 2.2	1.2 ± 1.2	20
0.158	10	0.019 ± 0.005	10 ± 2.6	0.06 ± 0.05	4 ± 2.8	0.9 ± 0.9	40
0.247	10	0.020 ± 0.005	9 ± 0.9	0.09 ± 0.04	7 ± 2.1	1.5 ± 1.3	10
0.396	10	0.017 ± 0.004	9 ± 0.5	0.10 ± 0.03	6 ± 2.3	1.0 ± 1.0	20
0.624	10	0.016 ± 0.002	9 ± 0.5	0.10 ± 0.03	8 ± 1.4	2.6 ± 1.3	0

Table 4. Mean (± 1 SD) weight gain, length increase, molts, and survival of red swamp crawfish (Procambarus clarkii) 25 - 35 mm TL at 22.0 C after 28 days following a 96-h static exposure to permethrin.

Exposure Concentration (µg / L)	N	Initial Total Weight (g)	Initial Total Length (mm)	Weight Gain (g)	Length Increase (mm)	Average Number of Molts	Mortality (%)
0	10	0.59 ± 0.12	30 ± 2.5	1.12 ± 0.28	11 ± 3.2	1.7 ± 1.2	0
0.099	10	0.60 ± 0.10	30 ± 1.9	1.44 ± 0.35	13 ± 4.2	2.4 ± 0.7	0
0.170	10	0.68 ± 0.09	31 ± 1.7	1.41 ± 0.29	13 ± 2.9	1.6 ± 0.8	10
0.292	10	0.62 ± 0.04	31 ± 1.7	1.31 ± 0.44	12 ± 4.3	2.1 ± 0.7	10
0.503	10	0.65 ± 0.11	30 ± 1.7	1.38 ± 0.39	13 ± 1.8	1.8 ± 1.1	20
0.684	10	0.67 ± 0.09	30 ± 1.7	1.61 ± 0.40	15 ± 3.5	2.5 ± 0.5	0
1.485	2	0.67 ± 0.20	30 ± 3.5	0.86 ± 0.18	10 ± 2.8	2.0 ± 0.0	0

Table 5. Mean (± 1 SD) weight gain, length increase, molts, and survival of red swamp crawfish (Procambarus clarkii) 45 - 55 mm TL at 22 .0 C after 28 days following a 96-h static exposure to permethrin.

Exposure Concentration (µg / L)	N	Initial Total Weight (g)	Initial Total Length (mm)	Weight Gain (g)	Length Increase (mm)	Average Number of Molts	Mortality (%)
0	10	2.25 ± 0.25	50 ± 1.3	3.60 ± 1.58	13 ± 4.6	1.5 ± 0.8	30
0.495	10	2.36 ± 0.28	50 ± 1.6	3.51 ± 1.54	12 ± 6.0	1.9 ± 0.6	10
0.653	10	2.47 ± 0.13	51 ± 0.8	3.66 ± 0.77	13 ± 2.7	1.9 ± 0.7	20
0.861	10	2.45 ± 0.24	50 ± 1.1	4.30 ± 1.10	16 ± 3.1	1.8 ± 0.4	0
1.138	10	2.28 ± 0.38	50 ± 2.4	3.83 ± 1.62	13 ± 5.0	1.9 ± 0.6	20
1.495	7	2.92 ± 0.54	52 ± 2.0	4.34 ± 1.29	15 ± 2.1	1.7 ± 0.5	17

mortality among crawfish with prior exposure to permethrin ranged from 0 to 30 % during the 28-day study.

In the 28-day post-exposure growth study, mortality of P. clarkii in the 65 - 75 mm TL size class was not evident. There were no differences in mean weight gain, total length, or molting frequency for P. clarkii with prior permethrin exposure. P. clarkii in the 65 - 75 mm TL size class gained an average of 0.9 g/week and grew an average of 1 mm/week (Table 6). In the 28 days following permethrin exposure in the acute toxicity test, P. clarkii in the 65 - 75 mm TL size class averaged 1 molt per crawfish.

Eighty-three percent of the immature male P. clarkii that were exposed to permethrin and 81 % of the control crawfish attained maturity in the 28-day growth study. All immature male crawfish of the 65 - 75 mm TL size class used in the 28-day growth study required a single molt to reach maturity.

The mean length of female and male P. clarkii used in the reproductive studies was 74 (± 2) mm TL. Sixty-nine percent of the females with prior exposure to permethrin and 63 % of the control females oviposited. Thirty-one percent of the egg clutches produced by permethrin-exposed females and 50 % of the egg clutches produced by control female P. clarkii hatched. Mean (± 1 SD) number of young produced per female was 138 ± 99 crawfish for the permethrin-exposed females and 106 ± 71 crawfish for control female P. clarkii. There were no significant differences in egg production or hatching among females exposed to permethrin and control female P. clarkii. Gross deformities were not apparent in third-instar P. clarkii in either the permethrin-exposed crawfish or the control groups.

Table 6. Mean (± 1 SD) weight gain, length increase, molts, and survival of red swamp crawfish (Procambarus clarkii) 65 - 75 mm TL at 22.0 C after 28 days following a 96-h static exposure to permethrin.

Exposure Concentration (µg / L)	N	Initial Total Weight (g)	Initial Total Length (mm)	Weight Gain (g)	Length Increase (mm)	Average Number of Molts	Mortality (%)
0	12	8.90 ± 1.15	70 ± 2.3	3.51 ± 0.85	6 ± 2.5	0.9 ± 0.3	0
0.653	12	8.85 ± 1.22	71 ± 2.6	3.92 ± 1.37	7 ± 3.0	0.9 ± 0.3	0
0.851	9	8.69 ± 1.17	70 ± 2.9	3.75 ± 1.70	6 ± 2.9	0.9 ± 0.3	0
1.119	3	9.50 ± 0.87	72 ± 1.2	3.63 ± 0.17	6 ± 1.5	1.0 ± 0.0	0

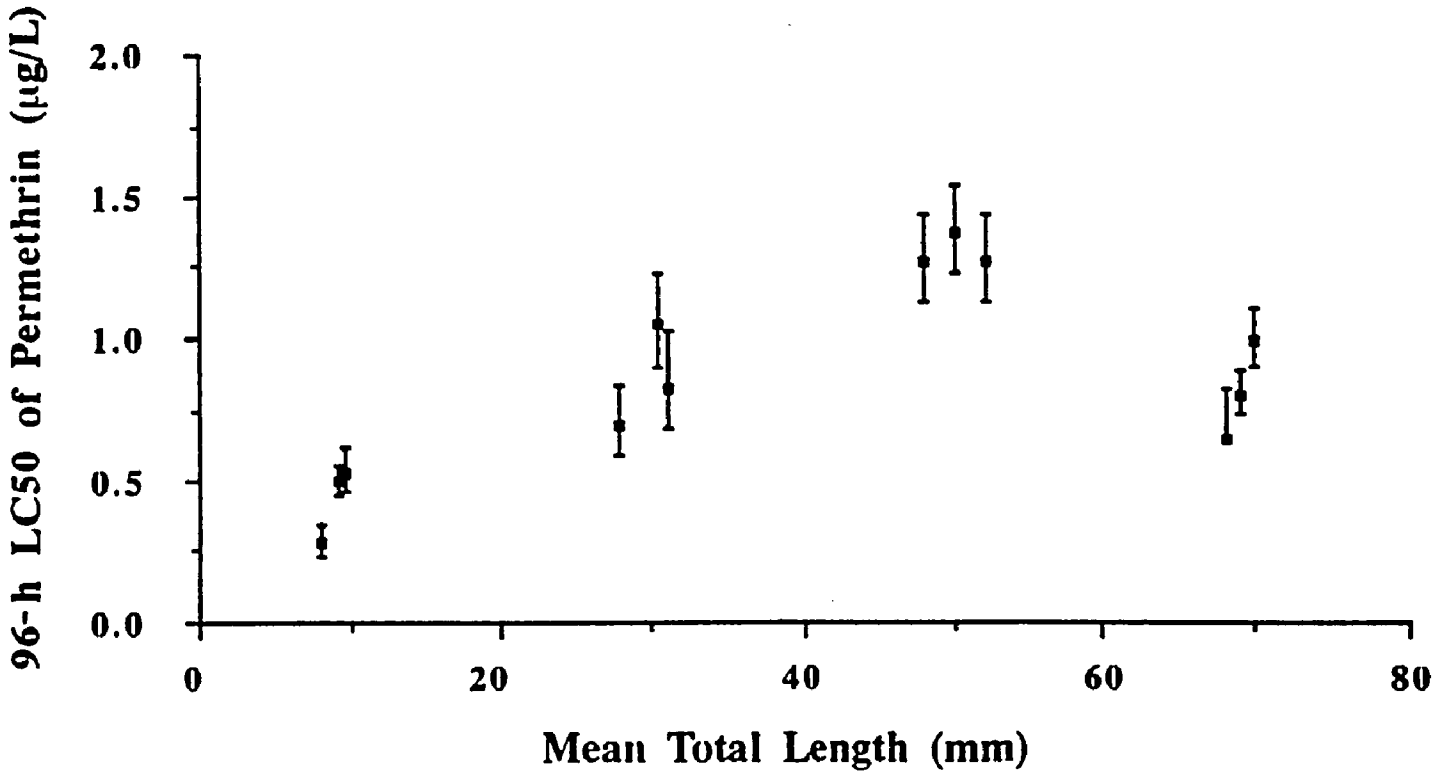
DISCUSSION

Permethrin is classified as a Type I synthetic pyrethroid analogue based on in vivo poisoning clinical signs in insects (Gammon et al. 1981). Physiochemical mode of action of Type I pyrethroids is complex and not completely understood, but clinical evidence indicates that this class of pyrethroids causes excitation and paralysis which results from repetitive firing of axons in the peripheral nervous system (Matsamura 1985). The clinical signs observed in crawfish exposed to permethrin were similar to those described in insects, which indicates that the toxic mode of action observed in pyrethroid-poisoned insects and crawfish may be similar. It is not known if the pathways of permethrin metabolism in crawfish are similar to those observed in insects.

P. clarkii exhibited a size-dose response for animals from 8 to 55 mm TL (Figure 2). The mean 96-h LC50's for P. clarkii in the 8 - 12 and 25 - 35 mm TL size classes (0.44 and 0.85 µg/L) were similar to those reported by other investigators. Jolly et al. (1977) reported the 96-h LC50 for 8 - 12 mm TL and 20 - 30 mm TL P. clarkii was 0.39 and 0.62 µg/L, respectively. Coulon (1982) obtained a 24-h LC50 of 0.49 µg/L for P. clarkii 18 - 27 mm TL. Relatively slight differences in results between the studies may have been affected by differences in experimental protocol and test conditions. The response of a population of organisms to toxicant exposure should be normally distributed if the experiment is repeated several times (Steel and Torrie 1980). Repetition of acute toxicity tests with size classes (including mature and immature organisms) that represent the population distribution of the species, should represent the potential impact of the toxicant on the population as a whole.

The increase in permethrin toxicity for the largest crawfish size class (65 - 75 mm TL) relative to the smaller size classes was unexpected because the relationship between size-class response and toxicant dose among the smaller crawfish would

Figure 2. The 96-h LC50 (and 95 % confidence limits) of permethrin for red swamp crawfish (Procambarus clarkii) 8 - 12, 25 - 35, 45 - 55, and 65 - 75 mm TL.


96-h LC50 of Permethrin (µg/L)
2.0
1.5
1.0
0.5
0.0
0
20
40
60
80
Mean Total Length (mm)

suggest larger organisms to be more tolerant. Mechanisms of differential uptake, absorption and excretion of permethrin probably vary among crawfish size classes and stages of maturity and could help to account for the responses observed in this study (Guarino 1987). Further research to determine the reasons for differences in toxicant susceptibility among size classes and among stages of maturity in P. clarkii is necessary.

Jewell and Winston (1989) reported that P. clarkii posesses an active mixed function oxidase system (MFO). The MFO system in crawfish (or any other organism) is responsible for the detoxification and elimination of xenobiotics (Khan et al. 1972; Lindstrom-seppa et al. 1983; Lindstrom-seppa and Hannienen 1986; Jewell and Winston 1989) ; however, with some xenobiotics (such as organophosphates) the MFO system may increase the toxicity of the compound by biotransforming it to a more actively toxic metabolite (Hodgson and Levi 1987). Mixed function oxidase activity varies with stages of maturity and molt cycles in marine crustacea (Lee 1981). The MFO system of mature P. clarkii may biotransform permethrin to a more lethal metabolite. Because no differences were observed in the toxicity of permethrin to immature male and female P. clarkii these data would indicate that some physiological changes associated with maturity and age increased the toxicity of permethrin to P. clarkii .

Sublethal exposure to permethrin at concentrations ranging from 0.624 to 1.495 µg/L had no deleterious effects on P. clarkii growth and survival during a period of 28 days, nor did it affect the onset of sexual maturity and reproduction. The absence of long-term or delayed deleterious effects of permethrin was probably not a result of duration of the exposure period. Jolly et al. (1977) exposed juvenile P. clarkii (20 - 30 mm TL) to an initial permethrin concentration of 0.1 µg/L for 10 weeks and reported growth and survival was not different from non-exposed crawfish. Permanent damage

to organ systems in crawfish that could reduce growth and retard onset of maturity, would occur only if the toxicant was absorbed, internally transported and retained at a target site in the proper concentration for a sufficient period of time to cause tissue damage. Coulon (1982) found that the environmental half-life of permethrin was 5.8 and 9.4 days in pond water and bottom sediment, respectively. Additionally, Coulon (1982) could not detect permethrin bioaccumulation in P. clarkii abdominal muscle or hepatopancreas (at a detection limit of 0.01 µg/g) when P. clarkii was exposed to 6.1 µg/L in earthen ponds. The toxicity of permethrin to crawfish is so high, it is probable that P. clarkii could not survive permethrin concentrations necessary to cause permanent tissue damage.

Permethrin is lethal to P. clarkii ranging in size from 8 to 75 mm TL at concentrations of 0.28 to 1.38 µg/L in exposures as long as 96 h.. Field application rates of permethrin to control insects range from 0.028 to 0.224 kg a.i./ha (ICI Americas Inc.). A 1 ha crawfish pond 46-cm deep treated at rates recommended for insect management would have permethrin concentrations of 6.1 to 48.1 µg/L. Based on the current study and past research, even the lowest field treatment level used for insect management would seriously impact crawfish populations. The acute toxicity tests in this study were conducted with laboratory prepared water with minimal concentrations of organic matter. Coulon (1982) found the toxicity of permethrin to red swamp crawfish may decrease as much as 100 % with the addition of sediment to exposure water. Coulon (1982) and Jarboe (1989, see section II of this study) found the toxicity of permethrin to crawfish decreased by more than 300 % when permethrin was applied to water in earthen ponds containing crawfish.

Metabolism of pesticides and other xenobiotics in mature red swamp crawfish and the influence of age and maturity on compound toxicity are areas of research which need to be investigated. If age and maturity do influence the toxicity of permethrin to P.

clarkii and the relationship is valid for other classes of xenobiotics both in the laboratory and field, a decrease in the number of mature red swamp crawfish during seasonal periods when they should be abundant may serve as a bioindicator of an impacted environment.

POPULATION MANAGEMENT OF RED SWAMP CRAWFISH (PROCAMBARUS CLARKII) BY DENSITY REDUCTION AND SUPPLEMENTAL FEEDING

OBJECTIVES

The ojectives of this study were to: (1) determine if density reduction and/or supplemental feeding could be used to induce growth of stunted crawfish populations in ponds; (2) examine the response of red swamp crawfish (Procambarus clarkii) populations in earthen ponds to permethrin application; and (3) characterize seasonal crawfish population dynamics which may be indicative of crawfish populations that stunt.

MATERIALS AND METHODS

Stocking of Crawfish Broodstock

Mature P. clarkii (mean size of 90 mm TL, 50:50 male:female ratio) were stocked in 15, 0.044 ha earthen ponds (55 m long x 8 m wide) 26 June 1987, at 171 kg/ha. The stocking density was two or three times higher than the density recommended for ponds in the first year of production (Avault and Huner 1985) because the intent was to produce high numbers of P. clarkii, characteristic of stunted populations. Brood crawfish were released in the water along pond embankments, and water was drained over 2 weeks (average of 15 cm per day) to stimulate burrowing by the crawfish. The ponds had been managed for crawfish production the previous year and some crawfish were present in addition to those stocked.

Pond Preparation

Ammonium nitrate was disked into pond bottoms at 24.6 kg N/ha, 2 weeks prior to rice seeding (Louisiana Cooperative Extension Service 1986). Water from a

100 m deep aquifer ("well water") was added to ponds 15 July 1987 until the water averaged 7 - 10 cm in depth. Pre-sprouted rice (Oryza sativa, Newbonnet variety) was water-seeded in all ponds at 172 kg seed/ha, and water was drained from the ponds within 48 hours (Louisiana Cooperative Extension Service 1986). Rice plants were top-dressed with ammonium nitrate at 30.8 kg N/ha 6 weeks after planting (Louisiana Cooperative Extension Service 1984). As rice grew, water was added to ponds and maintained at an average depth of 3 - 5 cm to minimize weed growth.

Vegetative biomass (dry weight) was quantified with area-transect sampling (Rice 1967) in early October, 2 weeks prior to filling the ponds with water for crawfish. Predominate vegetation types were identified, and the area covered by each type was then determined visually. A wooden quadrat (30.5 cm^2) was tossed into areas containing each vegetation type, and all plants (including roots) were collected from the area within the quadrat. The quadrat was tossed into each vegetation type of the pond a total of three times, and the three sub-samples were combined for further analysis. Depending upon the number of types of vegetation in the ponds, the total number of vegetation samples ranged from two to six samples per pond (six to 16 sub-samples).

Vegetation was rinsed free of soil with tap water, placed in aluminum foil, and air-dried in a forced-air oven at 60 C until the weight was near constant, cooled to room temperature, and weighed to the nearest 0.01 g (Brunson 1987). Vegetative biomass in each pond was estimated by multiplying the average dry weight of the sample by the area of the pond that vegetative type covered.

One month prior to permanent flooding of the ponds for crawfish production, a trapping lane, 1 m wide, was placed in the center of each pond along the length by cutting the rice at its base. Ponds were flooded for crawfish production to a depth of 0.5 m with well water aerated to near oxygen saturation on 20 - 27 October 1987. Dissolved oxygen (DO) and water temperature were measured in each pond twice a

week at 0600 to 0700 h with a polarographic oxygen meter (Model 57, Yellow Springs Instrument Co., Yellow Springs, Ohio).

Growth

In early November, and continuing at approximate monthly intervals thereafter, crawfish were captured in small-mesh traps (1.9-mm square mesh) and dip-nets (2.0-mm diamond mesh). A dip-net tow, which is generally selective for crawfish less than 40 mm TL (Romaire 1976), was made by pulling the dip-net along the pond bottom a distance of 1.2 m in six random locations in each pond. Four tows were made along the pond embankment and two tows were made in the center of the pond on each sampling date. Sample location of tows differed on each sampling period. Crawfish larger than 40 mm TL were captured with small-mesh traps with two-entrance funnels (Romaire 1976). Traps (two per pond) were baited with Purina Jumbo crawfish bait (Purina Mills, Inc., Shreveport, Louisiana) and gizzard shad (Dorosoma cepedianum) and the catch removed after 24 h. Crawfish caught in the small-mesh traps and dip-nets were counted and measured, identified to species, sexed, and the stage of maturity in males (Form I, mature; Form II, immature or juveniles) was determined. Crawfish were then returned to the pond.

The length data of crawfish collected with the small-mesh traps and dip-nets were combined by species for each sampling date and grouped in 5-mm size increments. The number of young-of-the-year (YOY) recruitment classes (cohorts) present on successive sampling dates were identified by quantitating the abundance of crawfish less than 15 mm TL from length-frequency histograms. YOY cohorts less than 15 mm TL were representative of recent spawning by females. Growth rates of YOY crawfish recruitment classes were estimated by changes in mean length between successive monthly sampling dates (Ricker 1975).

Crawfish Population Density Estimates

Crawfish population density was estimated in all ponds in January, February, March, and June 1988 using area-density sampling for crawfish less than 40 mm total length (TL- tip of rostrum to end of the telson), and the Schnabel mark-recapture procedure for crawfish larger than 40 mm TL (Everhart et al. 1976). Area-density estimates of crawfish less than 40 mm TL were determined by collecting crawfish with a dip-net . The area sampled by a dip-net tow was approximately 0.5 m^2. All crawfish within the area covered by the dip net tow were assumed to be captured (100 % capture efficiency). The density of crawfish less than 40 mm TL was determined by multiplying the mean number of crawfish captured per dip-net tow by 880 (440 m^2/ pond ÷ 0.5m^2/tow= 880)

At least 50 intermolt crawfish captured in small-mesh traps using the trapping procedure described in "Growth", were marked on the dorsal region of the carapace with water insoluble nail polish (Camougis and Hichar 1959) and returned to the pond within 30 minutes of capture. The process was repeated every two days over a 9-day sampling period, and the number of marked and unmarked crawfish captured were determined on each sampling day. The estimate of crawfish in the pond exceeding 40 mm TL ($\hat{N}$) was determined from:

$$\hat{N} = \frac{\Sigma(\text{Total Number Captured} * \text{Total Number Marked})}{\Sigma \text{Total Number Recaptured}}$$

Total crawfish population density in each pond was determined by adding the estimate from the area-density procedure with the estimate from the Schnabel mark-and-recapture procedure.

Experimental Treatments

Five experimental treatments with three replicate ponds per treatment were evaluated. The treatments were: (1) "high" crawfish density control, (2) "low" crawfish density control, (3) crawfish density reduced with permethrin, (4) crawfish density reduced with permethrin and supplemental feed provided, and (5) no crawfish density reduction and supplemental feed provided. Treatments were assigned to the 15 ponds following quantitative estimates of crawfish population densities made in January 1988 (see "Crawfish Population Estimates"). The ponds to which the high density and low density control treatments were assigned were those ponds with the three lowest and the three highest crawfish densities, respectively. The number of crawfish in these two treatments were not reduced by external inputs nor was supplemental feed used. The other three treatments of the study were assigned to the remaining nine ponds at random.

Crawfish populations in the two treatments receiving formulated feed were fed following population reduction in April (see "Crawfish Population Density Reduction). Crawfish that were fed (with or without density reduction) received a formulated crawfish ration (27 % crude protein, Appendix Table 13) at 28 kg/ha/day on 3 consecutive days per week beginning 15 April. Crawfish were fed on 30 days for a total of 840 kg of feed/ha (37 kg/pond). Feed was applied in shallow water along pond embankments and crawfish were not harvested on days that they were fed.

In the two "density reduced" treatments, crawfish populations were reduced to a targeted density of 4 to 6 crawfish/m^2 (Huner and Romaire 1978; Romaire et al. 1978; Lutz and Wolters 1986) with permethrin.

Crawfish Population Density Reduction

Permethrin (3-phenoxybenzyl (+) cis, trans, 3-(2,2-dichlorovinyl)-2, 2-dimethylcyclopropane carboxylate), a synthetic pyrethroid insecticide, was used to reduce crawfish population density. The specific formulation selected was Ambush ™ which contained 25.6 % permethrin as the active ingredient (Technical Information Bulletin, ICI Americas, Inc., Goldsboro, North Carolina).

Aquarium Study. "Field" toxicity tests on crawfish using water from experimental crawfish ponds assigned to the "density reduced" and "density reduced and fed" treatments were conducted on 5 - 6 April 1988. The following experimental procedures were conducted for each of the six ponds in which crawfish population density was reduced.

Ten liters of water from the top 10 cm of the water column were collected from each crawfish pond and transferred into eight, 19-L glass aquaria lined with polyethylene bags. Aquaria were placed on 1.2-m x 1.2-m wooden pallets and covered with plywood to prevent excessive heating. Crawfish and permethrin were added to aquaria containing pond water after 6 h.

Crawfish used in pond-water toxicity tests were collected from the ponds that were to have resident crawfish population density reduced. The mean size of crawfish used in the tests is presented in Table 7. Biomass loading of crawfish in each aquarium did not exceed 0.8 g/L (USEPA 1975), and the tests were conducted for 24 h to minimize the possibility of low DO stressing the crawfish and thereby making them more susceptible to permethrin toxicity. Additionally, permethrin toxicity tests conducted in the laboratory demonstrated that the death of crawfish exposed to lethal concentrations of permethrin usually occurred within 24 h (Jarboe 1989, see section I of this study).

Table 7. Mean (± 1 SD) size of Procambarus clarkii in permethrin field toxicity tests. The range of crawfish sizes used are presented in parentheses.

	Aquaria		Cages	
	N/aquaria	Total length (mm)	N/cage	Total length (mm)
			Density Reduced and Fed	
Replicate				
1	5	45 ± 12.2 (31 - 70)	30	60 ± 7.5 (30 - 80)
2	5	45 ± 6.7 (40 - 60)	30	57 ± 12.0 (36 - 90)
3	5	45 ± 13.3 (40 - 70)	30	60 ± 15.8 (41 - 80)
			Density Reduced	
Replicate				
1	5	51 ± 10.8 (36 - 70)	30	61 ± 11.5 (36 - 85)
2	5	32 ± 7.1 (35 - 40)	30	64 ± 11.7 (36 - 90)
3	5	29 ± 8.6 (35 - 40)	30	72 ± 11.2 (41 - 80)

A 1% stock solution (1,000 mg a.i./L) of permethrin was prepared in 1-L glass volumetric flasks using tap water as the solvent. The stock solution was diluted with tap water to 1.0 µg /ml active ingredient in 1-L glass volumetric flasks and an appropriate amount of diluted stock was added to aquaria to achieve final permethrin concentrations of 0, 2.0, 4.0, and 6.0 µg/L in aquaria. Each permethrin concentration was replicated twice. Permethrin was added to the pond water, stirred with a glass rod to mix the solution, and crawfish were immediately added to the aquaria. The DO and water temperature were measured at 0 and 24 h with a polargraphic oxygen meter and crawfish mortality at 24 h was recorded. Crawfish were considered dead if they failed to respond to a tactile stimulus.

Pond Study. The quantity of water in ponds to be treated with permethrin was calculated prior to application by multiplying pond area by mean water depth. Based on the information from the pond-water permethrin toxicity determinations, an appropriate amount of permethrin estimated to achieve the desired crawfish population reduction to 4 to 6 crawfish/m^2 was mixed with 18.9 L of tap water inside a backpack pesticide applicator and the solution was sprayed into ponds on 7 - 8 April 1988 (Appendix Table 14).

A composited pond water sample of 8 L was taken from several locations in each pond immediately before permethrin application. Total alkalinity, total hardness, pH, total organic carbon (TOC), conductivity, total solids (TS), and biochemical oxygen demand (BOD) was determined from the composited pond water sample. Total alkalinity (titrated with 0.02 N sulfuric acid) and total hardness (titrated with 0.01 M EDTA) in mg/L as $CaCO_3$ were determined according to procedures in APHA et al. (1985). The pH was determined with a glass electrode (Fisher Scientific Inc., New York, New York). Conductivity was measured with a conductivity meter (Model 33,

Yellow Springs Instrument, Co., Yellow Springs, Ohio). The TOC of pond water samples was measured with a Technicon Auto Analyzer (Model II, Technicon Inc., Terrytown, New York). The BOD of pond water was determined by incubation for 5 days at 20 C (APHA et al. 1985). The TS was determined by placing 100 ml of pond water in tared Erlenmeyer flasks, evaporating the samples to dryness in a forced-air convection oven at 100 C, and reweighing flask.

Mortality of crawfish in the ponds treated with permethin was estimated by putting 30 crawfish in each of two small-mesh wire cages in the ponds immediately prior to permethrin application (Table). Crawfish mortality in cages was determined at 24, 48, 72, and 96 hours following addition of permethrin.

The toxicity of permethrin to pond crawfish populations was determined from the differences in the population estimate 2 days prior to, and 7 days following permethrin application. Toxicity of permethrin to pond crawfish was compared among mature males and immature males, and female P. clarkii and P. acutus acutus in size classes ranging from 30 mm to 90 mm TL. The toxicity of permethrin was also compared between crawfish less than 40 mm TL and crawfish equal to or exceeding 40 mm TL.

Harvest

Crawfish were harvested on 4 consecutive days weekly with large-mesh traps (two-entrance funnels, 1.9-cm mesh size) placed in all ponds at a density of 90 traps/ha (4 traps/pond) (Romaire and Pfister 1983). Traps were baited between 0080 and 1100 h with a combination of cut gizzard shad (Dorosoma cepedianum) and Purina Jumbo formulated crawfish bait. Crawfish were removed from the traps after 24 h, counted and the total weight per trap was recorded.

Crawfish harvest was divided into pre-treatment and post-treatment intervals for a total of 41 harvest days. Pre-treatment harvest was conducted over 10 days from 15

March to 26 March 1988. Post-treatment ponds were trapped for a total of 31 days from 21 April through 15 June 1988.

Permethrin Residue Analysis

Abdominal muscle and hepatopancreas of crawfish from the six permethrin-treated ponds and an untreated control pond were analyzed for permethrin residue following chemical application. Fifteen to 25 crawfish were collected with baited large-mesh traps from each permethrin-treated pond at 7, 14, and 21 days post-pesticide application, and 15 - 25 crawfish were collected from a control pond on the same day. Crawfish were immediately placed on ice and transported to the laboratory were abdominal muscle was separated from the exoskeleton, wrapped in aluminum foil and stored at 0 C (Coulon 1982). Crawfish cephalothorax containing hepatopancreas was immediately wrapped in aluminum foil and frozen intact until analysis. Cephalothorax was thawed and the hepatopancreas was removed immediately prior to permethrin extractions.

Permethrin extractions and chromatographic analysis were conducted from 20 January to 29 February 1989 at the Institute for Environmental Studies, Louisiana State University, Baton Rouge, Louisiana according to procedures provided by ICI Americas (ICI Americas Information Bulletin 1986). All of the crawfish tissue collected on a specific sampling date from each pond was homogenized at 10,000 rpm in a stainless steel Waring blender. Individual tissue samples (about 1 g of hepatopancreas and 3 g of abdominal muscle, wet weight) were ground with anhydrous Na_2SO_4 and covered with dichloromethane (DCM). Tissue samples were then extracted three times by sonication in DCM. Extracts from the crawfish tissues were decanted through Na_2SO_4. Additional lipid was removed from hepatopancreas samples by adding 1 ml of ethyl acetate (personal communication, February 1989, Charles Henry, Research Associate,

Institute of Environmental Studies, Louisiana State University, Baton Rouge, Louisiana).

Hepatopancreas samples were heated to 40 C for 5 min; 5 ml of methanol was added to each sample; samples were cooled to - 70 C in a freezer; removed and the lipid was allowed to preciptate out of solution overnight at room temperature. Supernant from hepatopancreas and abdominal muscle extracts was concentrated to 200 µl by evaporating the organic solvent with N_2. Solvent was exchanged to hexane, and samples were passed through aromatic sulfonic acid (ASA) columns (J. T. Baker Chemical Co., Phillipsburg, New Jersey) for removal of additional lipid and impurities (personal communication, January 1989, Jay Means, Assistant Professor, Institute of Environmental Studies, Louisiana State University, Baton Rouge, Louisiana). Elutants were concentrated by evaporation with N_2 (50 µl for tail muscle and 1 ml for hepatopancreas) and analyzed by gas chromatography. Lipids collected in ASA columns were eluted with ethyl acetate and concentrated to a constant weight so that permethrin residue could be calculated on a lipid weight basis. Tissue vials containing precipitated hepatopancreas lipid were also concentrated to a constant weight for quantification. Procedural blanks (an extraction without tissue) were included with every set of sample extractions. Endrin (a chlorinated cyclodiene insecticide) was added to all samples (including controls and blanks) and permethrin was added to control samples prior to extraction to serve as internal standards. Recovery efficiency for extractions was 56.0 % for both abdominal muscle and hepatopancreas.

Samples were analyzed using a Hewlett-Packard 5890 gas chromatograph (Hewlett Packard Corp., Cupertino, California) equipped with a ^{63}Ni electron capture dector, splitless injection, a DB-5 capillary column (30 m x 0.25 mm I. D. x 0.25 µm film thickness) and helium carrier gas (linear velocity of 40 cm/sec). Injector and detector temperatures were set at 270 and 300 C, respectively. The oven was

programmed from 50 to 280 C with initial temperature of 50 C for 3 min; ramp to 250 C at 12 deg/min; ramp to 280 C at 6 deg/min (personal communication, January 1989, Debbie McMillan, Research Associate, Institute of Environmental Studies, Louisiana State University, Baton Rouge, Louisiana). Overall time of the run was 40 min. Based on analytical standards, the retention time of permethrin was 25.3 min with the cis permethrin isomer eluting approximately 0.19 sec before trans. The detection limit for abdominal muscle was 6.7 ng/g. Due to a high degree of matrix interference, the detection limit for hepatopancreas was 400.0 ng/g. Permethrin residue levels were calculated by comparing the peak height of the analyte to the peak height of a known amount of permethrin standard. The permethrin standard was supplied by ICI Americas Inc. The purity of the analytical standard was 95.0 %; 36.5 and 58.5 % cis and trans isomers, respectively.

Statistical Analysis

The experimental design used in the feeding and density reduction study for both environmental variables and crawfish population variables was a completely randomized design. Differences in treatment means for vegetative biomass, water temperature, and DO were compared using the analysis of variance. Data on crawfish population parameters were analyzed with the analysis of variance in a completely randomized design with a factorial arrangement of treatments and with sample date and treatment as the main effects. Linear contrasts for both the environmental and population variables were made between "low density control" versus the other four treatments; "low density control" versus "high density control"; "high density control" versus "fed" treatment; "density reduced" versus "density reduced and fed" treatment; "density reduced" treatments versus those treatments which were not reduced (not including the "low density control"); and "fed" treatments were compared to treatments receiving no feed (not including the "low density control"). The response variables evaluated with

the linear contrasts were total number harvested, total weight harvested, mean size, catch per trap-set (CPUE), percent Form I males, percent Form II/juvenile males, percent females, percent of crawfish exceeding 75 mm TL, TL of Form I males, population density of crawfish less than 15 mm TL, and growth rates of crawfish. Contrasts for these response variables were made prior to treatment, post-treatment, and pre- and post-treatment combined.

The concentration of permethrin lethal to 50 % of the crawfish in the 24-h pond water toxicity test was determined by simple linear regression of percent mortality against permethrin concentration (Rand and Petrochelli 1985).

RESULTS

Water Temperature, Dissolved Oxygen, and Forage Production

Water temperature ranged from a seasonal low of 2.3 C in January to a high of 28.0 C in June. Dissolved oxygen concentration ranged from a minimum of 0.6 mg/L in November to a high of 18.1 mg/L in January (Table 8). Total depletion of DO was not observed in any pond. Water temperature and DO concentration did not differ among the five treatments (P = 0.86 and 0.83, respectively).

Rice (complete plant) comprised 99 % of the total vegetative biomass. Control of weeds by water management was successful because production of volunteer vegetation was minor. Small stands (< 1 % of the total area coverage) of Cyperus spp., Sesbania spp., Scirpus spp., and Solidago sempervirens were present. Rice plants experienced no significant insect infestations, diseases, or nutritional problems, and panicle formation began in late November. Vegetative biomass ranged from 5,947 to 9,000 kg/ha (dry weight) and did not differ among the five treatments at the time ponds were flooded in fall (P = 0.33, Table 8).

Species Composition and Population Density

P. clarkii was the most abundant species of crawfish present in the ponds. P. clarkii comprised 72 to 100 % of the crawfish exceeding 40 mm TL in the five treatments. P. acutus acutus were present in all ponds except those assigned to the "low density control". P. acutus acutus comprised from 10 to 28 % of the crawfish exceeding 40 mm TL in treatments that had populations of white river crawfish. The abundance of P. acutus acutus in ponds remained consistent throughout the season in crawfish populations which were not treated with permethrin.

Crawfish population density (both species combined) for all treatments declined from January through June (Table 9). Crawfish density was highest with the initial estimate in early January (8 to 18 crawfish/m^2). Crawfish population density in

Table 8. Mean (± 1 SD) water temperature, dissolved oxygen, and forage biomass among treatments in crawfish density reduction and feeding study. Seasonal range of water temperature and dissolved oxygen are in parentheses.

Treatment	Water Temperature (C)	Dissolved Oxygen (mg / L)	Forage Biomass (kg / ha)
Low Density Control[1]	15.4 ± 6.2 (2.9 - 27.9)	5.8 ± 3.0 (0.6 - 15.8)	7,968 ± 2,582
High Density Control[2]	15.5 ± 6.1 (2.4 - 28.0)	5.5 ± 2.8 (0.8 - 13.2)	9,000 ± 554
Density Reduced	15.6 ± 6.1 (3.3 - 28.0)	5.8 ± 3.0 (0.9 - 14.4)	5,947 ± 532
Density Reduced and Fed	15.6 ± 6.2 (3.3 - 28.0)	5.5 ± 3.0 (0.6 - 14.5)	8,557 ± 2,104
Fed	15.6 ± 6.1 (2.3 - 26.5)	5.2 ± 3.0 (0.8 - 18.1)	7,538 ± 2,065

[1]Treatment with lowest crawfish population densities.

[2]Treatment with highest crawfish population densities.

Table 9. Mean (± 1 SD) crawfish density among treatments in the crawfish density reduction and feeding study as estimated from the combined results of the Schnabel mark-and-recapture and dip-net population estimates from January to June 1988.

Treatment	8 January 1988	7 February 1988	11 March 1988[1]	20 April 1988[2]	20 June 1988
	Density (N / m^2)				
Low Density Control[3]	8.4 ± 6.9	5.4 ± 8.7	4.0 ± 8.8	N. D.	5.8 ± 5.1
High Density Control[4]	18.0 ± 15.4	10.8 ± 5.9	9.2 ± 5.7	N. D.	8.7 ± 9.7
Density Reduced	16.4 ± 10.0	15.6 ± 9.5	14.2 ± 8.8	3.5 ± 0.5	4.1 ± 2.4
Density Reduced and Fed	17.0 ± 6.7	12.8 ± 6.6	11.5 ± 6.7	2.5 ± 1.2	4.7 ± 2.4
Fed	12.4 ± 3.8	8.5 ± 1.7	6.9 ± 2.9	N. D.	7.0 ± 4.7

[1]Population density prior to treatment application.

[2]Population density following treatment application not determined (N.D.).

[3]Treatment with lowest crawfish population densities.

[4]Treatment with highest crawfish population densities.

the "low density control", "high density control", and the "fed" treatments decreased about 50 % from January to March while population density in the "density reduced" and the "density reduced and fed" treatments decreased 12 and 41 %, respectively. Crawfish population density increased 25 to 100 % (1 - 2 crawfish/m^2) from March to June in the "low density control", "density reduced", and the "density reduced and fed" treatments. Population density in the "high density control" and the "fed" treatments remained relatively unchanged from March through June.

Abundance of Crawfish < 15 mm TL

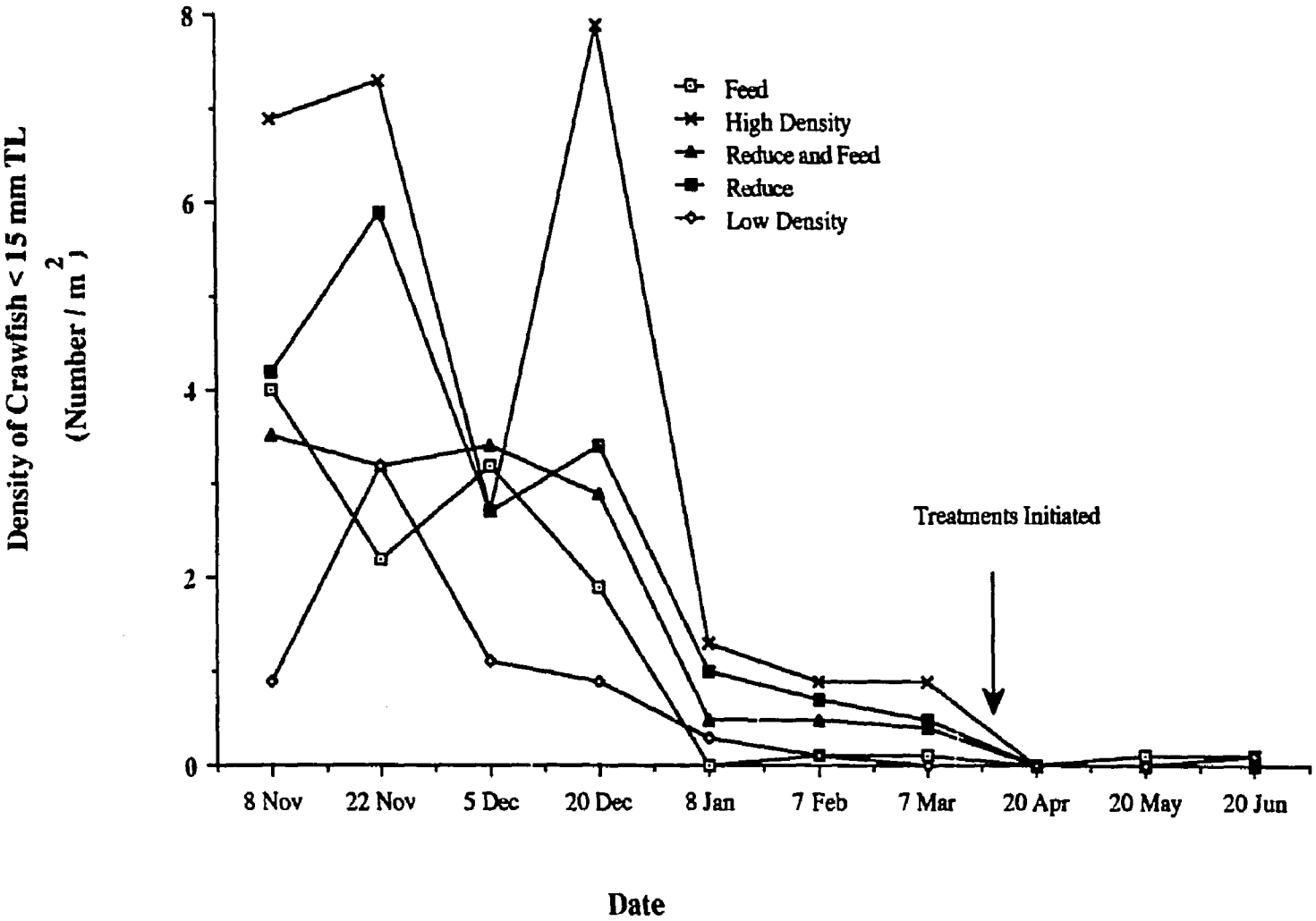
Crawfish less than 15 mm TL were captured in dip-net samples from all treatments from October to March but not in April through June. Peak density of crawfish less than 15 mm TL occurred in November and December. During these months, mean population density of crawfish less than 15 mm TL ranged from 8 crawfish/m^2 (4 crawfish/dip-net sweep) in ponds assigned to the "high density control" to less than 1 crawfish/m^2 in the "low density control". Mean population density of crawfish less than 15 mm TL decreased to 0 to 1 crawfish/m^2 in January and remained at this level through June (Figure 3).

Prior to initiation of treatments in April, ponds in the "low density control" treatment had fewer crawfish less than 15 mm TL (mean =1 crawfish/dip-net sweep) than all other treatments (mean = 3 crawfish/dip-net sweep, $P = 0.003$). Recruitment of crawfish less than 15 mm TL in "high density control" ponds exceeded the recruitment of crawfish in the other treatment ponds (mean = 4 crawfish/dip-net sweep, $P = 0.046$). The density of crawfish less than 15 mm TL post-treatment in April declined did not differ among treatments ($P = 0.68$).

Growth of Crawfish

The number of YOY recruitment classes (as determined by abundance of crawfish less than 15 mm TL) in the five treatments ranged from a low of one cohort per

Figure 3. Population density of crawfish less than 15 mm TL in the crawfish density reduction and feeding study from 8 November 1987 to 20 June 1988.


Density of Crawfish < 15 mm TL
(Number / m 2)
8
6
4
2
0
Feed
High Density
Reduce and Feed
Reduce
Low Density
Treatments Initiated
8 Nov
22 Nov
5 Dec
20 Dec
8 Jan
7 Feb
7 Mar
20 Apr
20 May
20 Jun
Date

pond in December to a high of five cohorts per pond in February. Mean growth of YOY recruitment cohorts was greatest in November and December, ranging from 3 to 7 mm per week (Table 10). Mean growth of crawfish declined to 1 to 2 mm per week from January through mid-June. The mean growth rate of YOY cohorts did not differ among the five treatments either prior to ($P = 0.45$) or post-treatment ($P = 0.30$).

Population Composition

Harvestable crawfish (those crawfish exceeding 75 mm TL) were present in the small-mesh traps samples from November through June. In pre-treatment samples, the percentage of crawfish exceeding 75 mm TL was greatest from 5 December through 7 February (Figure 4), and the composition of harvestable size crawfish in the small-mesh traps ranged from 44 to 86 %. The percentage of crawfish exceeding 75 mm TL from November to March did not differ among treatments ($P = 0.23$).

Following the initiation of harvest in March and application of treatments in April, the mean percentage of crawfish exceeding 75 mm TL in the "density reduced and fed" treatment exhibited the greatest change with an increase from 23 % in April to 62 % in June. The post-treatment percentage of crawfish exceeding 75 mm TL in the "low density control" averaged 40 % and it was significantly higher than the average of the other treatments (mean = 21 %, $P = 0.005$). In treatments where crawfish were fed a formulated ration, the percentage of crawfish exceeding 75 mm TL from April to June averaged 29 % and it was double that in the treatments in which crawfish were not fed (mean = 15 %, $P = 0.01$).

Mature male crawfish were present in small-mesh traps except from 20 December to 7 February (Figure 5). In November, the mean percentage of mature males captured in small-mesh trap samples ranged from 4 to 31 %. Mean percentage of mature males captured declined from 11 % in November to a low of 2 % in January. The percentage composition of mature males captured in small-mesh traps increased

Table 10. Mean (± 1 SD) growth rate of young-of-the-year red swamp crawfish (Procambarus clarkii) cohorts in the crawfish density and feeding study (mm TL/week). The range in the number of cohorts between treatment ponds is presented in parentheses.

	Treatment				
Time Period	Low Density Control	High Density Control	Density Reduced	Density Reduced and Fed	Fed
11/8 - 11/22/87	3 ± 1.2 (1 - 2)	7 ± 1.4 (1)	5 ± 2.3 (1 - 2)	6 ± 2.4 (1)	6 ± 2.2 (1)
11/22 - 12/5/87	5 ± 0.0 (1 - 2)	5 ± 0.8 (2)	3 ± 1.7 (1 - 3)	6 ± 0.5 (1 - 2)	4 ± 0.9 (1 - 2)
12/5 - 12/20/87	4 ± 0.5 (2 - 3)	3 ± 0.5 (2 - 3)	2 ± 0.8 (2 - 4)	3 ± 1.7 (1 - 3)	2 ± 1.2 (2 - 3)
12/20 - 1/8/88	3 ± 0.9 (2 - 4)	4 ± 0.8 (3 - 4)	3 ± 0.0 (3 - 4)	2 ± 0.9 (3 - 4)	2 ± 0.5 (2 - 4)
1/8 - 2/7/88	1 ± 0.5 (2 - 5)	1 ± 0.5 (2-5)	1± 0.0 (3 - 4)	1 ± 0.5 (1 - 4)	2 ± 0.0 (3)

Table 10. Mean (± 1 SD) growth rate of young-of-the-year red swamp crawfish (Procambarus clarkii) cohorts in the crawfish density and feeding study (mm TL/week). The range in the number of cohorts between treatment ponds is presented in parentheses (continued).

	Treatment				
Time Period	Low Density Control	High Density Control	Density Reduced	Density Reduced and Fed	Fed
2/7 - 3/11/88	2 ± 0.5 (2 - 4)	1 ± 0.5 (3 - 4)	2 ± 0.5 (3 - 5)	1 ± 0.5 (3 - 5)	2 ± 0.5 (4)
3/11 - 4/20/88	2 ± 0.5 (2 - 4)	1 ± 0.5 (3 - 4)	2 ± 0.5 (3 - 4)	2 ± 0.5 (3 - 5)	2 ± 0.0 (3 - 4)
4/20 - 5/20/88	1 ± 0.0 (1 - 3)	2 ± 0.8 (3 - 5)	2 ± 0.5 (1 - 4)	2 ± 0.0 (3 - 5)	2 ± 0.8 (3 - 4)
5/20 - 6/20/88	1 ± 0.0 (1 - 2)	1 ± 0.5 (2 - 5)	1 ± 0.0 (1 - 2)	1 ± 0.5 (1 - 4)	1 ± 0.5 (2 - 3)

Figure 4. Percentage of red swamp crawfish (Procambarus clarkii) exceeding 75 mm TL captured in small-mesh traps in the crawfish density reduction and feeding study from 8 November 1987 to 20 June 1988.

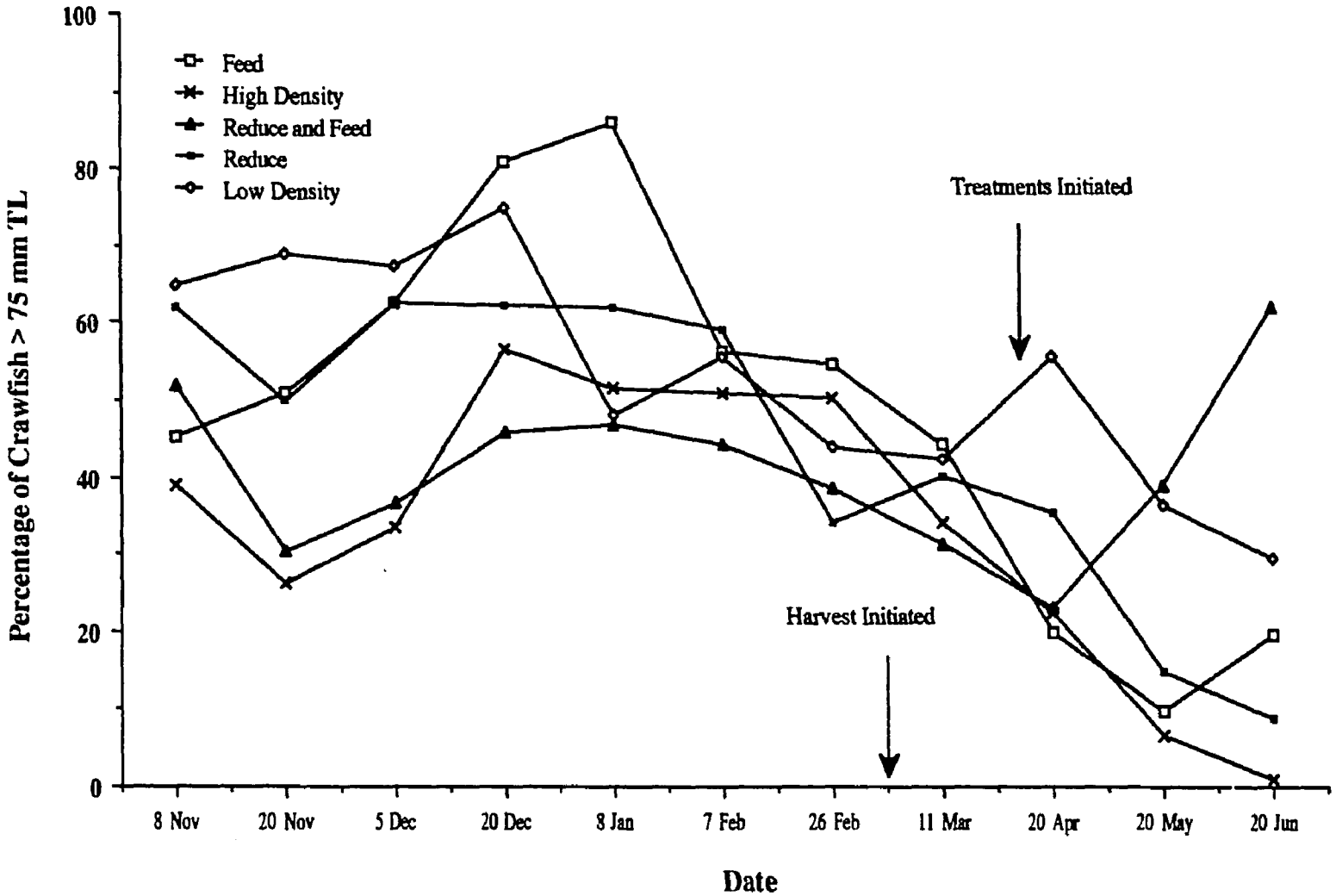
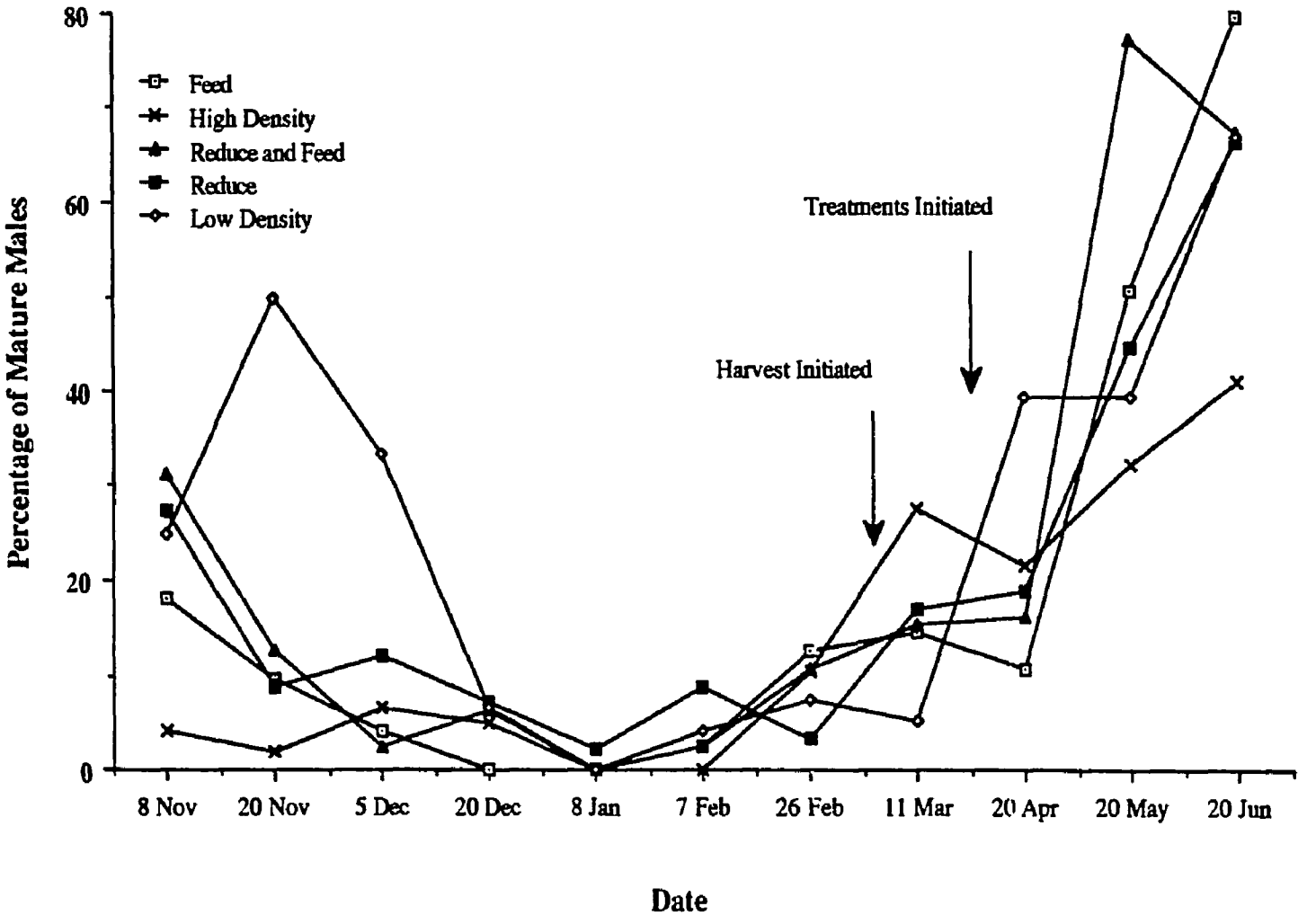

Feed
High Density
Reduce and Feed
Reduce
Low Density
Treatments Initiated
Harvest Initiated
Percentage of Crawfish > 75 mm TL
100
80
60
40
20
0
8 Nov
20 Nov
5 Dec
20 Dec
8 Jan
7 Feb
26 Feb
11 Mar
20 Apr
20 May
20 Jun
Date

Figure 5. Percentage of mature male (Form I) red swamp crawfish (Procambarus clarkii) captured in small-mesh traps in the crawfish density reduction and feeding study from 8 November 1987 to 20 June 1988.


Feed
High Density
Reduce and Feed
Reduce
Low Density
Treatments Initiated
Harvest Initiated
Percentage of Mature Males
80
60
40
20
0
8 Nov
20 Nov
5 Dec
20 Dec
8 Jan
7 Feb
26 Feb
11 Mar
20 Apr
20 May
20 Jun
Date

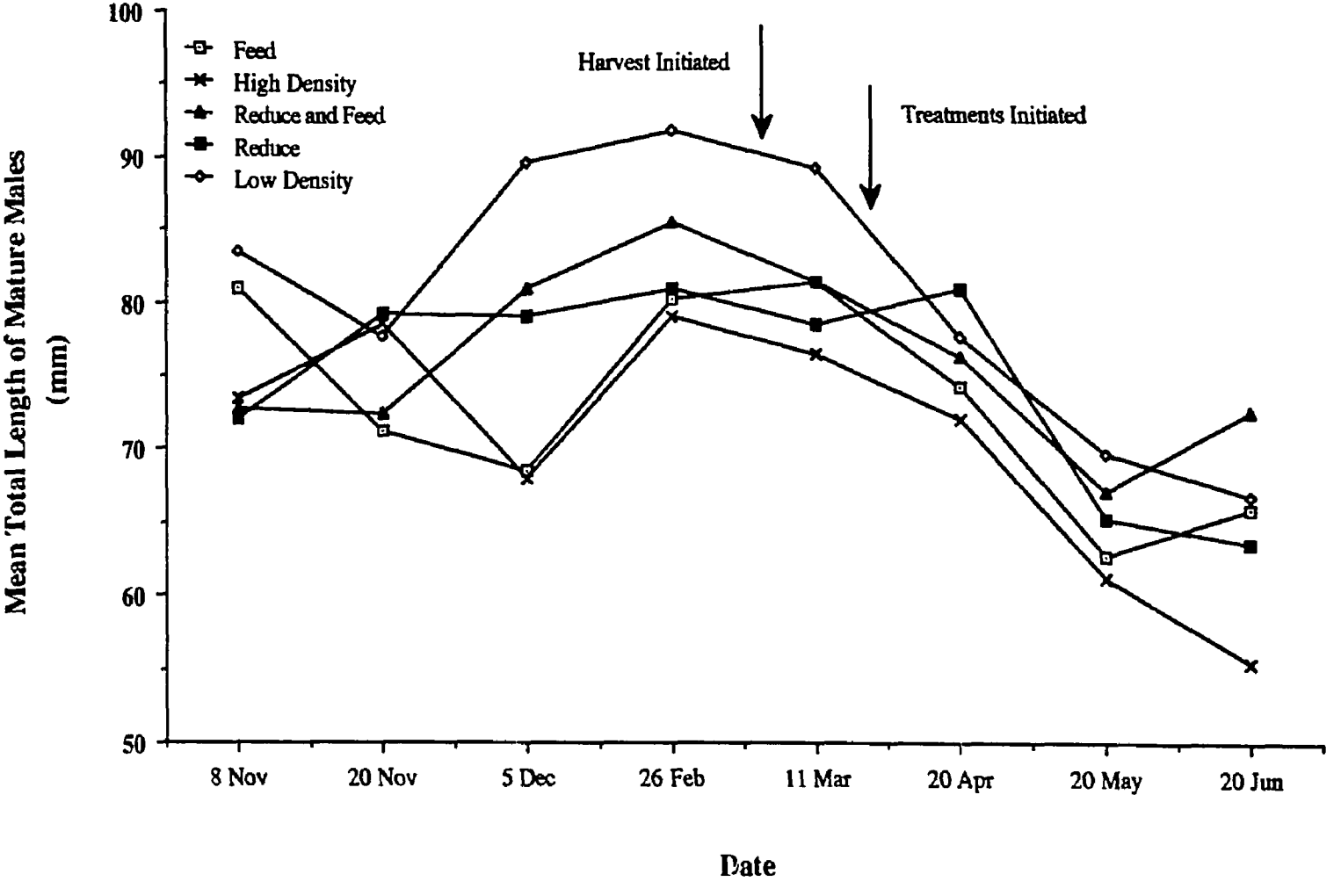
from January through June (mean = 80 %). The percentage of mature males in both pre-treatment and post-treatment samples did not differ significantly among treatments (P = 0.34).

Mean size of mature male red swamp crawfish was greatest in February and March, ranging from 77 mm TL in the "high density control" treatment to 92 mm TL in the "low density control" treatments (Figure 6). The mean size of mature males (average of all treatments) decreased from 76 mm in March to 65 mm TL in June. The mean size of mature males prior to permethrin application and/or feed application ranged from 77 to 87 mm TL, and the mean size did not differ among treatments (P = 0.31). The mean size of mature males fed formulated feed (mean = 70 mm TL) following treatment, was 8 % larger than crawfish in ponds that received no feed (mean = 65 mm TL, P = 0.01).

The abundance of immature male P. clarkii captured in small-mesh traps was greatest in January (49 % of all crawfish sampled) and declined to 11 % in June. Prior to treatment, the percentage of immature males in crawfish populations that were to receive feed averaged 40 %, 4 % more immature males than populations which were not to be fed (mean = 36 %, P = 0.26). The average percentage of immature male crawfish in treatments that were to undergo population density reduction was 39 %, only 2 % more immature males than ponds to be assigned to the "nonreduced" treatments (mean = 37 %, P = 0.43).

After permethrin application, the mean percentage of immature male crawfish in treatments ranged from 15 to 25 %. The mean percentage of immature male crawfish in the "non-fed" treatments was 23 %, and these treatments had 7 % more immature males than treatments where crawfish were fed the formulated ration (mean = 16 %, P = 0.03). The percentage of immature male crawfish in "reduced" and "nonreduced" treatments, after treatment application averaged 20 % and were not different from each

Figure 6. Mean total length of mature male (Form I) red swamp crawfish (Procambarus clarkii) in crawfish density reduction and feeding study from 8 November 1987 to 20 June 1988.


Mean Total Length of Mature Males (mm)
100
90
80
70
60
50
Feed
High Density
Reduce and Feed
Reduce
Low Density
Harvest Initiated
Treatments Initiated
8 Nov
20 Nov
5 Dec
26 Feb
11 Mar
20 Apr
20 May
20 Jun
Date

other (P = 0.74).

The percentage of female P. clarkii captured in small-mesh traps ranged from 49 to 67 % in the November through June production period. Prior to permethrin application, the percentage of females in the "fed" and "nonfed" treatments averaged 52 and 57 %, respectively (P = 0.62). The percentage of female crawfish in populations that were to have densities reduced averaged 53 %, and had 3 % fewer females than populations that were to remain untreated (mean = 56 %, P = 0.47). The percentage of females captured in small-mesh traps among the five treatments was not significantly different from each other (P = 0.28).

Following treatment application in April the percentage of female crawfish in treatments in which crawfish were fed a formulated ration averaged 67 %, and this was 5 % higher than treatments in which crawfish were not fed (mean = 62 %, P = 0.04). In treatments in which crawfish density was reduced the percentage of females captured in the small-mesh traps averaged 61 % and this was 5 % less than in treatments where crawfish populations were not reduced (mean = 66 %, P = 0.08). Ponds assigned to the "low density control" averaged 58 % female crawfish and there were fewer female crawfish in this treatment than all other treatments combined (P = 0.04).

Crawfish Harvest

The mean total weight of crawfish harvested from the five treatment ponds from 15 March to 26 March (pre-treatment application) ranged from 228 to 311 kg/ha, the mean total number of crawfish ranged from 13,908 to 18,767 crawfish/ha, and individual size ranged from 16.2 to 19.8 g/crawfish (Table 11). Crawfish harvest from "fed" and "nonfed" treatments averaged 280 kg/ha (P = 0.83) and the mean individual weight of crawfish from both treatment groups was 16.6 g (P = 0.80). Ponds in the "density reduced" treatments (mean = 252 kg/ha) produced 15 % less harvest than ponds assigned to "nonreduced" treatments (mean = 297 kg/ha, P = 0.36), and mean

Table 11. Mean (± 1SD) total weight, total number, individual size, and catch per unit effort (CPUE) of crawfish harvested from five treatments prior to permethrin exposure and/or feeding, 15 March to 26 March 1988.

Treatment	Total Weight (kg/ha)[1]	Total Number (N/ha)	Individual Size (g)	CPUE (kg / trap-set)
Low Density Control[2]	304 ± 83	15,317 ± 889	19.8 ± 5.4	0.30 ± 0.12
High Density Control[3]	285 ± 28	17,592 ± 2,177	16.2 ± 0.7	0.28 ± 0.20
Density Reduced	275 ± 91	15,775 ± 4,965	17.4 ± 0.3	0.27 ± 0.19
Density Reduced and Fed	228 ± 48	13,908 ± 1,658	16.4 ± 2.7	0.23 ± 0.12
Fed	311 ± 126	18,767 ± 7,181	16.4 ± 0.4	0.31 ± 0.31

[1]Crawfish harvested for 10 days (900 trap-sets/ha).

[2]Treatment with lowest crawfish population density.

[3]Treatment with highest crawfish population density.

individual size of crawfish from the two treatments was 16.5 g/crawfish (P = 0.44). The mean size of the crawfish captured from the "low density control" treatment was 19.8 g which was larger than crawfish harvested from the other four treatments (P = 0.1). The number of crawfish harvested from ponds that were to receive formulated feed and those treatments in which crawfish were not fed a formulated ration was similar averaging 16,337 and 16,683 crawfish/ha, respectively (P = 0.89). Crawfish populations assigned to "high density control" and "fed" treatments averaged 18,179 crawfish/ha and was 22 % higher than ponds where crawfish populations were to be reduced (mean = 14,180 crawfish/ha, P = 0.19).

Pre-treatment catch per trap-set (CPUE) ranged from 0.23 to 0.31 kg/trap-set (Table 11). CPUE of crawfish in ponds assigned to "fed" and "non fed" treatments averaged 0.28 kg/trap-set (P = 0.80). Ponds assigned to treatments that were to have crawfish populations reduced by permethrin had 17 % less CPUE (mean = 0.25 kg/trap-set) than treatments that were not to be reduced by permethrin application (mean = 0.30 kg/trap-set, P = 0.07).

The harvest of crawfish after treatments were initiated (April through June) ranged from 281 to 914 kg/ha and 19,817 to 64,542 crawfish/ha (Table 12). The yield of crawfish from ponds receiving formulated feed averaged 494 kg/ha, and was 64 % higher than the total weight of crawfish harvested from "nonfed" treatments (mean = 300 kg/ha, P = 0.18). Crawfish fed the formulated ration after permethrin exposure, had a 68 % greater harvest (mean = 35,470 crawfish/ha) than treatments where crawfish populations were not fed (mean = 21,171 crawfish/ha, P = 0.16). The average size of crawfish did not differ between "fed" and "nonfed" treatments (mean = 14.2 g/crawfish, P = 0.53). Treatments in which crawfish populations were reduced produced 13 % greater harvest (mean = 422 kg/ha) than treatments in which density was not reduced (mean = 372 kg/ha, P = 0.48). The number of crawfish harvested from populations

Table 12. Mean (± 1SD) total weight, total number, individual size, and catch per unit effort (CPUE) of crawfish harvested from five treatments after permethrin exposure and feeding, 21 April to 15 June 1988.

Treatment	Total Weight (kg/ha)[1]	Total Number (N/ha)	Individual Size (g)	CPUE (kg / trap-set)
Low Density Control[2]	914 ± 372	64,541 ± 22,351	13.9 ± 1.2	0.29 ± 0.23
High Density Control[3]	319 ± 44	22,525 ± 4,476	14.3 ± 1.3	0.10 ± 0.14
Density Reduced	281 ± 72	19,817 ± 5,994	14.3 ± 0.9	0.09 ± 0.06
Density Reduced and Fed	562 ± 254	38,483 ± 16,780	14.5 ± 0.3	0.18 ± 0.11
Fed	425 ± 258	32,458 ± 22,683	13.6 ± 1.3	0.14 ± 0.16

[1]Crawfish were harvested for 31 days (2790 trap-sets/ha).

[2]Treatment with lowest crawfish population densities.

[3]Treatment with highest population densities.

that were reduced averaged 29,150 crawfish/ha and was not different from "nonreduced" treatments (mean = 27,491 crawfish/ha, P = 0.49). The individual size of crawfish harvested from "population reduced" treatments was 14.4 g and crawfish harvested from the "nonreduced" treatments had a mean size of 13.9 g (P = 0.59, Table 12). The harvest of crawfish post-treatment from the "low density control" treatments averaged 914 kg/ha (64,542 crawfish/ha) and was significantly higher than all other treatments (P = 0.007, Table 12). The mean size of crawfish harvested from the "low density control" treatment was 13.9 g and was not different from the other four treatments (P = 0.82).

The average CPUE of crawfish captured from all ponds post-treatment ranged from 0.09 to 0.30 kg/trap-set (Table 12). Treatments in which crawfish were fed formulated feed had a CPUE of 0.16 kg/trap-set and was 78 % higher than treatments not receiving feed (mean = 0.09 kg/trap-set, P = 0.001). The CPUE of crawfish populations that were reduced by permethrin exposure averaged 0.14 kg/trap-set, 17 % higher than the CPUE of "nonreduced" treatments (mean = 0.12 kg/trap-set, P = 0.15). The average CPUE of crawfish from the "low density control" was 0.29 kg/trap-set and was greater than the mean CPUE of the other four treatments (P = 0.001).

Total harvest of crawfish from March through June ranged from 557 to 1,217 kg/ha (35,592 to 79,858 crawfish/ha, Table 13). Treatments in which crawfish received a formulated feed produced 32 % more weight (mean = 763 kg/ha) than treatments in which the crawfish populations were not fed (mean = 580 kg/ha, P = 0.32). "Fed" treatments (mean = 51,808 crawfish/ha) had a 37 % higher crawfish harvest than treatments receiving no formulated feed (mean = 37,854 crawfish/ha, P = 0.23). The average size of crawfish harvested from "fed" treatments was 4 % less (mean = 14.8 g) than crawfish captured from "nonfed" treatments (mean = 15.4 g, P = 0.32). The total weight of crawfish harvested from "population reduced" (mean = 673

Table 13. Mean (± 1SD) total weight, total number, individual size, and catch per unit effort (CPUE) of crawfish harvested from the crawfish density reduction and feeding study, 15 March to 15 June 1988.

Treatment	Total Weight (kg/ha)[1]	Total Number (N/ha)	Individual Size (g)	CPUE (kg / trap-set)
Low Density Control[2]	1,218 ± 449	79,858 ± 22,957	15.0 ± 1.7	0.30 ± 0.20
High Density Control[3]	604 ± 35	40,117 ± 5,050	15.1 ± 1.1	0.15 ± 0.17
Density Reduced	557 ± 157	35, 591± 10,652	15.7 ± 0.3	0.14 ± 0.13
Density Reduced and Fed	790 ± 301	52,392 ± 17,647	14.9 ± 0.7	0.19 ± 0.11
Fed	736 ± 372	51,225 ± 29,207	14.7 ± 0.9	0.18 ± 0.22

[1]Crawfish harvested for 41 days (3690 trap-sets/ha).

[2]Treatment with lowest crawfish population densities.

[3]Treatment with highest crawfish population densities.

kg/ha) and "nonreduced" (mean = 670 kg/ha) treatments were similar (P = 0.60). Treatments in which crawfish populations were "reduced" had an average harvest of 43,992 crawfish/ha (mean size = 15.2 g) and "nonreduced" treatments produced 45,671 crawfish/ha (mean size = 15.2 g, P = 0.49). The mean total yield of crawfish from the "low density control" treatment was 1,217 kg/ha and was higher than the total weight of crawfish harvested from the other treatments (P = 0.02). Overall, the number of crawfish harvested from the "low density control" treatment was 79,858 crawfish/ha, significantly higher than the average of the remaining treatments (mean = 44,831 crawfish/ha, P = 0.017).

The highest mean CPUE (0.69 kg/trap-set) among all treatments occurred in mid-March and a second peak (0.53 kg/trap-set) was observed in April, the week following application of permethrin and formulated feeds. Treatments in which crawfish received formulated feed had 19 % higher CPUE (mean = 0.19 kg/trap-set) than treatments where crawfish were not fed (mean = 0.16 kg/trap-set, P = 0.32). "Density reduced" and "nonreduced" treatments both had mean CPUE of 0.16 kg/trap-set from March through June (P = 0.60). Ponds assigned to the "low density control" treatment averaged 0.30 kg/trap-set which was higher than the average of the other four treatments (mean = 0.16 kg/trap-set, P = 0.02).

The Effects of Permethrin on Crawfish Populations

Mean water temperature ranged from 19.8 to 23.6 C and DO ranged from 5.1 to 7.4 mg/L in the 24 h pond water, permethrin acute toxicity tests. The 24-h LC50 of permethrin to crawfish in aquaria containing pond water ranged from 1.0 to 3.1 µg/L (Table 14).

The TS of pond water varied as much as 100 %, ranging from 103 mg/L to 207 mg/L. Total alkalinity ranged from 160 to 332 mg/L as $CaCO_3$, and total hardness of pond water ranged from 146 to 306 mg/L as $CaCO_3$. At the time of pesticide

Table 14. Percent mortality and estimated 24-h LC50 of crawfish exposed to permethrin in 24-h pond-side static acute toxicity tests.

Replicate	Permethrin Concentration (µg / L) 0	2	4	6	Estimated LC50 (µg / L)
		Percent Mortality of Crawfish in Aquaria			
		Density Reduced and Fed Treatment			
1	0	50	100	100	2.0
2	0	0	90	100	3.1
3	0	30	100	100	2.3
		Density Reduced			
1	0	100	100	100	1.0
2	0	40	100	100	2.1
3	0	30	100	100	2.3

application (0800 to 1200 h), pond water temperature ranged from 18.8 to 19.6 C, DO ranged from 2.0 to 5.7 mg/L, and the pH ranged from 7.4 to 7.7 (Table 15). The BOD ranged from 1.3 mg/L to 2.6 mg/L and TOC ranged from 2.0 to 7.0 mg C/L.

Based on the results of the field acute toxicity tests, permethrin concentrations of 1.0 to 3.0 µg permethrin/L of pond water were required to effect crawfish population density reductions.

Permethrin was applied to the "density reduced" and "density reduced and fed" treatment ponds between 0800 and 1200 h on 7 - 8 April. Crawfish exoskeletons were observed floating in the water column, and clinical signs indicative of a synthetic pyrethroid poisoning in crawfish were witnessed within 6 h of permethrin administration. Crawfish were observed initially wandering hyperactively along pond margins. Hyperactivity was rapidly followed by ataxia in most, but not all observed crawfish. Many crawfish left the water briefly, but returned and none were observed crossing embankments to other ponds. Crawfish mortality in ponds was documented 7 h after permethrin application. Nontarget fish (bullheads, sunfish, and mosquitofish), reptiles (snakes, turtles, and frogs), and mammals (nutria and muskrat) were present in the ponds at the time of treatment, but no mortality was observed among these organisms on either the day of permethrin application or later.

Mortality of crawfish maintained in wire cages ranged from 0 to 44 % (Table 16). Mortality among crawfish in cages occurred in the first 24 h following permethrin application and, no mortality occurred thereafter through 96 h. Some cannibalism of crawfish in cages occurred so mortality resulting from permethrin exposure may be lower than what was observed.

The number of crawfish captured in small-mesh traps and dip-net samples conducted 12 - 13 days post-application indicated that crawfish population size was reduced by 54 to 83 % in permethrin-treated ponds (Table 16). Mortality among

Table 15. Water quality characteristics in experimental crawfish ponds in the crawfish density reduction and feeding study prior to permethrin application on 7 - 8 April 1988.

	Total Solids (mg/L)	Total Alkalinity (mg/L)	Total Hardness (mg/L)	Water Temperature (C)	Dissolved Oxygen (mg/L)	pH	BOD (mg/L)	TOC (mg C/L)
				Density Reduced and Fed				
Replicate								
1	207	332	306	19.5	5.7	7.7	1.4	6.7
2	108	270	249	19.1	2.6	7.4	1.7	2.0
3	110	192	173	18.8	2.9	7.7	2.6	5.8
				Density Reduced				
Replicate								
1	103	185	170	19.6	2.5	7.7	1.4	2.6
2	111	160	146	18.6	2.0	7.7	2.0	6.4
3	110	313	280	18.8	3.8	7.6	1.3	7.0

Table 16. Mortality of Procambarus clarkii in cages and ponds in the crawfish density reduction and feeding study following permethrin application.

	Permethrin Concentration (µg/L)	Mortality of Caged Crawfish[1] (%)	Mortality of Pond Crawfish[2] (%)
		Density Reduced and Fed	
Replicate			
1	2.0	0.0	83.1[3]
2	2.5	44.1	78.6
3	2.0	16.6	70.0[3]
		Density Reduced	
Replicate			
1	1.0	0.0	54.4
2	2.0	10.8	80.4
3	3.0	39.5	79.8

[1]Total percent mortality in 96 h.
[2]Total percent mortality in 7 d.
[3]Value includes mortality of Procambarus acutus acutus.

crawfish less than 40 mm TL in permethrin-treated ponds averaged 75 % (range: 55 to 84 %). Mortality of crawfish less than 40 mm TL was greater than the mortality among crawfish exceeding 40 mm TL in five of the six treated ponds (Table 17). The average mortality among crawfish exceeding 40 mm TL was 62 % (range: 42 to 79 %).

Mature male red swamp crawfish experienced the highest mortality of the crawfish exceeding 40 mm TL. Mature males had a mean mortality of 67 % (range: 33 to 100 %), females had a mean mortality of 65 % (range: 43 to 86 %), and immature males had a mean mortality of 51 % (range: 30 to 72 %). Form I male P. clarkii had higher mortality than either female or immature male P. clarkii in three of the six ponds that were treated with permethrin (Table 18).

White river crawfish (P. acutus acutus) comprised 25 and 22 % of the crawfish populations exceeding 40 mm TL in replicates 1 and 3 of the "density reduced and fed" treatment, respectively, prior to permethrin application. Immediately following permethrin application (and throughout the remainder of the study), no white river crawfish were captured in either pond, thus permethrin administered to these ponds was apparently toxic to 100 % of P. acutus acutus.

Permethrin Residue Analysis

Results of gas chromatographic analysis of crawfish abdominal muscle and hepatopancreas are presented in Tables 19 and 20, respectively. No permethrin residues were detected above or below the detection limits established for this study in either the crawfish abdominal muscle (6.7 ng/g detection limit) or the hepatopancreas (400 ng/g detection limit) of the treatment or control ponds at 7, 14, or 21 days following permethrin administration.

Table 17. Estimated percent decrease of Procambarus clarkii and P. acutus acutus populations in ponds treated with permethrin in crawfish density reduction and feeding study on 7 - 8 April 1988 .

	Prior to Permethrin Application[1]		After Permethrin Application[2]			
	Population Size of Crawfish/Pond		Population Size of Crawfish/Pond			
Replicate	< 40 mm TL	≥ 40 mm TL	< 40 mm TL	% Decrease	≥ 40 mm TL	% Decrease
			Density Reduced and Fed			
1	5,427	422	880	83.8	146	65.5
2	3,666	802	733	80.0	329	59.1
3	3,373	842	1,026	69.9	188	78.0
			Density Reduced			
1	3,327	555	1,466	54.6	275	50.6
2	9,239	1,110	1,760	80.9	642	42.2
3	8,653	865	1,759	79.7	177	79.5

[1] The date of the pre-application population estimate was 11 March 1988.

[2] The date of the post-application population estimate was 20 April 1988.

Table 18. Estimated percent decrease in Form I male, Form II male, and female Procambarus clarkii in ponds treated with permethrin in crawfish density reduction and feeding study on 7 - 8 April 1988.

	The Number of Crawfish per Pond Prior to Permethrin Application[1]			The Number of Crawfish per Pond After Permethrin Application[2]					
Replicate	Form I Male	Form II Male	Female	Form I Male	Decrease (%)	Form II Male	Decrease (%)	Female	Decrease (%)
				Density Reduced and Fed					
1	52	104	266	19	64.0	78	66.0	91	65.6
2	28	341	433	19	33.3	139	59.1	170	60.7
3	79	300	463	0	100.0	84	78.0	100	78.2
				Density Reduced					
1	42	183	330	23	46.1	78	42.9	147	55.4
2	125	289	696	39	68.7	203	29.8	399	42.7
3	192	133	540	22	88.5	82	38.9	74	86.3

[1] The date of the pre-application population estimate was 11 March 1988.

[2] The date of the post-application population estimate was 20 April 1988.

Table 19. Gas chromatographic analysis for permethrin residue in composite Procambarus clarkii hepatopancreas samples (1 injection/sample) 7, 14, and 21 days following permethrin applications to treatment ponds in crawfish density reduction and feeding study.

	Application Rate (µg / L)	Days Post-Application 7	14	21
		Residue Level (µg / g)		
		Density Reduced and Fed		
Replicate				
1	2.0	ND[2]	ND	ND
2	2.5	ND	ND	ND
3	2.0	ND	ND	ND
		Density Reduced		
Replicate				
1	1.0	ND	ND	ND
2	2.0	ND	ND	ND
3	3.0	ND	ND	ND
Control[1]	0.0	ND	ND	ND

[1] Control crawfish hepatopancreas obtained from pond 14, an untreated pond.

[2] Permethrin was not detected (ND) at a detection level of 400.0 ng/g.

Table 20. Gas chromatographic analysis for permethrin residue in composite Procambarus clarkii abdominal muscle samples (1 injection/sample) 7, 14, and 21 days following permethrin applications to treatment ponds in crawfish density reduction and feeding study.

	Application Rate (µg / L)	Days Post-Application 7	14	21
		Residue Level (µg / g)		
		Density Reduced and Fed		
Replicate				
1	2.0	ND[2]	ND	ND
2	2.5	ND	ND	ND
3	2.0	ND	ND	ND
		Density Reduced		
Replicate				
1	1.0	ND	ND	ND
2	2.0	ND	ND	ND
3	3.0	ND	ND	ND
Control[1]	0.0	ND	ND	ND

[1] Control crawfish abdominal muscle obtained from pond 14, an untreated pond.

[2] Permethrin was not detected (ND) at a detection level of 6.7 ng/g.

DISCUSSION

The Effect of Feeding and Density Reduction on Production of P. clarkii.

Several factors can result in crawfish "stunting" at sizes smaller than that acceptable by markets. Avault et al. (1974) attributed stunting to overcrowding of crawfish populations. Large populations of crawfish rapidly deplete the food supply, and the lack of forage retards growth and stimulates individuals in the population to mature at small sizes (Avault et al. 1974, de la Bretonne and Fowler 1976, Huner and Romaire 1978, Huner and Barr 1984).

Research on crawfish populations have documented densities at which crawfish populations can stunt. Forester (1976) and Clark et al. (1975) reported P. clarkii stunted at 65 - 75 mm TL at densities of 4 - 6 crawfish/m^2 in ponds where alligatorweed (Alternanthera philoxeroides) and/or smartweed (Polygonum spp.) were planted as food for crawfish. Goyert and Avault (1978) reported maturation of P. clarkii at sizes near 75 mm TL when reared in tanks at densities of 20 crawfish/m^2 and fed a crustacean ration. Romaire et al. (1978) reported severe stunting of P. clarkii at densities of 12 crawfish/m^2 in ponds in which inorganic fertilizers or agricultural vegetative products such as rice and bahiagrass hay were applied at rates as high as 4,000 kg of hay/ha.

Crawfish populations are dynamic and population density at the time that stunting is manifested is not an accurate estimate of the density at which stunting occurs (Romaire and Lutz 1989). Momot and Romaire (1983) found that P. clarkii populations in ponds in which greater than 45 % of the young-of-the-year (YOY) population exceeded 75 mm TL 12 weeks post-flooding, produced 25 to 30 % higher yield than populations in which less than 10 % of the crawfish exceeded 75 mm TL; however, the average size of crawfish produced from the higher yielding ponds was only 1 g larger (mean = 22 g) than those harvested from the lower yielding ponds (mean

= 21 g). In the current study some of the ponds which had the highest percentage of crawfish exceeding 75 mm TL 12 weeks post-flooding (75 - 80 % of the crawfish captured in the small mesh trap samples greater than 75 mm TL) had relatively low yield (604 kg/ha) and small crawfish (mean size = 15.1 g). The discrepancy between the results of these studies may be caused by the presence of a relatively high standing crop of holdhover crawfish in the ponds from the previous production season. Forage in ponds with high densities of holdover crawfish is depleted ealier than ponds having few or no holdover crawfish (Jarboe, personal observation; personal communication from L. de la Bretonne, Aquaculture Specialist, Louisiana Cooperative Extension Service, Baton Rouge, Louisiana). In ponds that have holdover populations of crawfish (such as the current study), crawfish population density (number and weight/m^2) 4 to 6 weeks after permanent flooding may be indicative of crawfish populations that are overcrowded relative to food resources and will likely stunt. The "high density" ponds displayed the mid- to late-season characteristics of stunted crawfish populations (such as large numbers of small crawfish) that were not evident in the "low density" ponds of this study. This study indicates that ponds with a population density of 16 - 18 crawfish/m^2 (52.5 g/m^2) inclusive of high recruitment (7 - 8 YOY/m^2) from November to January and a rice vegetative biomass of 9,000 kg/ha at the time of fall flooding may have crawfish populations that stunt at an unacceptable market size unless some external input such as supplemental feeding and/or density reduction is implemented as a management strategy.

Growth of YOY P. clarkii in production ponds ranges from 1 to 7 mm per week and the rate is largely dependent on crawfish density, type and quality of forage, onset of maturity, and environmental conditions, particularly temperature and DO (Romaire 1976, Goyert and Avault 1978, Huner and Barr 1984, Avault and Huner 1985, Lutz and Wolters 1986, Romaire and Lutz 1989, and de la Bretonne and Romaire 1989).

An identifiable increase in YOY growth was not documented in response to density reduction or supplemental feeding. In addition to the reasons previously mentioned for the lack of a measurable increase in crawfish growth to the external stimuli (density reduction and/or supplemental feeding), it is possible that by grouping YOY crawfish into 5-mm size classes, small variations in the response of YOY growth rate to treatment were masked. In future studies, crawfish length to the nearest mm may help to detect differences of YOY growth in response to the management practices of density reduction and supplemental feeding.

The size at which procambarid crawfish mature is an indicator of the severity of stunting in crawfish populations (Huner and Romaire 1978, Huner and Barr 1984, Romaire and Lutz 1989). In this study, size of mature male P. clarkii and their percent composition of the pond populations ranged from 70 - 110 mm TL and 4 - 50 %, respectively in November 1987. Mature males present in the ponds in the first 2 - 3 months post-flooding were holdovers from the previous season. The decline in abundance of mature male P. clarkii from December 1987 through February 1988 was attributed to death of older mature crawfish, Form I males that molted to Form II stage, and burrowing by males for reproduction (Huner and Barr 1984, Romaire and Lutz 1989, and de la Bretonne and Romaire 1989). The "high density control" treatment produced significantly smaller mature male P. clarkii (mean size of 71 mm TL) than the other four treatments, and the "low density control" treatment produced the largest mature males (mean size of 81 mm TL), indicating a size-at-maturity, population density-dependent inverse relationship. Most of the crawfish in this study that were in the range of harvestable size were 68 mm to 78 mm TL. The large-mesh traps (1.9-cm dia hexagonal mesh) are selective for crawfish greater than 70 mm to 75 mm TL (Romaire 1989). Slightly higher growth rates of crawfish in the "low density control" ponds that could not be detected statistically likely resulted in a higher standing crop of

crawfish exceeding 75 mm TL. As a result, catch was greater in the "low density control" ponds.

Rice is the most common forage planted for crawfish in Louisiana (Brunson et al. 1988). Consumption of rice by large numbers of crawfish can rapidly deplete the supply of forage. In this study, forage was depleted in all ponds by February, but because vegetation was not sampled throughout the study it was not possible to quantitatively correlate forage depletion and crawfish population density to the onset of stunting. The results of this study do indicate that rate of growth of YOY crawfish decreases in relation to the abundance of available vegetative forage. Research which correlates forage depletion to the onset of stunting should be conducted. The ability to predict rates of forage depletion based on density estimates of crawfish would assist the culturist in determining when management techniques (such as the addition of formulated feeds or population density reduction) would be beneficial in maintaining acceptable growth of crawfish in ponds.

Investigations on providing P. clarkii formulated rations to maintain growth are limited (Smitherman et al. 1967; Clark et al. 1975). Crawfish have been stocked into ponds or pools and fed pelleted rations formulated for either finfish (Smitherman et al. 1967) or for crawfish (Clark et al. 1975). Research on supplemental feeding of pelleted rations to P. clarkii have produced inconsistent results, but previous investigators tended to view their findings as promising for the production of more and larger crawfish in ponds depleted of vegetative forage.

Smitherman et al. (1967) evaluated the supplemental feeding of a formulated ration (crude protein level unspecified) to P. clarkii stocked at a size of 7 - 11 mm TL and densities of 2.5 to 5.0 crawfish/m^2 in earthen ponds. Smitherman et al. (1967) concluded there was no increase in crawfish production compared to crawfish populations in ponds in which inorganic fertilizer was added to stimulate food

production. Smitherman et al. (1967) reported that many of the crawfish matured, burrowed and were not available for harvest, but the average weight of the harvested crawfish (12.9 g) indicated the potential value of supplemental feeding for the production of crawfish. Clark et al. (1975) fed crawfish pelleted fish rations (35 % crude protein) or a combination of pelleted fish rations and an extruded ration (32 % crude protein) formulated for crawfish. Pools were stocked with YOY P. clarkii at densities ranging from 3.1 to 6.2 crawfish/m^2 (less than 10 mm TL, mean weight = 0.1 g, and 25 - 35 mm TL, mean weight = 2.0 g) and crawfish were fed 3 % body weight/day with the amount fed adjusted weekly to compensate for growth. The growth and production was 40 to 41 % greater for crawfish fed the pelleted and extruded feeds (Clark et al. 1975) compared to populations in fertilized pools or crawfish receiving either alligatorweed or smartweed.

In the present study, P. clarkii were fed a pelleted ration formulated for crawfish (Appendix Table 13). An increase in the percentage of harvestable crawfish, and an increase in size at maturity of male P. clarkii was observed in crawfish populations which either had densities reduced by permethrin in April or populations that received formulated feed, but percentage and harvestable crawfish and size of mature males were highest in crawfish populations which were both density reduced and supplementally fed.

Initially, crawfish in the treatments receiving formulated feed were fed at a rate of 3.0 % of the estimated crawfish biomass; however, this amount was not adequate to distribute feed evenly, so the amount of feed was increased to 28 kg/ha/day (2 to 11 times the original levels) to reduce the competition among crawfish for feed pellets. Following the increase in feeding rate, crawfish exoskeletons (evidence of recent molting) were observed in ponds after the first day of each weekly feeding. This study indicates that feeding P. clarkii a 27 % crude protein feed (at a density of 5 - 8

crawfish/m^2 and 24.6 to 39.6 g/m^2) can increase the number of harvestable size crawfish. If feeding had been initiated prior to April and at feeding levels greater than 28 kg/ha/day three days weekly, the response of crawfish to feed may have been greater. Research needs to be conducted which establishes proper feeding rates taking into consideration a crawfish population composed of different size classes (nutritional requirements of crawfish at different sizes), forage and formulated feed interactions, and the best time of the season to begin feeding to maximize crawfish growth.

In the present study and past research associated with the use of formulated rations as supplemental feed for crawfish, feeding rates were based upon estimated crawfish standing crops. The studies were conducted in small ponds and crawfish standing crops were determined either by time-consuming population estimates, or from an extrapolation of population size based upon initial stocking density. A crawfish culturist with a 20 ha crawfish pond may not have the manpower nor the training to conduct extensive mark-and-recapture population estimates. Thus, the approach to establishing feeding rates for crawfish in a commercial facility must be simple. A method could involve an integrated regime of supplemental feeding followed by periodic population sampling using small-mesh traps. The culturist could measure the growth response of the crawfish population to feeding by constructing length-frequency histograms and documenting the change in size classes of crawfish according to feeding rate. If no positive shift in the mean size of crawfish is noted in the histogram then the culturist could increase feeding rates until responses in growth were observed. Management of crawfish populations in this manner would allow for continued growth of crawfish throughout the segment of the production season when the ponds are deficient of forage. This form of population management would be amenable to application with micro-computers so that once a program was developed, the culturist could determine feeding rate from data collected from small-mesh traps, enter the

crawfish lengths into a data base, and use the computer program to determine feeding rates.

Based on data from my study, the best conditions for sustained crawfish harvest in ponds which forage is depleted or is projected to be depleted is to reduce population density to 3 to 5 crawfish/m^2 by March if necessary and possibly feed crawfish a formulated ration. However, at this time it is not economically feasible to feed pond crawfish a formulated ration at a level of 28 kg/ha on three days per week with current culture techniques without a concomitant decrease in other production costs (de la Bretonne and Romaire 1989). In Louisiana, a system of grading crawfish according to size was implemented in the 1988 - 1989 season to take advantage of the high prices paid by foreign markets for large crawfish (greater than 30 g each). If successful management techniques for the production of crawfish greater than 30 g are developed that utilize formulated feed and/or density reduction, the higher price paid for the larger crawfish may make use of formulated feed economically feasible.

Although the percentage of crawfish larger than 75 mm TL, and size was greater in the "density reduced and fed" treatment, few crawfish exceeding 90 mm TL (about 21 g) were evident. de la Bretonne and Fowler (1976) and Lutz and Wolters (1986) speculated that adult populations of "stunted" crawfish result from negative genetic selection caused by current harvesting techniques which select the fastest growing crawfish in the population. Many crawfish not harvested mature at an earlier age and smaller size thus increasing the number of smaller crawfish in the population. Bosworth (1989) demonstrated there was no negative selection for growth in P. clarkii populations, but did show that age at maturity of male P. clarkii was a trait that was heritable. If the gene pool is dominated by earlier maturing individuals, then regardless of how much crawfish density is reduced and the amount they are fed, it may not be possible to produce large crawfish following harvest of the faster growing individuals.

The crawfish yields from ponds in this study ranged from 557 to 1,218 kg/ha for 41 trap-set days (3,690 trap-set days/ha). The production of crawfish in this study was consistent with the average state-wide harvest of crawfish from open ricefield ponds in Louisiana. Harvests generally range from 450 to 2,800 kg/ha (mean = 1,000 kg/ha) in ricefield ponds that are harvested 120 to 180 days (3,600 to 5,000 trap-set days/ha) per production season (Romaire 1989).

Population density reduction in combination with a program of supplemental feeding of formulated crustacean rations can be an effective management strategy to increase crawfish growth and increase the harvest of crawfish from forage-depleted ponds. Although the yield of crawfish and the percentage of crawfish larger than 75 mm TL were increased from treated experimental ponds relative to untreated ponds, efforts to produce high numbers of crawfish exceeding 90 mm TL were not successful.

The Influence of Permethrin Applications on Crawfish in Ponds

In laboratory acute toxicity determinations it has been established that permethrin ellicts a rapid toxic response and is lethal to P. clarkii at low concentrations. Jolly and Avault (1978) determined the 96-h LC50 for 8 - 12 mm TL and 20 - 30 mm TL juvenile P. clarkii to be 0.39 and 0.62 µg/L, respectively. Coulon (1982) estimated the 24-h LC50 of juvenile P. clarkii (18 - 27 mm TL) to be 0.49 µg/L. P. clarkii that were 8 - 10, 25 - 35, 45 - 55, and 65 - 75 mm TL had 96-h LC50's of 0.44, 0.85, 1.34, and 0.83 µg/L, respectively (Jarboe 1989, see section I of this study). The static acute toxicity tests of Jolly and Avault (1978), Coulon (1982), and Jarboe (1989, see section I of this study) were conducted in the laboratory at Louisiana State University and had water of similar quality (total alkalinity, total hardness, pH, water temperature); however, in field conditions other factors can influence the toxicity of permethrin.

Permethrin is a chlorinated compound which has an octanol/water partition coefficient of 3.48 (Verschueren 1983). Permethrin is lipophilic, and it tends to be

rapidly adsorbed to sediment in earthen ponds (Coulon 1982). Adsorbance of a toxicant to suspended matter would restrict its uptake and subsequent translocation to the target site(s) within the organism. The surface pond water used in the pond-side bioassays did contain suspended matter, and the 24-h LC50 for crawfish in pond water were 2 to 6 times higher than results obtained in the laboratory using dechlorinated, hardness-adjusted tap water. Coulon (1982) observed an increase in the LC50 of permethrin to P. clarkii in 24-h acute toxicity tests which were designed to examine the effects of various soil types on the toxicity of permethrin to P. clarkii and Ictalurus punctatus.

The use of a pond-side acute toxicity test utilizing surface water was a simple and rapid method for determining the toxicity of permethrin crawfish in "pond" water. The format of this bioassay may be useful for estimating the toxicity of other xenobiotics to P. clarkii in field conditions. Limitations imposed by biomass loading in aquaria restricted the number of crawfish used in the field bioassay. The ratios among crawfish size classes held in the aquaria were not totally representative of the size structure of crawfish in the pond. A better determination of permethrin toxicity to crawfish, outside of actual pond applications, would be accomplished by using more aquaria containing pond water and crawfish, or by using larger aquaria so that more crawfish could be incorporated into the acute toxicity tests.

Placement of crawfish in wire cages is a method which has been used to determine the impact of xenobiotics on wild crawfish populations (Hendrick and Everett 1965, Leonhard 1977, and Coulon 1982). When the estimated mortality in pond populations was compared to mortality in cages, the cages were not effective in determining the impact of permethrin on resident crawfish populations. Coulon (1982) reported that permethrin applied into earthen ponds was rapidly adsorbed to pond sediment. Cages which contained crawfish were positioned so that only a small area of the cage was in contact with bottom sediment. The reduction in contact between

crawfish and sediment could have restricted the uptake of permethrin from the water/sediment interface into crawfish thus reducing mortality. Another possible reason for the reduced mortality in caged crawfish may have been cage design and construction materials. The mesh size of the cage (1.9 mm dia) may have restricted the horizontal movement of permethrin through the cage; thereby, restricting the exposure of crawfish to waterborne permethrin. To determine the reason for the reduced mortality of caged crawfish, water and sediment samples would have to be obtained from inside and around the cages, and analyzed for permethrin following application. More developmental research is necessary to design a cage for research purposes which takes into account the activities of a benthic invertebrate so that the organism can be effectively exposed to environmental concentrations of toxicants.

At concentrations of 1.0 to 3.0 µg/L permethrin is acutely toxic to resident P. clarkii and may be one of the most toxic insecticides to crawfish in field conditions (Hendrick and Everett 1965, Hendrick et al. 1966, Hughes 1966, Chang and Lange 1967, Hyde et al. 1972, Ekanem 1981, Romaire 1983, and France 1986). Comparison of crawfish mortality between permethrin-treated ponds is difficult because the application rate of permethrin varied. Mortality among crawfish in ponds indicated that the crawfish did not respond to permethrin in a typical dose-dependent manner. Organic matter concentration (as measured by BOD and TOC) varied between ponds and could have bound permethrin prior to uptake by crawfish. Refugia, such as burrows, may have minimized permethrin exposure to many crawfish. At the time of permethrin application, treated ponds were devoid of macrophytes and the ponds were "overpopulated" with crawfish relative to forage resources. Physiological stress as a result of nutritional deficiencies and overpopulation may have caused the crawfish to respond to the chemical exposure in a manner other than a typical dose-response relationship. At the concentrations of permethrin used in this study, mortality among

crawfish populations which are not overpopulated and which have an abundant food supply could be different.

Permethrin toxicity exhibited by P. clarkii in ponds indicate there are some distinct trends supported by laboratory observations. In four of the six ponds treated with permethrin, P. clarkii smaller than 40 mm TL had higher mortalities than larger crawfish; a finding which is consistent with laboratory studies (Jolly and Avault 1978, and Jarboe 1989, see section I of this study). Mature P. clarkii males were more sensitive to the toxic effects of permethrin in ponds than either female or juvenile male P. clarkii of the same size; a finding consistent with laboratory studies (Jarboe 1989, see section I of this study). The response of mature female P. clarkii to permethrin was not examined in this study. Further research to elucidate the effects of permethrin on both mature male and female P. clarkii are necessary to understand the impact of this compound. Physical and biochemical factors that may influence the toxicity of permethrin to adult and juvenile P. clarkii are reviewed by Jarboe (1989, see section I of this study).

The hypersensitivity of P. acutus acutus to permethrin was first reported by Coulon (1982). In Coulon's study, identical numbers of caged P. clarkii and P. acutus acutus were placed in earthen ponds juxtapose and treated with 4.0 µg/L of permethrin. The application resulted in 65 and 100 % mortality to red swamp and white river crawfish, respectively. In the current study, 100 % mortality of pond populations of P. acutus acutus occurred at permethrin applications of about 2.0 µg/L. Although it is apparent that P. acutus acutus is more sensitive to permethrin than P. clarkii, it is not known why and this requires further examination.

P. acutus acutus and P. clarkii occupy the same environment and have similar ecological niches (Huner and Barr 1984). Romaire and Lutz (1989) reported that commercial crawfish ponds dominated by P. acutus acutus have lower yield potential

than ponds dominated by P. clarkii . In Louisiana, high catches of P. acutus acutus with P. clarkii often lowers the profit margin of the crawfish culturist because P. acutus acutus have lower market value than P. clarkii (Avault and Huner 1985). In production ponds where both species are found, use of permethrin at low concentrations may be an effective means of eradicating P. acutus acutus populations but not P. clarkii.

Coulon (1982) detected permethrin residue in the tail muscle of crawfish placed in ponds treated at 16 µg/L, 7 days following application; however, in this study no permethrin residue was detected in either crawfish abdominal muscle or hepatopancreas at 7, 14, and 21 days, post-treatment at exposure rates of 1.0 to 3.0 µg/L. The detection limits set in this study may have been too high to measure any residue. At low application levels (1.0 - 3.0 µg/L) permethrin may not be available for uptake because it has a very short residence time in pond water and sediment. The half-life of permethrin is 5.8 days in pond water and 9.4 days in pond sediment under the environmental conditions described by Coulon (1982).

In laboratory bioassays, reproduction and growth of P. clarkii that survived exposure to permethrin were not affected (Jarboe 1989, see section I of this study). Following permethrin treatment, subsequent sampling over three months indicated that P. clarkii which survived exposures grew and reproduced, and population density increased within a month of permethrin application, although not to previous levels.

Permethrin exposure at concentrations of 1.0 to 3.0 µg/L in pond water initiated crawfish mortality within 6 h of application. Mortality within crawfish populations indicated the toxicity of permethrin to crawfish in ponds was influenced by crawfish size, sex, and stage of maturity. Permethrin at the environmental concentrations examined in this study does not appear to bioconcentrate or have any apparent sublethal or chronic toxic affects in surviving crawfish. Further research on the effects of permethrin in crawfish should focus on how this compound is metabolized and what the

biologic mechanism is that makes P. acutus acutus more sensitive to permethrin than P. clarkii.

Permethrin was used to experimentally effect crawfish density reduction. Alternative means of pond crawfish density reduction should be investigated. Before any of the management techniques implimented in this research (such as supplemental feeding of formulated rations, or population reduction) can be recommended for use on a commercial scale, more research is necessary to determine nutritional requirements, feeding rates, and other feeding strategies which are optimum for pond production of red swamp crawfish.

CONCLUSIONS

1. The mean static acute 96-h LC50 of permethrin to red swamp crawfish 8 - 12 mm TL (0.017 g), 25 - 35 mm TL (0.64 g), 45 - 55 mm TL (2.45 g), and 65 - 75 mm TL (8.98 g) was 0.44, 0.85, 1.30, and 0.81 µg/L, respectively. Permethrin toxicity did not significantly differ among immature males or mature male and female P. clarkii of the similar size in laboratory static acute toxicity tests. Crawfish surviving exposures exhibited no differences in post-exposure growth, onset of sexual maturity, or production of viable young when compared to non-exposed controls. Permethrin did not cause any detectable teratogenic effects in crawfish.

2. The 24-h LC50 of permethrin to crawfish (31 to 70 mm TL) in aquaria containing pond water ranged from 1.0 to 3.1 µg/L. The exposure of caged crawfish was not an effective means of determining the toxicity of permethrin to pond crawfish populations. Pond crawfish responded to permethrin within 6 h of application and crawfish mortality in ponds which had been treated with permethrin occurred within 7 h. Crawfish less than 40 mm TL were more susceptible to permethrin poisoning than larger crawfish. Form I male P. clarkii were more sensitive to permethrin than either immature males or female crawfish of the same size. Pond populations of P. acutus acutus were more susceptible to permethrin than P. clarkii. Permethrin does not bioconcentrate to levels greater than 6.7 ng/g and 400.0 ng/g, respectively, in either abdominal muscle or hepatopancreas of P. clarkii at the application levels of 1.0 to 3.0 µg/L.

3. Crawfish densities of 16 - 18 crawfish/m^2 inclusive of 7 - 8 YOY crawfish/m^2 from November to January is an indicator that a pond will have a stunted crawfish population the following spring. Feeding P. clarkii a formulated feed increased

the percentage of crawfish exceeding 75 mm TL in the population. The mean size of Form I mature male crawfish was significantly increased by the feeding of a formulated ration. The yield and number of crawfish, and the CPUE in high density crawfish ponds was significantly increased by feeding a formulated feed. Ponds with 5 to 8 crawfish/m^2 (inclusive of YOY crawfish) from November to January had greater yields (number and weight of crawfish), larger individual crawfish, and higher CPUEs than ponds with high densities of crawfish or ponds in which the high density crawfish populations had been reduced and/or received a formulated feed. A combination of population density reduction and the supplemental feeding of a formulated feed was the most effective means of stimulating crawfish growth in high-density stunted crawfish ponds.

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APPENDIX

Appendix Table 1. Mortality of Procambarus clarkii 8 - 12 mm TL at various concentrations of permethrin in 96-h definitive tests (replicate 1). The number of crawfish per test concentration was 30 (N = 30).

Permethrin	% Crawfish mortality after						
(µg / L)	6 h	12 h	18 h	24 h	48 h	72 h	96 h
0.990	100	100	100	100	100	100	100
0.625	67	77	77	77	77	77	77
0.394	10	33	33	33	33	33	33
0.248	0	0	0	0	0	0	0
0.158	0	0	0	0	0	0	0
0.099	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0

Appendix Table 2. Mortality of Procambarus clarkii 8 - 12 mm TL at various concentrations of permethrin in 96-h definitive tests (replicate 2). The number of crawfish per test concentration was 15 (N = 15).

Permethrin	% Crawfish mortality after						
(µg / L)	6 h	12 h	18 h	24 h	48 h	72 h	96 h
0.990	100	100	100	100	100	100	100
0.625	100	100	100	100	100	100	100
0.394	73	73	73	73	73	73	73
0.248	40	47	47	47	47	47	47
0.158	13	20	20	20	20	20	20
0.099	7	7	7	7	7	7	7
0	0	0	0	0	0	0	0

Appendix Table 3. Mortality of Procambarus clarkii 8 - 12 mm TL at various concentrations of permethrin in 96-h definitive tests (replicate 3). The number of crawfish per test concentration was 15 (N = 15).

Permethrin	% Crawfish mortality after						
(µg / L)	6 h	12 h	18 h	24 h	48 h	72 h	96 h
0.990	100	100	100	100	100	100	100
0.625	33	73	73	73	73	73	73
0.394	0	20	20	20	20	20	20
0.248	0	0	0	0	0	0	0
0.158	0	0	0	0	0	0	0
0.099	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0

Appendix Table 4. Mortality of Procambarus clarkii 25 - 35 mm TL at various concentrations of permethrin in 96-h definitive tests (replicate 1). The number of crawfish per test concentration was 18 (N = 18).

Permethrin	% Crawfish mortality after						
(µg / L)	6 h	12 h	18 h	24 h	48 h	72 h	96 h
1.485	44	78	83	89	89	89	89
0.864	17	22	28	28	28	28	28
0.503	6	6	6	6	6	6	6
0.292	0	0	0	0	0	0	0
0.170	0	0	0	0	0	0	0
0.099	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0

Appendix Table 5. Mortality of Procambarus clarkii 25 - 35 mm TL at various concentrations of permethrin in 96-h definitive tests (replicate 2). The number of crawfish per test concentration was 18 (N = 18).

Permethrin	% Crawfish mortality after						
(µg / L)	6 h	12 h	18 h	24 h	48 h	72 h	96 h
1.485	73	100	100	100	100	100	100
0.864	13	73	80	80	80	80	80
0.503	0	7	13	13	13	13	13
0.292	7	7	7	7	7	7	7
0.170	0	0	0	0	0	0	0
0.099	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0

Appendix Table 6. Mortality of Procambarus clarkii 25 - 35 mm TL at various concentrations of permethrin in 96-h definitive tests (replicate 3). The number of crawfish per test concentration was 18 (N = 18).

Permethrin	% Crawfish mortality after						
(µg / L)	6 h	12 h	18 h	24 h	48 h	72 h	96 h
1.485	87	93	93	93	93	93	93
0.864	13	60	67	67	67	67	67
0.503	7	7	7	13	13	13	13
0.292	0	0	0	0	0	7	7
0.170	0	0	0	0	0	0	0
0.099	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0

Appendix Table 7. Mortality of Procambarus clarkii 45 - 55 mm TL at various concentrations of permethrin in 96-h definitive tests (replicate 1). The number of crawfish per test concentration was 18 (N = 18).

Permethrin (µg / L)	% Crawfish mortality after						
	6 h	12 h	18 h	24 h	48 h	72 h	96 h
1.980	6	50	78	89	89	89	89
1.495	0	11	22	33	50	61	61
1.138	0	22	33	39	39	39	39
0.861	0	6	6	11	11	11	11
0.653	0	0	0	0	6	6	6
0.495	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0

Appendix Table 8. Mortality of Procambarus clarkii 45 - 55 mm TL at various concentrations of permethrin in 96-h definitive tests (replicate 2). The number of crawfish per test concentration was 18 (N = 18).

Permethrin	% Crawfish mortality after						
(µg / L)	6 h	12 h	18 h	24 h	48 h	72 h	96 h
1.980	6	61	83	100	100	100	100
1.495	0	17	44	50	56	56	56
1.138	0	17	22	33	39	39	39
0.861	0	6	11	22	22	22	22
0.653	0	0	11	11	11	11	11
0.495	0	0	6	6	6	6	6
0	0	0	0	0	0	0	0

Appendix Table 9. Mortality of Procambarus clarkii 45 - 55 mm TL at various concentrations of permethrin in 96-h definitive tests (replicate 3). The number of crawfish per test concentration was 18 (N = 18).

Permethrin	% Crawfish mortality after						
(µg / L)	6 h	12 h	18 h	24 h	48 h	72 h	96 h
1.980	6	61	83	100	100	100	100
1.495	0	17	44	50	56	56	56
1.138	0	17	22	33	39	39	39
0.861	0	6	11	22	22	22	22
0.653	0	0	11	11	11	11	11
0.495	0	6	6	6	6	6	6
0	0	0	0	0	0	0	0

Appendix Table 10. Mortality of Procambarus clarkii 65 - 75 mm TL at various concentrations of permethrin in 96-h definitive tests (replicate 1). The number of crawfish per test concentration was 12 (N = 12).

Permethrin	% Crawfish mortality after						
(µg / L)	6 h	12 h	18 h	24 h	48 h	72 h	96 h
2.475	41	83	100	100	100	100	100
1.921	8	83	100	100	100	100	100
1.465	0	50	75	92	100	100	100
1.119	0	25	67	92	100	100	100
0.851	0	25	42	42	67	67	67
0.653	0	0	8	8	8	8	8
0	0	0	0	0	0	0	0

Appendix Table 11. Mortality of Procambarus clarkii 65 - 75 mm TL at various concentrations of permethrin in 96-h definitive tests (replicate 2). The number of crawfish per test concentration was 12 (N = 12).

Permethrin (µg / L)	% Crawfish mortality after						
	6 h	12 h	18 h	24 h	48 h	72 h	96 h
2.475	25	83	100	100	100	100	100
1.921	83	83	100	100	100	100	100
1.465	0	75	75	92	100	100	100
1.119	0	25	67	67	83	83	83
0.851	0	17	33	42	75	75	75
0.653	0	17	42	50	50	50	50
0	0	0	0	0	0	0	0

Appendix Table 12. Mortality of Procambarus clarkii 65 - 75 mm TL at various concentrations of permethrin in 96-h definitive tests (replicate 3). The number of crawfish per test concentration was 12 (N = 12).

Permethrin	% Crawfish mortality after						
(µg / L)	6 h	12 h	18 h	24 h	48 h	72 h	96 h
2.475	8	50	92	100	100	100	100
1.921	8	92	100	100	100	100	100
1.465	0	50	67	100	100	100	100
1.119	0	8	33	42	75	75	75
0.851	0	0	17	25	25	25	25
0.653	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0

Appendix Table 13. Proximate analysis[1] of the Dupont formulated crawfish feed (E. I. du Pont de Nemours & Co., Inc., Orange, Texas).

Components	Percent Dry Weight
Protein	27.0
Crude Fiber	7.0
Lipid	6.0
Ash	14.0

FEED COMPONENTS

Fish Meal, Crustacean Meal, Soybean Meal, Grain Products, Grain By-Products, Animal By-Products, Fish Oil, Fish By-Products, Binder, Soy Lecithin, Brewers Yeast, Vitamin C, Niacin, Choline, Vitamin A, Vitamin D, Vitamin E, Vitamin K, Vitamin B, Vitamin B2, Vitamin B6, Inositol, Vitamin B12, Folic Acid, PABA, D-Calcium Pantothenate, Biotin, D-Calcium Phosphate, Copper Sulfate, Zinc Oxide, Magnesium Oxide, Potassium Chloride, Stabilizing Agents

[1]Proximate analysis determined at the Feed and Fertilizer Laboratory, Louisiana State University, Baton Rouge, Louisiana

Appendix Table 14. Water volume and the amount of permethrin administered to treated ponds in the crawfish density reduction and feeding study on 7 - 8 April 1988.

	Pond Volume (L)	Amount of Permethrin Administered (mg a. i.)
	Density Reduced and Fed	
Replicate		
1	157,991	316
2	153,537	384
3	200,832	402
	Density Reduced	
Replicate		
1	250,076	250
2	230,950	462
3	219,319	658

VITA

Herman Henry Jarboe was born on August 19, 1954 in Louisville, Kentucky the son of Doris Jean and Dr. Charles Harry Jarboe. He attended Ballard High School in Louisville, Kentucky and graduated August 1972.

After graduating from high school he enlisted in the United States Army were he served as a wheeled and tracked vehicle mechanic. He was honorable discharged with the rank of Specialist Fourth Class in October 1975.

He was admitted into the University of Louisville in January 1976. On May 1981 he graduated from the University of Louisville, with a Bachelors of Science degree in Zoology.

He was admitted into the Department of Wildlife and Fisheries at Missippi State University, Starkville Mississippi, on August 1982. In August 1984 he graduated from Mississippi State University with a Master of Science degree in Wildlife and Fisheries and the title of his thesis was "Evaluation of excess vitamin E supplementation in channel catfish (Ictalurus punctatus) production feeds".

In August 1985 he was admitted into the doctoral program in Department of Forestry, Wildlife, and Fisheries at Louisiana State University and is currently pursuing his Ph. D. The title of his dissertation is "Population management of red swamp crawfish (Procambarus clarkii) by density reduction and/or supplemental feeding.

He was married to Ann Leslie Looney on April 1, 1989

DOCTORAL EXAMINATION AND DISSERTATION REPORT

Candidate: Herman H. Jarboe

Major Field: Wildlife and Fisheries Science

Title of Dissertation: Toxicity of permethrin to Procambarus clarkii, and the effects of permethrin-induced density reduction and supplemental feeding on stunted crawfish populations

Approved:

Robert P. Romaire
Major Professor and Chairman

F. Glen Kemby
Dean of the Graduate School

EXAMINING COMMITTEE:

Mark K Johnson

Dudley D. Culley

Gary W. Whitledge

Richard Cordry

Robert C. Reigh

Date of Examination:

15 November 1989