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## Water Quality Dynamics in Commercial Crawfish Ponds and Toxicity of Selected Water Quality Variables to *Procambarus clarkii*

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WATER QUALITY DYNAMICS IN COMMERCIAL  
CRAWFISH PONDS AND TOXICITY OF  
SELECTED WATER QUALITY VARIABLES TO Procambarus clarkii

A THESIS

Submitted to the Graduate Faculty of the  
Louisiana State University and  
Agricultural and Mechanical College  
in partial fulfillment of the  
requirements for the degree of  
Master of Science

in

The School of Forestry, Wildlife, and Fisheries

by

Thomas Michael Hymel  
B.S., Clemson University, 1981  
May 1985



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## ABSTRACT

Water quality in five southern Louisiana commercial crawfish (Procambarus clarkii and P. acutus acutus) ponds was monitored from November 1982 through May 1983. Two ponds were planted with rice (Oryza sativa) and three ponds had volunteer terrestrial and semi-aquatic vegetation as crawfish forage.

Water quality was poorest in November, April, and May when water temperatures exceeded 18°C. However, only dissolved oxygen (DO) attained concentrations low enough (<1.0 mg/liter) to be acutely toxic to crawfish. Free carbon dioxide (CO<sub>2</sub>) attained levels (12.0 mg/liter) that could potentially harm crawfish during periods of low DO (<2.0 mg/liter). The temperature, pH, free CO<sub>2</sub>, nitrate, total phosphorus, biochemical oxygen demand (BOD), chlorophyll a, total hardness, and total alkalinity did not differ significantly among rice ponds and ponds with volunteer vegetation. The DO, nitrite, total nitrogen, and chemical oxygen demand (COD) were highest in rice ponds (P<0.05). The soluble inorganic phosphorus (SIP) and conductivity were highest in ponds with volunteer vegetation (P<0.05).

Free CO<sub>2</sub>, SIP, conductivity, and total alkalinity levels were positively correlated with an increase in macrophytic biomass (P<0.10). Nitrite, total nitrogen, COD, BOD, turbidity, temperature and DO decreased with increased vegetative biomass. Crawfish catch was positively correlated with total ammonia, BOD, COD, and water temperature, and negatively correlated with total phosphorus, total nitrogen, SIP, total alkalinity, and DO.

The toxicity of hydroxyl and hydrogen ions (i.e., pH), ammonia, and nitrite to juvenile P. clarkii was determined in static acute toxicity

tests. The 96-hour LC50's of hydroxyl and hydrogen ions to P. clarkii were  $10^{-3.88}$  moles  $\text{OH}^-$ /liter (pH 10.12) and  $10^{-3.21}$  moles  $\text{H}^+$ /liter (pH 3.21). The 96-hour LC50's of ammonia and nitrite to P. clarkii were 2.65 mg  $\text{NH}_3$ -N/liter and 5.94 mg  $\text{NO}_2$ -N/liter, respectively.

## INTRODUCTION

Culture of crawfish (Procambarus clarkii and P. acutus acutus) is the only large-scale crustacean aquaculture industry in the U.S. Most of the approximately 50,000 hectares of commercial crawfish farms are located in the southeastern U.S., principally in Louisiana. In 1983, nearly 40,000 hectares of commercial crawfish ponds in Louisiana produced a crop with a wholesale value of \$45 million (Louisiana Cooperative Extension Service 1983). Procambarid crawfish are also farmed in Mississippi, Texas, and South Carolina. Crawfish yields from ponds presently range from 200 to 4,000 kg per hectare depending upon the type and intensity of management, but few data are available on what potential maximum yields could be attained with improved management.

Water quality is the most important environmental factor limiting crawfish yields in commercial ponds (Huner and Barr 1984). Poor water quality may cause crawfish kills resulting in substantial economic losses to the producer. Moreover, crawfish do not feed or grow well and may become predisposed to stress-related diseases in ponds with poor water quality (Scott 1984). The relationship between water quality and crawfish production, however, has not been extensively investigated.

Poorest water quality reportedly occurs soon after flooding ponds in the fall (September through November) when decomposition of vegetation - the principal forage for crawfish - results in low concentrations of dissolved oxygen (Avault et al. 1975). Dissolved oxygen (DO) should be maintained above 2.0 mg/liter for optimal crawfish production (Romaine 1983) and significant mortality can occur when the DO is less than 1.0 mg/liter (Melancon and Avault 1976). Although low DO is well recognized as a factor limiting crawfish production, little

research has been conducted on the potential deleterious effects of other water quality variables, such as ammonia, nitrites and pH, on crawfish. Crawfish farmers often experience "pond failures" in which most or all crawfish die from unknown causes. Although mortality is generally attributed to low DO, other water quality variables could be responsible.

Water quality research in crawfish culture is limited. Most studies were conducted in small experimental ponds (0.05 surface hectare or less) and water quality was of secondary interest. Day (1983) measured temporal trends in DO, water temperature, chemical oxygen demand (COD), pH, total alkalinity, and total hardness in a study designed to evaluate crawfish forages. Miltner (1980) recorded water temperature and DO in experimental ponds planted with rice (Oryza sativa) or millet (Echinochloa frumentacea). Chien (1980) measured monthly trends in inorganic nitrogen, total nitrogen, soluble orthophosphate, and total phosphorus in rice-crawfish, double-cropping experiments. Johnson (1980) monitored the changes in DO, water temperature, pH, total alkalinity and turbidity in a crawfish forage study.

Several investigators have conducted laboratory studies to determine the toxicity of selected water quality variables to P. clarkii and P. acutus acutus. Melancon and Avault (1977) evaluated the effects of low DO on newly hatched and juvenile P. clarkii. Johnson (1982) determined the acute toxicity of carbon dioxide, ammonia, nitrite, and hydrogen sulfide to newly hatched and adult P. acutus acutus in 24-hour exposure tests. McMahon and Morgan (1981) determined the tolerance of

adult P. clarkii to highly acid and alkaline waters (i.e., low and high pH, respectively).

No research has been conducted on temporal changes in water quality in commercial crawfish ponds, nor has the relationship between type and quantity of vegetative biomass and water quality in commercial crawfish ponds been investigated. Furthermore, there is a paucity of information on the relationship between changes in water quality and its effect on crawfish catch. Currently, water quality management recommendations to commercial crawfish farmers are based principally on "best-guess estimates" or "rules of thumb" and not sound scientific evidence. This often results in inadequate management of water and added expense to the crawfish farmer.

Baseline investigations are needed to determine temporal changes in water quality in crawfish ponds and to ascertain the relationship between water quality and crawfish production. The purposes of this study were: (1) to obtain data on temporal changes in water quality and nutrient levels in five commercial crawfish ponds and to relate these changes to differences in type and quantity of vegetation and to the crawfish catch; and (2) to determine the acute toxicity of hydroxyl and hydrogen ions (i.e., pH), ammonia, and nitrite to juvenile P. clarkii.

# WATER QUALITY DYNAMICS IN COMMERCIAL CRAWFISH PONDS

## Objectives

1. To measure temporal changes in 16 water quality variables in five commercial crawfish ponds.
2. To ascertain if water quality in the ponds is correlated with type and quantity of vegetation.
3. To determine if changes in crawfish catch are correlated with temporal variation in water quality in the ponds.

## Description of the Study Area

Five commercial crawfish ponds located within 2 km of Henderson, Louisiana (west of the Atchafalaya Basin) were used in this study (Figure 1). The ponds were built in 1980 by St. Martin Land Company and contained a resident crawfish population. Ponds ranged in size from 14.6 to 23.1 hectares and averaged 60 cm in depth when filled with water. Two ponds (R1 and R2) were planted with rice (Oryza sativa var. Saturn) in April 1982 and the grain was harvested in September. Rice straw and stubble were left in the ponds as crawfish forage. The other three ponds (B1, A1 and A2) were not planted with a cover crop. Rather, the ponds were colonized "naturally" from May through September 1982, and volunteer terrestrial and semi-aquatic plants served as the principal forages for crawfish. The dominant plants present were alligatorweed (Alternanthera philoxeroides), smartweeds (Polygonum spp.), water primrose (Ludwigia peploides), and coffee weed (Sesbania

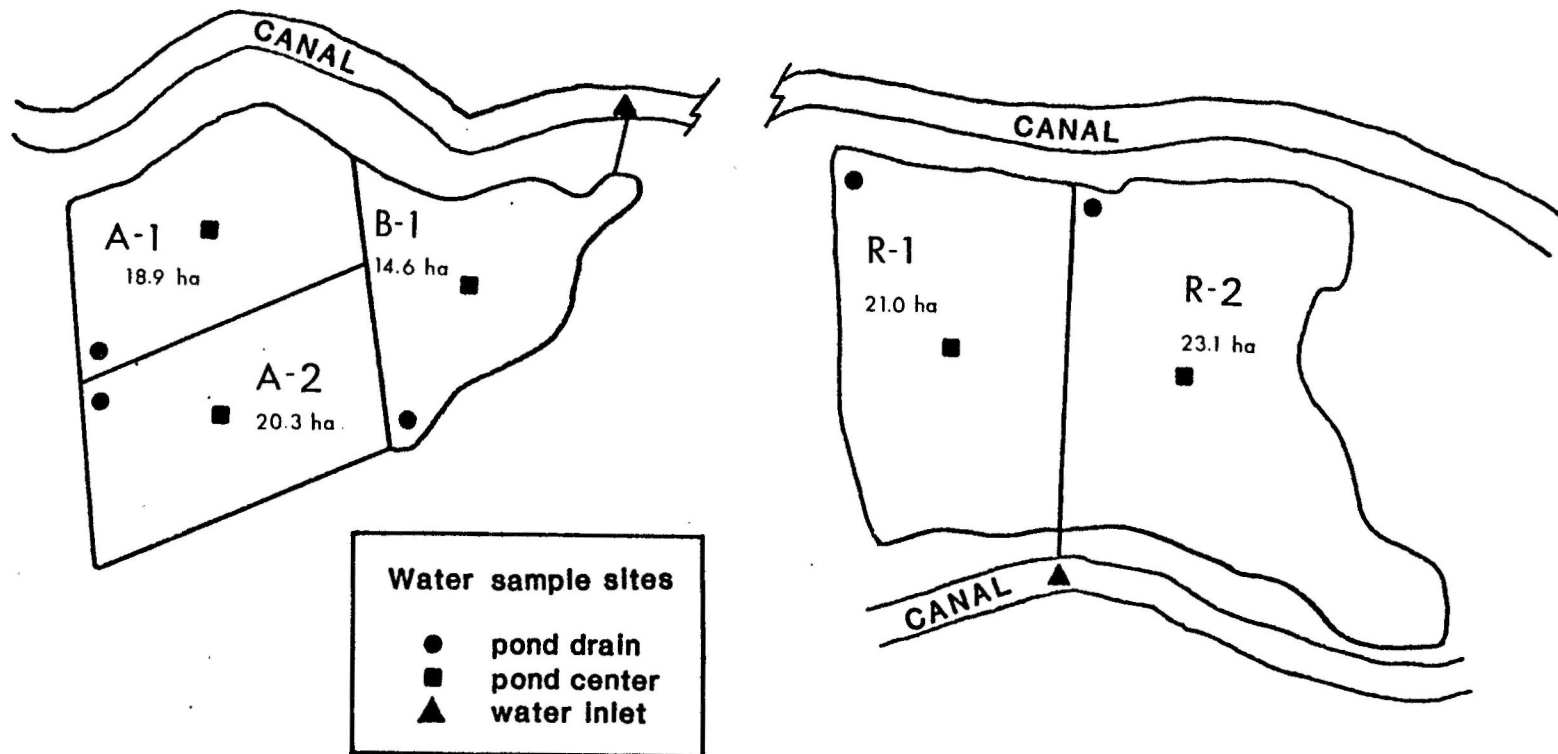


Figure 1. Five commercial crawfish ponds near Henderson, Louisiana, that were used in the water quality study. Two ponds (R-1 and R-2) were planted with rice and three ponds (B-1, A-1, and A-2) contained volunteer vegetation.

macrocarpa). Other plants included sedges (Cyperus spp.) and annual grasses (Panicum spp.).

Prior to flooding the ponds in October, lanes were established in each pond by mowing a 4-m wide swath of vegetation along longitudinal transects spaced about 15 m apart. The cut material was baled into 1,000 kg units and left in the pond. The lanes served as areas to place traps and allowed easy access to traps by boat. The surface area of each pond and number of traps fished were as follows:

<u>Pond</u>	<u>Vegetation type</u>	<u>Area (hectare)</u>	<u>No. of traps/hectare</u>
R1	Rice	21.0	37
R2	Rice	23.1	40
B1	Volunteer vegetation	14.6	62
A1	Volunteer vegetation	18.9	30
A2	Volunteer vegetation	20.3	37

### Materials and Methods

#### Water quality

The ponds were filled from 16-20 October 1982 with water pumped from canals (20 m wide x 3 m deep) adjacent to the study site (Figure 1). Water samples were taken weekly from the water supply canals and in all ponds from 1 November 1982 (10 days after filling) through 25 May, 1983. Samples were collected between 0730 and 0830 hours at a permanent station near the center and drain of each pond and at a single location adjacent to the water inlet in each canal (Figure 1). The water column was equally represented in the sample by submerging a 2-liter polyethylene bottle to the pond bottom and slowly raising it so that as



it reached the surface, it had just filled. Dissolved oxygen and water temperature were measured in situ at the surface, mid-depth, and bottom at each pond station and at the surface of the canals with a polarographic oxygen meter with thermistor (Yellow Springs Instrument Model 58) . The water samples were transported to a water chemistry laboratory at LSU, Baton Rouge, where analytical work was initiated by 1100 hours.

The following water quality analyses were conducted according to established analytical procedures (Boyd 1979; APHA et al. 1980): total ammonia, nitrate, nitrite, total nitrogen, pH, chemical oxygen demand (COD), free carbon dioxide, soluble inorganic phosphorus, total phosphorus, total hardness, and total alkalinity. Additional analyses included biochemical oxygen demand (BOD), chlorophyll a, turbidity, and conductivity. Methods of chemical and instrumental analyses are presented in Table 1. Total ammonia, nitrate, nitrite and total nitrogen were expressed as equivalent weights of nitrogen (i.e., mg N/liter). Soluble inorganic phosphorus and total phosphorus were expressed as equivalent weights of phosphorus (i.e., mg P/liter). Total hardness and total alkalinity are presented as  $\text{CaCO}_3$  equivalents (Boyd 1982).

#### Vegetative biomass

One week prior to flooding ponds, 21 to 30 vegetation samples were collected from random locations in each pond to estimate the above-ground vegetative biomass (Vollenweider 1969). A quadrat ( $0.1 \text{ m}^2$ ), constructed from 1-cm diameter steel rod, was used to determine the boundaries of each sample. All vegetation within the quadrat was

Table 1. Water quality variables and methods of analysis.

Variable	Instrument or method of analysis
Water temperature	Thermistor
Dissolved oxygen	Polarographic probe
Hydrogen ion concentration	Glass electrode (pH meter)
Free CO <sub>2</sub>	Ion specific electrode
Total ammonia-nitrogen	Ion specific electrode
Nitrite-nitrogen <sup>a</sup>	Diazotization of sulfanilic acid
Nitrate-nitrogen <sup>a</sup>	Phenoldisulfonic acid method
Soluble inorganic phosphorus <sup>a</sup>	Stannous chloride reduction
COD <sup>a</sup>	Dichromate oxidation
BOD <sup>b</sup>	Incubation at 20°C for 120 hours
Total hardness <sup>a</sup>	EDTA titration
Total alkalinity <sup>a</sup>	Titration with 0.02 N H <sub>2</sub> SO <sub>4</sub>
Conductivity	Probe
Chlorophyll <u>a</u> <sup>a</sup>	Acetone extraction/spectroscopy
Total phosphorus <sup>a</sup>	Persulfate digestion
Total nitrogen	Persulfate digestion/Ion specific electrode
Turbidity <sup>b</sup>	Nephelometry

<sup>a</sup> After Boyd (1979).<sup>b</sup> After APHA et al. (1980).

clipped at the soil surface, placed in a plastic bag, and returned to LSU for analysis. The vegetation was dried for 24 hours at 100°C (Vollenweider 1969) in a gravity convection oven (Precision Model 17) and then weighed to the nearest 0.1 g on a Mettler Model 1200 top-loading balance. Data were multiplied by a factor of 0.01 to provide an estimate of the vegetative biomass in  $\text{kg/m}^2$  (dry weight basis).

#### Water management

The ponds were managed according to the discretion of the pond manager. After ponds were initially flooded, each pond was periodically flushed with canal water that was aerated (or partially deoxygenated if the canal water was supersaturated with oxygen) to 90 to 100% oxygen saturation by allowing water to fall through an aeration box in each pond. The aeration box consisted of two, 2-m W x 2-m L expanded metal screens (1.27-cm square mesh) spaced 0.75 m apart vertically and supported by a wooden frame. The ponds were generally flushed when DO in ponds declined below 1 to 2 mg/liter. Thus, water management practices in each pond varied and the degree to which differences in management practices influenced water quality is difficult to assess.

#### Crawfish catch

Detailed records on the crawfish harvest from each pond were maintained by the pond manager and made available for this study. Commercial harvest of crawfish in ponds began in mid-November and was terminated in May. Ponds were drained in June. Poor weather, labor shortages, and low prices for crawfish precluded daily harvesting in ponds. Likewise, these same factors resulted in the ponds being fished

with differential frequency and intensity. Harvest data were standardized for each pond by calculating the crawfish catch per unit trap effort (kg crawfish/trap/day) from the commercial harvest records. The catch per unit trap effort (CPUE) was determined by dividing the weight (kg) of crawfish caught in a pond on a given day by the number of traps fished in the pond on that same day.

### Statistical analyses

An analysis of variance (completely randomized design) was used to test for statistical differences between water quality in the canals (source water) and in the ponds (Steel and Torrie 1980). Analysis of covariance (completely randomized design) was employed for each of the 16 water quality variables to determine differences in water quality between the two vegetation types, adjusted for differences in the quantity of vegetative biomass. Vegetative biomass served as the covariable in the analyses.

Temporal changes in water quality were ascertained by incorporating sampling date as a split-plot (i.e., repeated measures design) in the analysis of covariance. Duncan's new multiple range test (Steel and Torrie 1980) was used to test for significant differences among treatment means, i.e., source water versus pond water, or rice versus volunteer vegetation. Differences in treatment means were declared significant at  $\alpha \leq 0.05$ .

Simple linear correlation analysis (Draper and Smith 1965) was used to determine the degree and direction of correlation between vegetative biomass and water quality, as well as the correlation between water quality and crawfish CPUE. Because only five observations of vegetative

biomass (one observation per pond) could be used in the correlation analysis, a correlation was declared significant at  $\alpha \leq 0.1$ . However, correlations between water quality variables and CPUE were declared significant at  $\alpha \leq 0.05$ . All statistical analyses were made with the Statistical Analysis System (SAS) software package on a IBM 3083 computer.

### Results and Discussion

#### Vegetative biomass

The productivity of a crawfish pond depends largely upon the quantity and nutritive quality of forage available to crawfish throughout the growing season. If an adequate food supply is unavailable, crawfish growth may be reduced and in severe cases stunting may occur. In either case, crawfish of poor market quality can lead to economic losses.

Vegetation biomass in the five crawfish ponds was as follows:

<u>Pond</u>	<u>Vegetation type</u>	<u>N</u>	<u>kg dry matter/m<sup>2</sup></u> ( $\bar{x} \pm SD$ )
R-1	Rice	30	2.04 $\pm$ 0.75
R-2	Rice	30	1.56 $\pm$ 0.79
B-1	Natural vegetation	25	2.34 $\pm$ 1.00
A-1	Natural vegetation	25	2.34 $\pm$ 1.01
A-2	Natural vegetation	21	2.60 $\pm$ 1.09

The three ponds colonized by volunteer vegetation contained 29% greater biomass ( $\bar{x} = 2.41 \text{ kg/m}^2$ ) than did the two ponds planted with rice ( $\bar{x} = 1.71 \text{ kg/m}^2$ ;  $P < 0.01$ ). Vegetative biomass from 0.5 to 5 kg dry matter/m<sup>2</sup>

were reported in experimental crawfish ponds (Johnson 1980; Miltner 1980) and in commercial ponds (Nassar 1982) where rice or volunteer vegetation served as crawfish forage. The amount of vegetation depends upon plant species and maturity, nutrient content of the soil, climate, and management of the vegetation (e.g., insect and weed control, fertilization, etc.).

Vegetation is the principal forage for pond-raised crawfish. Crawfish feed directly on vegetation and associated periphyton, but more significantly on microbial enriched plant detritus (Goldman et al. 1974; Avault et al. 1975; Momot et al. 1978). LaCaze (1981) reported that 20% of the crawfish diet consists of animals including oligochaetes, insect larvae, snails, and other immobile species. The amount of a specific forage that will maximize crawfish production by providing sufficient feed while minimizing poor water quality has not yet been determined. Too little forage will result in crawfish "stunting" at undesirable market sizes while an overabundance of forage will cause poor water quality (Huner and Barr 1984). The forage system used in crawfish culture contrasts sharply with other commercial aquaculture systems (e.g., channel catfish and rainbow trout) in which fish are fed expensive, high-protein rations.

In Louisiana, about 30% of the 40,000 hectares devoted to crawfish culture contain rice as a forage crop (Huner and Barr 1984). However, 50 to 70% of the crawfish farmers plant no crop but rather encourage growth of annual terrestrial grasses and sedges and emergent, semi-aquatic vegetation such as alligatorweed, smartweed, and water primrose. Some crawfish farmers plant millet, sorghum, or other cereal grain crops for crawfish feed.

The chemical and structural composition, especially the carbon:nitrogen (C:N) ratio, may vary greatly between plant species, and changes in C:N ratio occur as some species mature (Boyd 1974). Vegetation with high nitrogen and low fiber (i.e., narrow C:N ratio) are generally more nutritious but decay more rapidly than plants with low nitrogen and high fiber (i.e., wide C:N ratio) (Goyert et al. 1975). The chemical and structural composition of plants as well as water temperature, DO and invertebrate community structure significantly influence the rate of vegetative decomposition (Hanlon 1982).

Although vegetation is the principal food for crawfish, it can also have serious water quality implications. The impact on water quality is related principally to type and quantity of vegetation in ponds. Rice is frequently planted to minimize poor water quality because it is a semi-aquatic plant with a wide C:N ratio which results in slow decomposition (Miltner 1980). Ponds populated with many plant species with correspondingly different rates of decay may influence water quality differently than ponds planted with rice.

## Water quality

### Water temperature

Water temperatures in ponds were within the range suitable for crawfish growth and survival. Shortly after flooding ponds in early November, water temperature ranged from 16.0 to 24.0°C ( $\bar{x} \pm SE = 19.7 \pm 0.7^\circ\text{C}$ ) (Figure 2). Water temperature declined significantly in December and by mid-January had reached a low of 7.0 to 12.0°C ( $\bar{x} \pm SE = 9.5 \pm 0.2^\circ\text{C}$ ). Water warmed significantly in late February, and by May water temperature had increased to 22.0 to 29.0°C ( $\bar{x} \pm SE = 25.1 \pm$

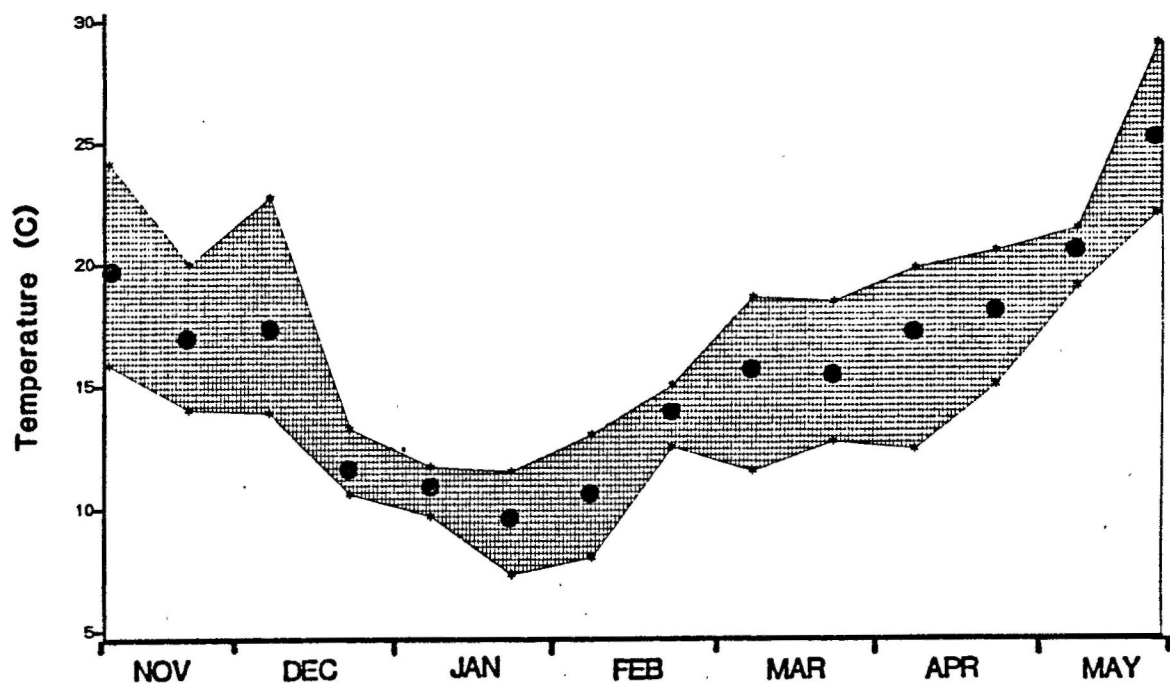


Figure 2. Temporal variation in water temperature in five commercial crawfish ponds near Henderson, Louisiana. Dots are means and upper and lower solid lines are maximum and minimum concentrations in the ponds.



0.5°C). No significant difference in temperature was found between canal water and pond water (Table 2). Similarly, no difference in water temperature was noted between ponds planted with rice and those colonized by volunteer vegetation (Table 2).

Water temperature declined significantly with an increase in vegetative biomass ( $r = -0.87$ ;  $P < 0.1$ ), probably due to shading of the water column by the standing vegetation. Sculthorpe (1967) reported a cooling effect in pond water resulting from self-shading by dense stands of emergent macrophytes. Dense quantities of standing macrophytes (i.e., erect vegetation extending above the water surface) in November and December cooled pond waters by limiting light penetration and reduced water turbulence and circulation which tend to distribute heat (Cole 1975).

The ponds were thermally stratified from late fall through mid-winter, but no stratification was evident in late winter and spring (Table 3). Water temperatures between surface and bottom varied as much as 1.8°C from November to mid-January though the difference from surface to bottom was usually no greater than 0.2°C (Table 3). When standing vegetation was depleted by mid-January, shading was eliminated and water circulation was improved which resulted in uniform temperatures from surface to bottom from late January through May (Table 3). Similarly, bottom water temperatures in late fall and winter were 1 to 2°C cooler than at the surface in experimental crawfish ponds (0.04 hectare) with standing vegetation (Chein 1980; Miltner 1980). After standing vegetation was depleted in January, water temperature was uniform from surface to bottom in these experimental ponds. Conversely, experimental ponds in which vegetation had been mowed or disked into the pond bottom

Table 2. Water quality in supply canals and in five commercial crawfish ponds either planted with rice or vegetated naturally. Statistical differences in means were determined with Duncan's new multiple range test (N=52 to 150).<sup>1</sup>

Water quality variable	Canal		Type of vegetation			
			Rice		Natural vegetation	
<hr/>						
<u>Overall Mean</u>						
Temperature (C)	15.7	a	15.5	a	15.2	a
DO (mg/liter)	7.7	a	6.6	b	5.1	c
pH	6.9	a	6.9	a	6.9	a
Free CO <sub>2</sub> (mg/liter)	0.7	b	1.0	a	1.2	a
Total alkalinity (mg/liter)	50.0	b	61.0	a	65.0	a
Total hardness (mg/liter)	58.0	b	68.0	a	69.0	a
Conductivity (μmhos/cm)	122.0	c	141.8	b	160.0	a
Total ammonia (mg N/liter)	0.14	a	0.18	a	0.13	a
Nitrite (mg NO <sub>2</sub> -N/liter)	0.03	b	0.05	a	0.01	c
Total nitrogen (mg N/liter)	4.4	b	5.4	a	3.8	b
SIP (μg P/liter)	29.0	a	28.0	b	29.0	a
Total phosphorus (mg P/liter)	0.18	b	0.20	a	0.20	a
COD (mg/liter)	27.0	c	41.0	a	31.0	b
BOD (mg/liter)	2.95	a	3.39	a	3.05	a
Turbidity (NTU)	87.0	b	188.0	a	37.0	c
Chlorophyll <u>a</u> (μg/liter)	26.0	a	19.0	b	15.0	b

<sup>1</sup> Horizontal comparison only. Means with same letter are not significantly different (P>0.05).

Table 3. Water temperature and dissolved oxygen stratification on selected sample dates in two commercial crawfish ponds (Pond B-1, natural vegetation and Pond R-1, rice) near Henderson, Louisiana.

Location of sample	Sampling date and pond number									
	10 Nov 1982 B-1	10 Nov 1982 R-1	14 Dec 1982 B-1	14 Dec 1982 R-1	19 Jan 1983 B-1	19 Jan 1983 R-1	9 Mar 1983 B-1	9 Mar 1983 R-1	11 May 1983 B-1	11 May 1983 R-1
<u>Temperature (C)</u>										
S <sup>a</sup>	17.0	16.6	22.3	22.7	9.8	7.5	18.5	17.1	22.5	22.3
M	16.9	16.6	21.8	22.6	9.8	7.5	18.5	17.1	22.5	22.3
B	16.9	16.3	20.5	22.5	9.8	7.5	18.5	17.1	22.5	22.3
<u>Dissolved oxygen (mg/liter)</u>										
S	4.60	4.14	4.27	7.74	4.24	7.90	2.10	4.00	3.50	6.00
M	4.58	3.86	1.80	5.73	4.24	7.90	2.10	4.00	3.50	6.00
B	4.26	3.78	0.49	4.10	4.24	7.90	2.10	4.00	3.50	6.00

<sup>a</sup> Sampling locations were surface (S), mid-depth (M), and bottom (B).

prior to adding water had uniform temperatures from top to bottom in fall, winter and spring (Chein 1980).

Procambarid crawfish can survive temperatures from 0 to 35°C but 22 to 27°C is considered optimal for growth (Johnson 1983). Taylor (1984) reported P. clarkii had a thermal preference of 22.0°C. Mobile organisms such as crawfish actively seek temperatures that enhance or optimize growth or reproduction (Reynolds 1977). Crawfish growth is inhibited and activity is minimal below 10°C (Johnson 1983; Huner and Barr 1984) and P. clarkii cannot survive sustained temperatures exceeding 38°C (Hobbs and Hall 1974).

Water temperatures from mid-November through April were  $7 \pm 3^\circ\text{C}$  below that considered optimum (22-27°C) for the growth of P. clarkii (Figure 2). Optimum temperatures occurred principally during early November and in May. Maximum water temperatures (29°C in May) never approached lethal limits (38°C).

Most crawfish ponds are flooded in September and October to take advantage of optimal water temperatures for crawfish growth and so that marketable crawfish will be available by late November (Huner and Barr 1984). However, the practice of filling crawfish ponds with water before mid-October frequently causes poor water quality. When water temperatures exceed 26°C, vegetation decomposes rapidly which may cause severe DO depletion and crawfish death (Day 1983; Johnson 1983). If flooding is delayed until the maximum ambient water temperature is stabilized at 20°C, then the cooler water will reduce vegetative decomposition and minimize DO depletion (Chien 1980), but it may slow crawfish growth.

### Dissolved oxygen

Equilibrium concentration (i.e., 100% gas saturation) of DO in water at sea level ranges from 14.15 mg/liter at 0°C to 7.53 mg/liter at 30°C (Boyd 1982). Major inputs of DO include that released in photosynthesis by phytoplankton and macrophytes and gains from atmospheric diffusion.

Dissolved oxygen exhibited significant seasonal variation in the ponds, and it increased with decreasing water temperature and vice-versa (Figures 2 and 3). The DO concentration in the five ponds averaged ( $\bar{x} \pm SE$ )  $4.0 \pm 0.5$  mg/liter in November and ranged from 0.6 to 8.3 mg/liter (6.1 to 83.2% DO saturation). The average DO increased from  $7.1 \pm 0.5$  mg/liter in mid-December to  $9.1 \pm 0.4$  mg/liter by early February as a consequence of cold water temperatures. The DO ranged from 2.7 to 13.0 mg/liter (24.7 to 107.2% DO saturation) during winter. As water temperatures increased in the spring, the DO generally declined (Figures 2 and 3) and ranged from 0.3 to 9.9 mg/liter (3.4 and 94.6% DO saturation). The mean DO in May was  $4.2 \pm 0.5$  mg/liter. Although the water temperature between surface and bottom did not differ more than 2.0°C from November through mid-January, the DO differed as much as 3.8 mg/liter between the surface and bottom on some dates in both rice and naturally vegetated ponds (Table 3). No DO stratification was observed after mid-January because cold water temperatures (<10°C) and a paucity of standing vegetation allowed for complete mixing of the water column.

Dissolved oxygen was significantly higher in the canals ( $\bar{x} = 7.7$  mg/liter) than in the ponds ( $P < 0.05$ ; Table 2). The canals had higher DO because they contained little or no decomposing macrophytes. The DO was 23% lower in the naturally vegetated ponds ( $\bar{x} = 5.1$  mg/liter) than in

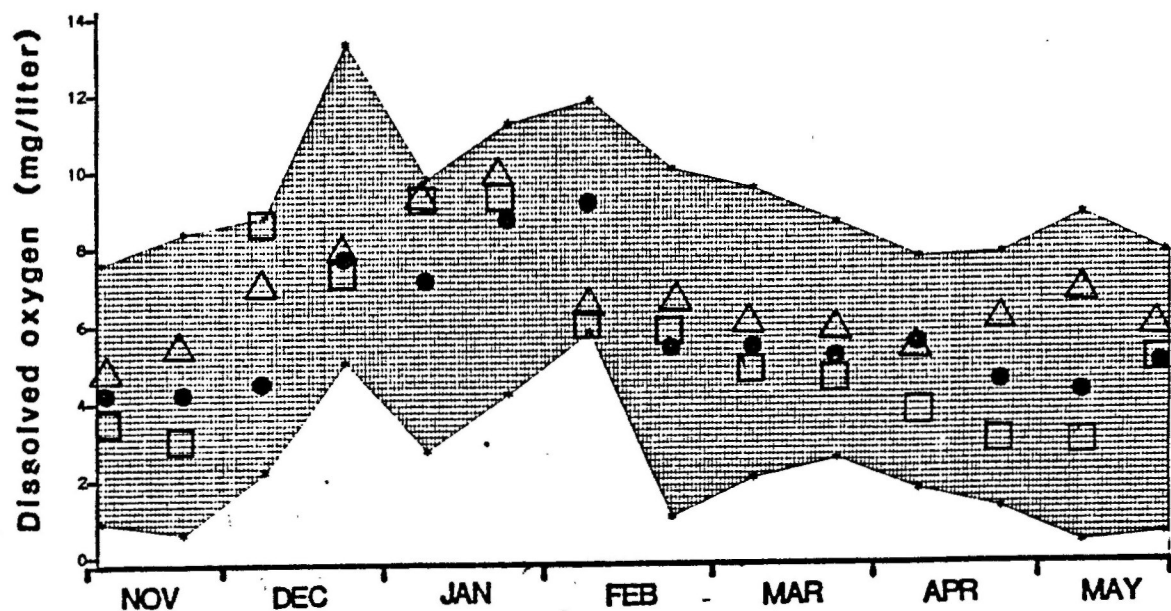


Figure 3. Temporal variation in dissolved oxygen (DO) in five commercial crawfish ponds near Henderson, Louisiana. Triangles and squares are means of rice and naturally vegetated ponds, respectively. Dots are means of all ponds combined and upper and lower solid lines are maximum and minimum concentrations for the ponds.

ponds planted with rice ( $\bar{x}=6.6$  mg/liter;  $P<0.05$ ). This decrease was due principally to a greater amount of vegetation in naturally vegetated ponds and was not necessarily a function of vegetation type. The naturally vegetated ponds had 29% more vegetation than ponds with rice and the DO decreased significantly with an increase in vegetative biomass ( $r= -0.87$ ,  $P<0.1$ ). In addition, dense stands of Sesbania spp. and large mats of filamentous algae in the naturally vegetated ponds effectively impeded wind and water circulation which could have decreased DO through reduced atmospheric diffusion.

Low DO in commercial crawfish ponds occurs most frequently in the first six weeks after flooding because elevated water temperatures ( $>26^{\circ}\text{C}$ ) result in rapid decay of large amounts of vegetation. The DO increases in winter and early spring because the colder water temperature reduces respiration of the aquatic biota (including decomposers) and the equilibrium concentration of DO is increased. Similarly, low DO is prevalent in April and May because higher water temperatures increases oxygen consumption by the biota and the equilibrium concentration of DO is decreased. The temporal trends in DO in the five commercial crawfish ponds were very similar to trends in DO in experimental (0.04 hectare) crawfish ponds (Chien 1978, 1980; Johnson 1980; Miltner 1980).

Dissolved oxygen should be maintained above 2.0 mg/liter for optimal crawfish production (Romaine 1983) and significant mortality of crawfish occurs when the DO drops below 1.0 mg/liter (Melancon and Avault 1977). From November through May the DO concentration in the five ponds was less than 2.0 mg/liter on 12% of the sampling dates ( $n = 131$ ), and it was below 1.0 mg/liter 8% of the time. The DO was

less than 2.0 mg/liter most frequently in November and in May and was less frequent in the colder months of December, January and March (Table 4). Dissolved oxygen concentration below 1.0 mg/liter occurred most frequently in November, February and May (Table 4). Low DO was more prevalent in the naturally vegetated ponds than in rice ponds (Table 4). About 80 and 90% of the occurrences of DO less than 2.0 and less than 1.0 mg/liter, respectively, occurred in the ponds with volunteer vegetation ( $P < 0.01$ ). Low DO was most frequent when water temperatures exceeded 18°C.

Newly hatched P. clarkii (9-12 mm TL) are not tolerant of low DO. Newly hatched and juvenile P. clarkii begin to die at DO concentrations less than 1.10 and 0.85 mg/liter, respectively (Melancon and Avault 1976). The 96-hour LC50 of newly hatched P. clarkii ranges from 0.75 to 1.10 mg/liter. Conversely, juvenile crawfish (31-35 mm TL) had a 96-hour LC50 of 0.49 mg/liter (Melancon and Avault 1976). Mortality of crawfish can occur in submerged traps when DO is 1.0 mg/liter or less (Avault et al. 1975). Low DO places extreme physiological stress on crawfish and thereby reduces feeding and growth. Prolonged DO stress may predispose crawfish to parasitic infections and disease outbreaks (Scott 1984). The DO in the five ponds periodically attained stressful levels and some crawfish mortality may have occurred.

Inadequate concentrations of DO are most frequently alleviated by adding oxygenated water to the ponds or by aerating existing pond water by recirculation. Zeman (1982) demonstrated in experimental ponds (<1 hectare) that DO concentrations could be maintained above 2 or 3 mg/liter during the fall and spring by implementing a management program of exchanging water in the ponds with fresh, oxygenated water from



Table 4. Occurrence of dissolved oxygen less than or equal to 1.0 or 2.0 mg/liter in five commercial crawfish ponds. Rice was planted in two ponds and three ponds were allowed to naturally vegetate. Data are expressed as a percentage of the total sampling dates per month.

Month	Dissolved oxygen		Dissolved oxygen	
	< 1 mg/liter		< 2 mg/liter	
	Rice	Natural vegetation	Rice	Natural vegetation
% occurrence				
Nov	6	18	13	18
Dec	0	6	7	6
Jan	0	0	0	0
Feb	0	13	0	13
Mar	0	0	0	0
Apr	0	6	0	25
May	0	12	0	18

another source or by recirculation of existing pond waters. The success of a management program to increase DO in crawfish ponds depends on the exchange (flushing) or recirculating capacity of the pumping system. The Louisiana Cooperative Extension Service (Baker 1983) recommends that a pumping system for crawfish ponds have a capacity of 940 liters per minute per hectare so as to allow for a complete exchange of water in 3.5 days. The ponds used in this study had this exchange capacity, but the aerated water must be circulated through the entire pond to be most effective. Some crawfish farmers construct baffle levees within the pond to effect better circulation of oxygenated water. The ponds used in this study had interior borrow ditches adjacent to the levees and these ditches probably directed much of the fresh water directly to the drains and not throughout the entire area of the pond. The dense stands of vegetation at flooding also provided resistance to water circulation such that attempts to increase DO by water exchange were often unsuccessful.

### Free CO<sub>2</sub>

The free CO<sub>2</sub> is the amount of CO<sub>2</sub> dissolved as a gas in water and does not include the CO<sub>2</sub> bound in bicarbonates (HCO<sub>3</sub><sup>-</sup>) and carbonates (CO<sub>3</sub><sup>2-</sup>). Equilibrium concentration of free CO<sub>2</sub> in water at sea level ranges from 1.10 mg/liter at 0°C to 0.42 mg/liter at 30°C (Boyd 1982). The major input of free CO<sub>2</sub> in pond water is the CO<sub>2</sub> excreted in respiration by microorganisms and other aquatic biota, such as macrophytes, plankton, crawfish, etc.

Free CO<sub>2</sub> was highest in November when concentrations ranged from 1.0 to 12.0 mg/liter ( $\bar{x} \pm SE = 3.0 \pm 0.7$  mg/liter) (Figure 4). Free CO<sub>2</sub>

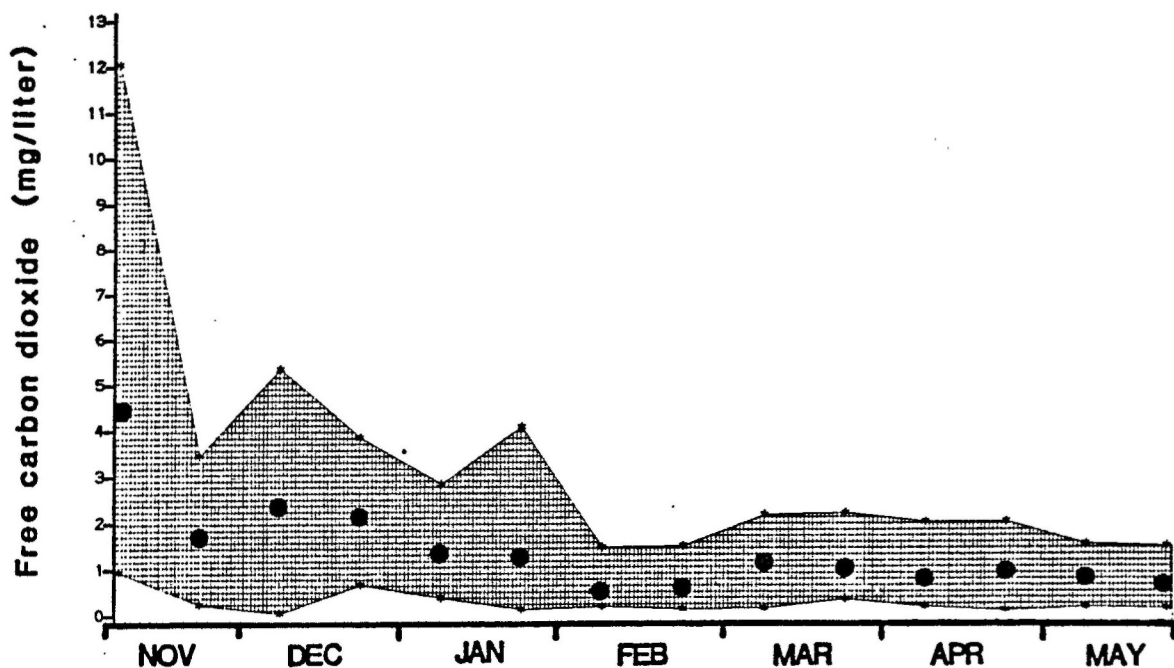


Figure 4. Temporal variation in free carbon dioxide (CO<sub>2</sub>) in five commercial crawfish ponds near Henderson, Louisiana. Dots are means and upper and lower solid lines are maximum and minimum concentrations in the ponds.

decreased gradually to a low of 0.3 to 1.5 mg/liter in early February and remained at a relatively low and stable concentration ( $\bar{x} \pm SE = 0.9 \pm 0.1$  mg/liter) through late May (Figure 4). The canal water ( $\bar{x}=0.7$  mg/liter) contained 36% less free  $CO_2$  than the pond waters ( $\bar{x}=1.1$  mg/liter) ( $P<0.05$ ; Table 2), because the canals had a paucity of macrophytes that were subject to decomposition. The free  $CO_2$  in rice ponds did not differ from naturally vegetated ponds (Table 2). Free  $CO_2$  significantly increased with an increase in vegetative biomass ( $r=0.70$ ;  $P<0.1$ ). This increase in  $CO_2$  was apparently a result of increased respiration by microbes associated with increased vegetative substrate.

Free  $CO_2$  in ponds was highest in the fall when large quantities of vegetation and warm water temperatures ( $>26^\circ C$ ) stimulated decomposition and a release of large amounts of  $CO_2$ . Free  $CO_2$  declined in winter because reduced water temperature decreased decomposition and respiration. The free  $CO_2$  did not increase with warm temperatures in the spring because of a slight increase in pH, and most of the terrestrial macrophytes had decomposed.

High concentrations of free  $CO_2$  interfere with respiration in aquatic animals by inhibiting gas exchange (Storer and Usinger 1965). The toxicity of free  $CO_2$  increases when DO is low ( $<2$  mg/liter) (Boyd 1979). Carbon dioxide produced in metabolic processes leaves the organism by diffusion at the gill-water interface and an increase in free  $CO_2$  in the water impedes outward diffusion. In fishes, internal accumulation of  $CO_2$  limits oxygen uptake by the hemoglobin and can result in suffocation. Free  $CO_2$  levels up to 10 mg/liter at dawn may be tolerated by fish provided DO exceeds 4.0 mg/liter (Parks et al. 1975). Moreover, most species will survive for several days in water containing

up to 60 mg CO<sub>2</sub>/liter provided DO is plentiful (Hart 1944, Haskell and Davies 1958). In general, water supporting good fish populations contains less than 5 mg/liter of free CO<sub>2</sub>. Reliable information on the toxicity of CO<sub>2</sub> to crawfish is not available. However, potential problems with high CO<sub>2</sub> may occur within the first month after flooding crawfish ponds because free CO<sub>2</sub> concentrations are high and DO is generally low.

### pH

The pH in the five ponds varied little from November through May and typically ranged from 6.5 to 7.5 ( $\bar{x} \pm SE = 6.9 \pm 0.3$ ) (Figure 5). A steep decline in pH to 5.7-6.3 occurred in all ponds during early January. Intense rainfall (Table 5) may have been responsible for the pH drop. About 33 cm of rainfall, or about 50% of the volume of each pond, fell during a 10-day period in early January. Rainfall that is saturated with free CO<sub>2</sub> typically has a pH of 5.0-5.5 (Trewartha 1968), and Feagley and Cremers (1984) reported rainfall in many areas of southern Louisiana to have a pH as low as 4.1. Generally, a seasonal increase in pH occurs in eutrophic waters during spring, summer, and fall, particularly when water temperature exceeds 25°C (Boyd 1979, 1982). The seasonal rise in pH results because the CO<sub>2</sub>, an acidic compound, assimilated in photosynthesis exceeds that released by respiration. No seasonal increase in pH occurred in the five crawfish ponds, nor has any seasonal increase in pH been observed in experimental crawfish ponds (Zeman 1982; Day 1983). No difference in pH was found between canal water and pond water (Table 2). Similarly, no difference

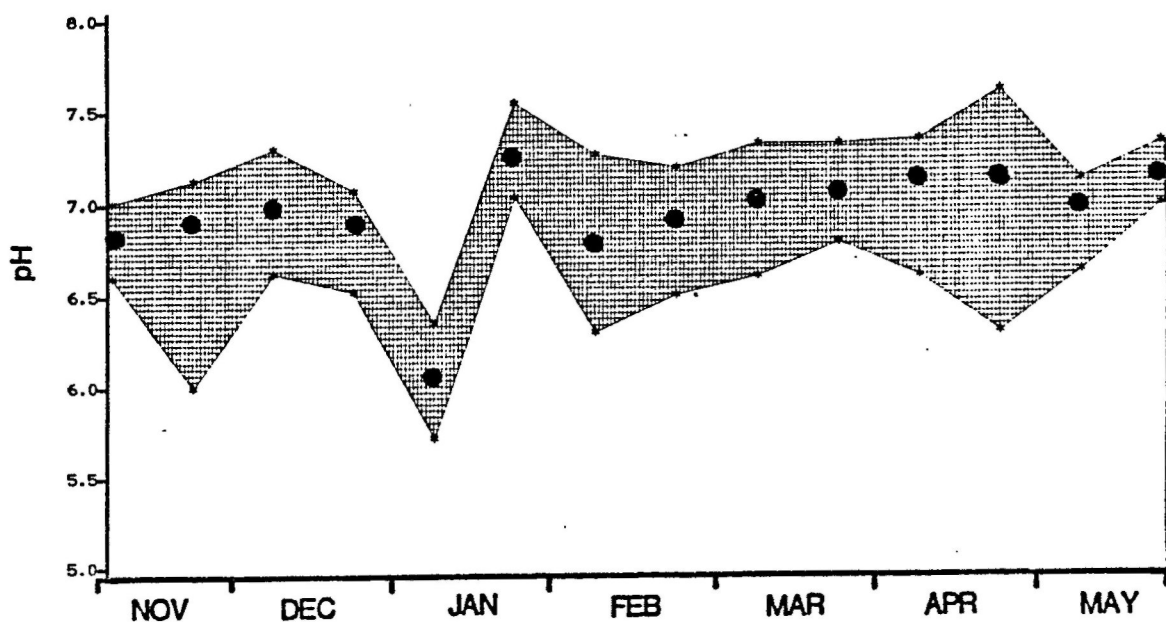


Figure 5. Temporal variation in pH in five commercial crawfish ponds near Henderson, Louisiana. Dots are means and upper and lower solid lines are maximum and minimum concentrations in the ponds.

Table 5. Monthly rainfall from November 1982 through May 1983 at five commerical crawfish ponds near Henderson, Louisiana. Data collected from rain gauge adjacent to the ponds.

Month	Rainfall (cm)
Nov	16.6
Dec	32.0
Jan	39.6
Feb	13.7
Mar	17.2
Apr	8.2
May	21.6

in pH was noted between ponds with rice and naturally vegetated ponds (Table 2). There was no correlation between pH and vegetative biomass.

The pH in the ponds was generally within levels (pH 6.5-9.0 at daybreak) reported most conducive for fish production (Boyd 1979, 1982). Similar pH levels (6.5-7.7) were reported in small (<1 hectare) experimental crawfish ponds planted with soybeans or rice (Zeman 1982; Day 1983). The relative stability in pH in the five ponds can be attributed principally to the total alkalinity (>50 mg/liter as  $\text{CaCO}_3$  equivalent). Water with a total alkalinity that exceeds 20 mg/liter is a strong buffer to changes in pH because bicarbonate ( $\text{HCO}_3^-$ ) and carbonate ( $\text{CO}_3^{2-}$ ) ions neutralize increases in  $\text{H}^+$  or  $\text{OH}^-$  (Boyd 1979, 1982).

#### Total alkalinity

Total alkalinity is the the total titratable bases in water expressed as  $\text{CaCO}_3$  equivalent. In most natural waters, bicarbonate ( $\text{HCO}_3^-$ ) and carbonate ( $\text{CO}_3^{2-}$ ) are the predominant bases. Total alkalinity of natural waters range from less than 5 to several hundred mg/liter. If total alkalinity of waters is too low (<20 mg/liter), phytoplankton growth may be limited by inadequate supplies of carbon dioxide needed for photosynthesis (Boyd 1979, 1982). Production of aquatic biota increases with increased alkalinity. The increase in production is not due to higher concentrations of alkalinity alone but is also caused by higher levels of phosphorus and other essential nutrients which increase with alkalinity. Water with high alkalinity is more strongly buffered against changes in pH.



Total alkalinity was greatest in November and December and ranged from 64 to 132 mg/liter as ( $\bar{x} \pm SE = 90 \pm 5.0$  mg/liter) (Figure 6). Total alkalinity decreased through late January when it ranged from 39 to 69 mg/liter ( $\bar{x} \pm SE = 50 \pm 1.6$  mg/liter). Total alkalinity exhibited large fluctuations from February through May and ranged between 39 and 114 mg/liter ( $\bar{x}=50$  mg/liter).

Total alkalinity in canals ( $\bar{x}=50$  mg/liter) was significantly lower ( $P<0.05$ ) than either rice ( $\bar{x}=61$  mg/liter) or naturally vegetated ( $\bar{x}=65$  mg/liter) ponds (Table 2). The total alkalinity of ponds with rice did not significantly differ from ponds with natural vegetation. Total alkalinity was positively correlated with vegetative biomass ( $r=0.89$ ;  $P<0.1$ ). Alkalinity increased with an increase in vegetation because more free  $CO_2$  was released (from decomposition) in ponds with greater macrophytic biomass. The free  $CO_2$  reacts with alkaline earth carbonates, such as  $CaCO_3$  or  $CaMg(CO_3)_2$ , to increase the concentrations of  $HCO_3^-$  and  $CO_3^{2-}$ .

Total alkalinity was always above the minimum concentration of 20 mg/liter considered necessary for good fish production (Boyd 1979, 1982). Fish production in fertilized ponds did not increase as the total alkalinity increased from 20 to 120 mg/liter (Boyd and Walley 1975). However, fish production increased substantially as the total alkalinity increased from 0 to 20 mg/liter. The total alkalinity in crawfish ponds with less than 20 mg/liter can be increased by liming. Procedures for liming fish ponds are outlined by Boyd (1979).

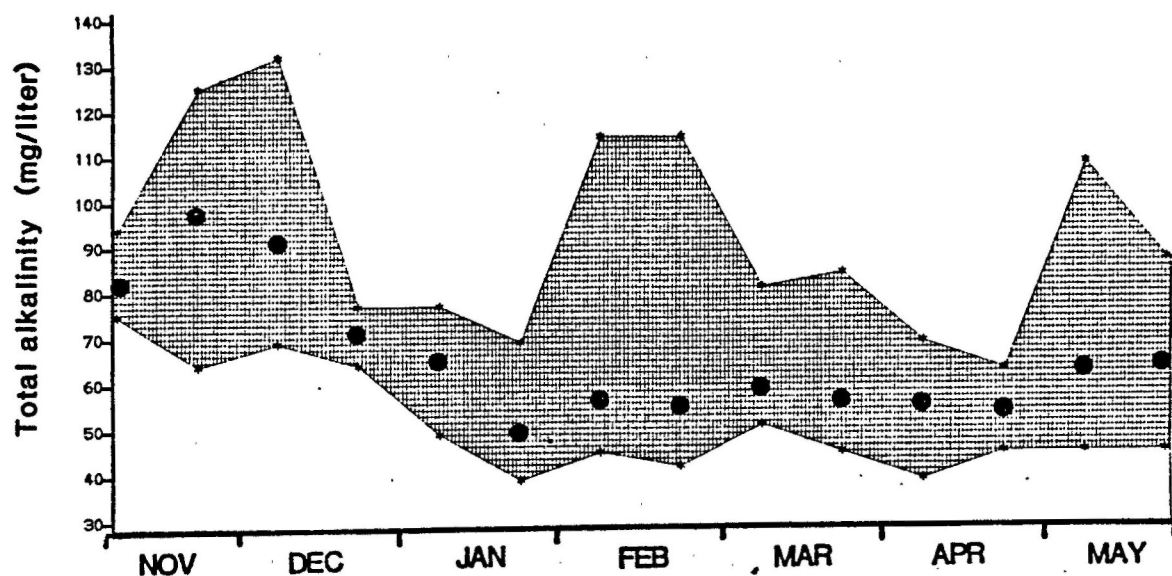


Figure 6. Temporal variation in total alkalinity in five commercial crawfish ponds near Henderson, Louisiana. Dots are means and upper and lower solid lines are maximum and minimum concentrations in the ponds.

### Total hardness

Total hardness is the concentration of divalent metallic ions (e.g.,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Mn}^{2+}$ ) in water, expressed as mg/liter of  $\text{CaCO}_3$  equivalent. Total hardness is usually of similar magnitude to total alkalinity because the anions of alkalinity (e.g.,  $\text{HCO}_3^-$ ,  $\text{CO}_3^{2-}$ ) and the cations of hardness (e.g.,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ) are normally derived from the solution of carbonate minerals such as calcite ( $\text{CaCO}_3$ ) or dolomite ( $\text{CaMg}(\text{CO}_3)_2$ ) (Boyd 1982). The hardness and alkalinity of pond waters reflect the carbonate contents of rocks and soils of watersheds and bottom muds.

Total hardness in the ponds exhibited temporal variations similar to total alkalinity. Total hardness in the ponds ranged from 72 to 105 mg/liter ( $\bar{x} \pm \text{SE} = 85 \pm 1.6$  mg/liter) in November and then declined to range from 50 to 75 mg/liter ( $\bar{x} \pm \text{SE} = 67 \pm 1.5$  mg/liter) by January (Figure 7). The water hardness increased moderately through late winter and spring and by May ranged between 70 and 140 mg/liter ( $\bar{x} \pm \text{SE} = 100 \pm 4.0$  mg/liter). The decrease in water hardness in winter can be attributed mostly to the dilution of pond water by large amounts of rain in December and January (Table 5), but also in part to decreased vegetative decomposition and subsequent reduction in the release of divalent cations (particularly  $\text{Mg}^{2+}$  and  $\text{Ca}^{2+}$ ) from tissues of vegetation. However, the pond waters ( $\bar{x}=68$  mg/liter) contained significantly more total hardness than the canals ( $\bar{x} = 58$  mg/liter;  $P<0.05$ ), which was probably due in part to the release of  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  from decomposing vegetation. The total hardness in rice ponds ( $\bar{x}=68$  mg/liter) did not differ from ponds with natural vegetation ( $\bar{x}=69$  mg/liter) (Table 2). Total hardness increased with an increase in

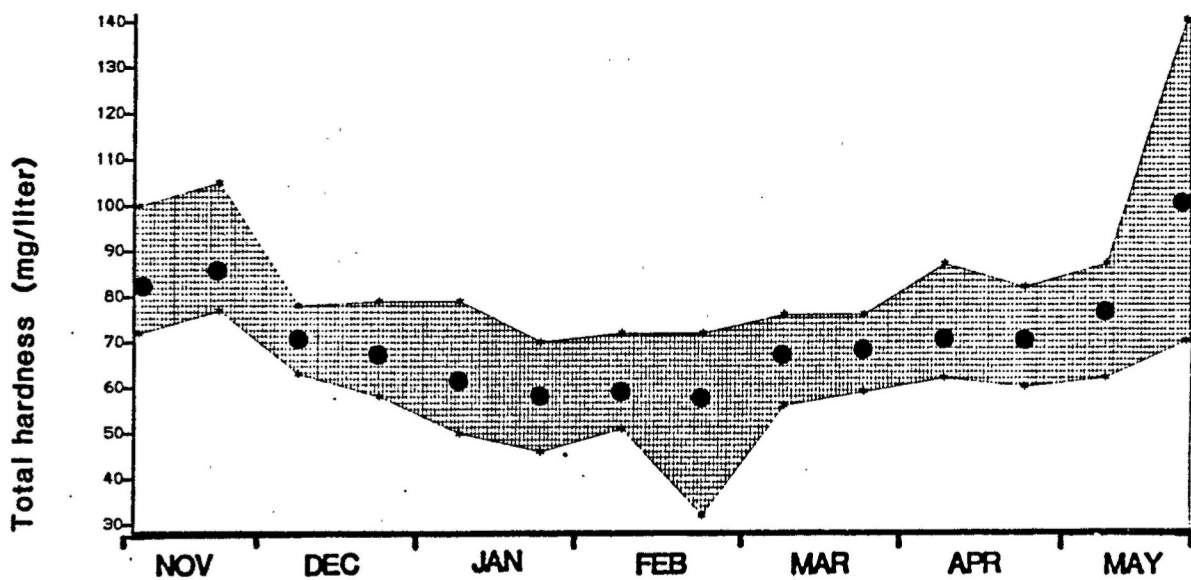


Figure 7. Temporal variation in total hardness in five commercial crawfish ponds near Henderson, Louisiana. Dots are means and upper and lower solid lines are maximum and minimum concentrations in the ponds.

vegetative biomass ( $r=0.48$ ;  $P<0.1$ ) because greater amounts of divalent cations were released as vegetation decomposed.

The total hardness of pond waters should be above 20 mg/liter for optimum production of cultured aquatic animals (Boyd 1982). The total hardness in the five ponds exceeded the 17 mg/liter reported necessary for good survival of juvenile P. clarkii (Smitherman et al. 1967). The hardness was also greater than the 50 mg/liter required for good crawfish production, but slightly below the 100 mg/liter suggested for optimum crawfish production (de la Bretonne et al. 1969). Total hardness in crawfish ponds can be increased by liming. Procedures for liming fish ponds are given by Boyd (1982).

### Conductivity

Conductivity is a numerical expression of the ability of water to carry an electric current. The conductivity depends on the total concentration of the ions (e.g.,  $\text{Na}^+$ ,  $\text{Cl}^-$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ , etc.) dissolved in water and the temperature at which the measurement is made (APHA et al. 1980). Distilled water has conductivity near 0  $\mu\text{mhos/cm}$  while freshwaters normally have conductivities of 20 to 1500  $\mu\text{mhos/cm}$  (Boyd 1979).

Highest conductivity in the five ponds occurred in November with levels from 175 to 250  $\mu\text{mhos/cm}$  ( $\bar{x} \pm \text{SE} = 210 \pm 4.5 \mu\text{mhos/cm}$ ) (Figure 8). The conductivity declined to a low of 100 to 150  $\mu\text{mhos/cm}$  ( $\bar{x} \pm \text{SE} = 156 \pm 2.7 \mu\text{mhos/cm}$ ) in January, it increased during the spring, and by May concentrations of 125 to 250  $\mu\text{mhos/cm}$  ( $\bar{x} \pm \text{SE} = 159 \pm 4.2 \mu\text{mhos/cm}$ ) were measured. The conductivity of canal water ( $\bar{x}=122 \mu\text{mhos/cm}$ ) was significantly lower than pond waters ( $P<0.05$ ), and naturally vegetated

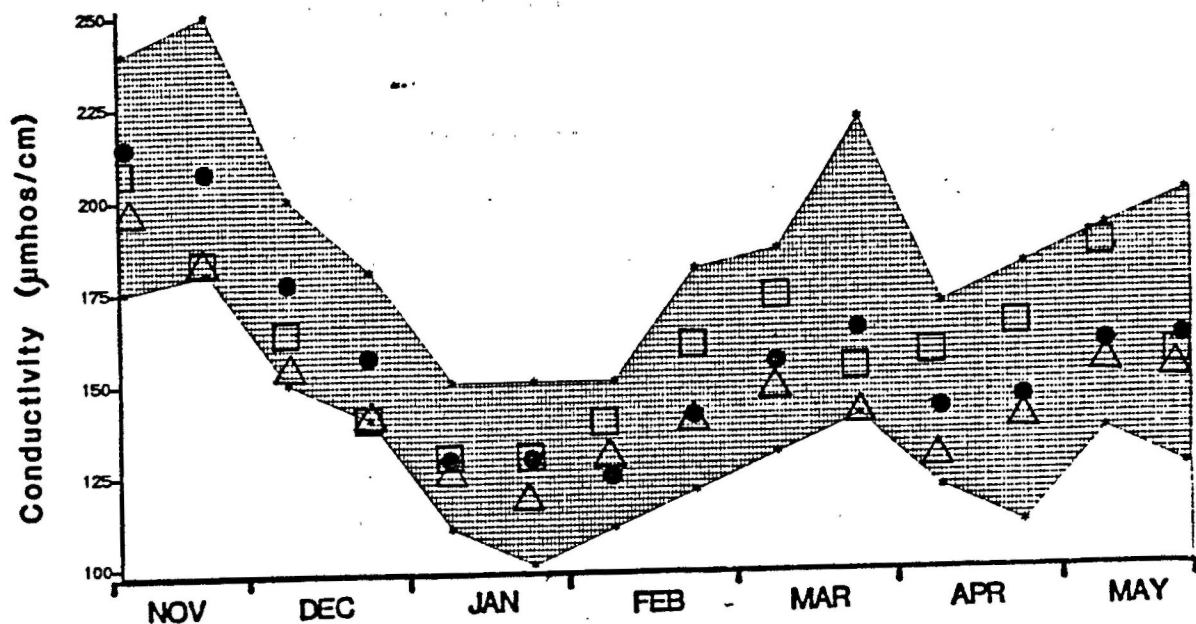


Figure 8. Temporal variation in conductivity in five commercial crawfish ponds near Henderson, Louisiana. Triangles and squares are means of rice and naturally vegetated ponds, respectively. Dots are means of all ponds combined and upper and lower solid lines are maximum and minimum concentrations for the ponds.

ponds ( $\bar{x}=160 \mu\text{mhos/cm}$ ) had greater conductivity than did ponds with rice ( $\bar{x}=142 \mu\text{mhos/cm}$ ;  $P<0.05$ ) (Table 2). The conductivity was positively correlated with vegetative biomass ( $r=0.83$ ;  $P<0.1$ ).

The high conductivity in the fall probably resulted from release of ions (e.g.,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{PO}_4^{3-}$ ,  $\text{NH}_4^+$ , etc.) from decomposing vegetation. Conductivity was greatest in naturally vegetated ponds because they contained 29% more vegetative biomass than did rice ponds. The decrease in conductivity during the winter was probably due to a decrease in ions from reduced vegetative decomposition and from significant dilution of pond waters by large quantities of rainfall (Table 5). The conductivity increased from February to May from an increase in ions released as a result of vegetative decomposition caused by higher water temperatures.

Conductivity can be used as a simple and rapid estimate of total hardness (APHA et al. 1980). Conductivity of the pond waters was positively correlated with total hardness ( $r=0.58$ ;  $P<0.01$ ), and conductivity and total hardness exhibited similar temporal trends (Figures 7 and 8). If conductivity in freshwater crawfish ponds remains above  $125 \mu\text{mhos/cm}$ , then sufficient water hardness ( $>50 \text{ mg/liter as CaCO}_3$ ) is probably present for good crawfish production. However, the relationship between conductivity and total hardness should be empirically determined on a case by case basis before conductivity is used as an estimate of total hardness.

### Nitrogen

Nitrogen is present in water in inorganic forms such as ammonia, nitrate, and nitrite, and in organic forms such as amino acids or complex particulate organic matter (Alexander 1977). Most of the

nitrogen in ponds is present in organic matter (Boyd 1982) and nitrogen concentration in aquatic biota may comprise 1 to 10% of dry weight (Goldman and Horne 1983). Certain species of nitrogen such as ammonia and nitrates are essential nutrients to primary producers and microbial decomposers. However, these same forms of nitrogen, if present in sufficient quantities, may be toxic to aquatic animals.

Total ammonia (TA-N). The principal nitrogenous compound excreted by aquatic organisms is ammonia (Campbell 1973). Ammonia may attain concentrations that are deleterious to fish and it can reduce fish production (Boyd 1979). Total ammonia nitrogen is a measure of both un-ionized ( $\text{NH}_3$ ) and ionized ( $\text{NH}_4^+$ ) ammonia. The relative proportion of each species can be determined from the temperature and pH (Trussell 1972). As pH increases, the amount of  $\text{NH}_3$  increases and that of  $\text{NH}_4^+$  decreases. Un-ionized ammonia is toxic, but the ionized species is non-toxic (Robinette 1976).

The TA-N ranged between 0.01 and 0.16 mg/liter ( $\bar{x} \pm \text{SE} = 0.05 \pm 0.001$  mg/liter) from November through January with little variation among the ponds (Figure 9). TA-N increased from February through May with significant variation among the ponds. In early May, TA-N ranged from 0.02 to 0.92 mg/liter ( $\bar{x} \pm \text{SE} = 0.34 \pm 0.06$  mg/liter). No difference in the concentration of TA-N was found between canal water ( $\bar{x}=0.14$  mg/liter) and ponds planted with rice ( $\bar{x}=0.18$  mg/liter) or natural vegetation ( $\bar{x}=0.13$  mg/liter) (Table 2). No correlation was found between TA-N levels and vegetative biomass.

The TA-N concentrations in the five commercial crawfish ponds were generally lower than those measured in experimental crawfish ponds. Zeman (1982) reported TA-N levels between 0.0 and 4.0 mg/liter ( $\bar{x}=1.6$



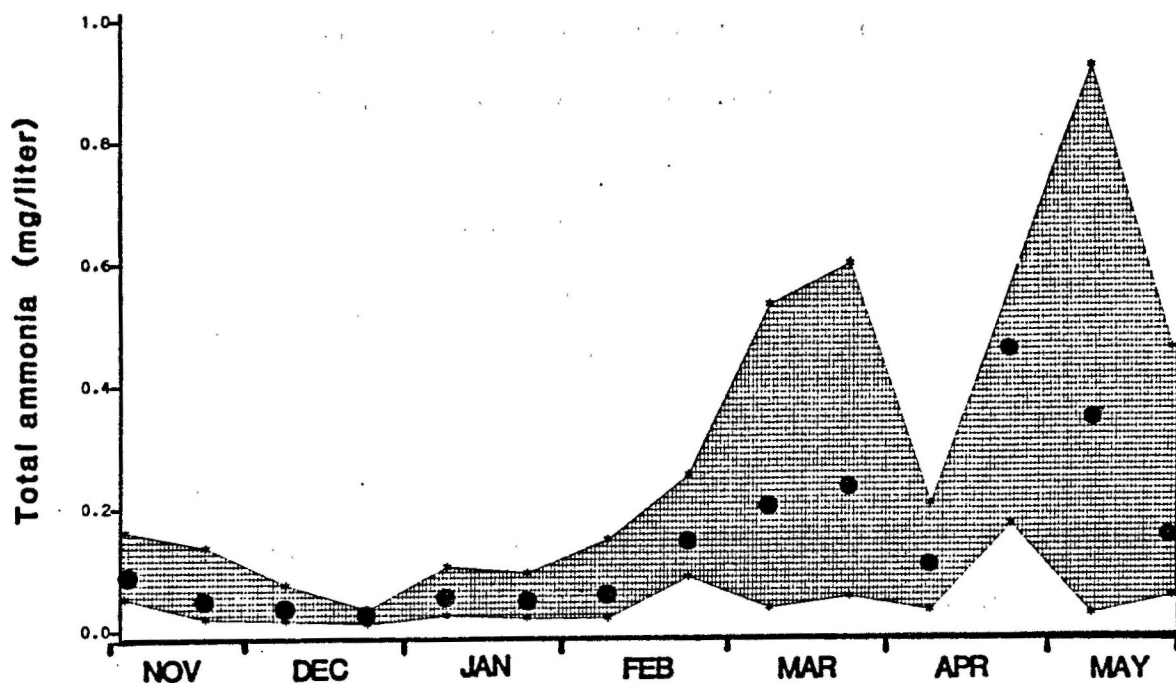


Figure 9. Temporal variation in total ammonia (TA-N) in five commercial crawfish ponds near Henderson, Louisiana. Dots are means and upper and lower solid lines are maximum and minimum concentrations in the ponds.

mg/liter) in crawfish ponds less than 0.4 hectare in surface area. The TA-N in the five crawfish ponds was lower than concentrations generally reported in catfish production ponds where the TA-N ranged from 0.02 to 7.34 mg/liter (Tucker and Boyd 1979; Brown and Boyd 1982).

The un-ionized ( $\text{NH}_3$ ) ammonia concentrations were probably not high enough to adversely affect the growth and health of crawfish in the five crawfish ponds. The maximum TA-N concentration was 0.9 mg/liter at pH 7.2 and 22°C. This concentration corresponds to 0.007 mg  $\text{NH}_3$ -N/liter. I concluded that  $\text{NH}_3$ -N levels below 0.06 mg/liter should pose no problem to crawfish provided the pH does not exceed 8.5 for an extended period (see page 89 of this thesis).

Nitrite. Nitrite ( $\text{NO}_2^-$ ) is an intermediate ion formed during the nitrification of ammonia. Nitrite accumulates when nitrification is inhibited by nitrous acid ( $\text{HNO}_2$ ) and un-ionized ammonia (Anthonisen et al. 1976). Most problems with nitrite toxicity to aquatic animals occur in closed recirculating systems or laboratory holding facilities (Spotte 1979), although nitrite accumulation can occur in the anaerobic sediments of earthen ponds from the denitrification of nitrate ( $\text{NO}_3^-$ ) (Boyd and Hollerman 1980). Nitrite can attain levels toxic to fish or stress fish to such degree that decreased growth and predisposition to disease occurs. Concentrations of nitrite are seldom appreciable in fish ponds except when dissolved oxygen is low (Boyd 1979).

Temporal trends in nitrite from November through May were similar to total ammonia. Nitrite concentration in the ponds ranged between 0.0 and 0.06 mg  $\text{NO}_2$ -N/liter ( $\bar{x} \pm \text{SE} = 0.03 \pm 0.001$  mg/liter) from November through January (Figure 10). Considerable variation in nitrite levels (range: 0.0 to 0.20 mg/liter) occurred from February through May as

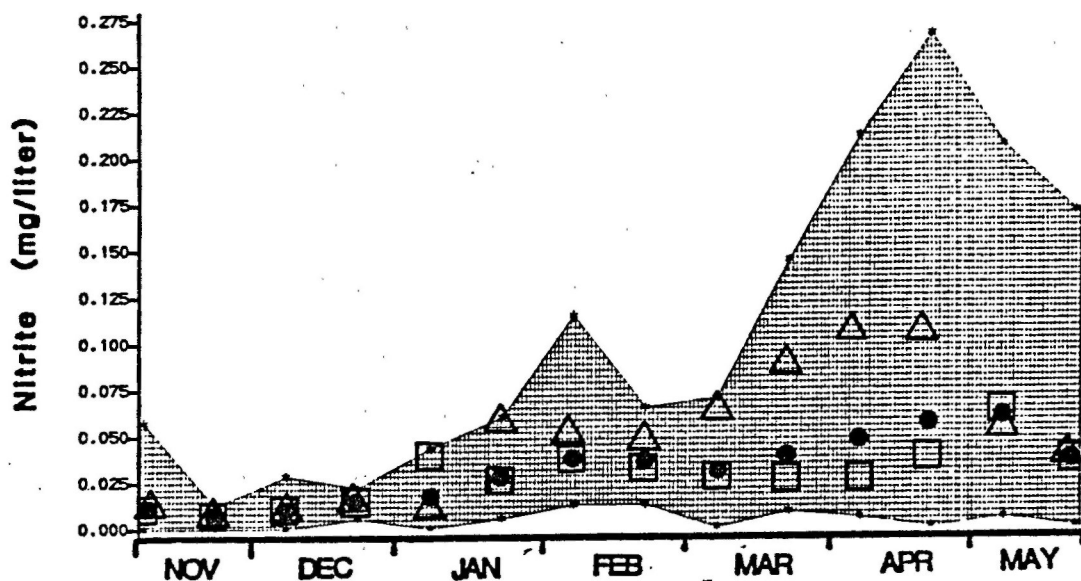


Figure 10. Temporal variation in nitrite ( $\text{NO}_2\text{-N}$ ) in five commercial crawfish ponds near Henderson, Louisiana. Triangles and squares are means of rice and naturally vegetated ponds, respectively. Dots are means of all ponds combined and upper and lower solid lines are maximum and minimum concentrations for the ponds.

nitrite concentrations increased. By May, nitrite averaged  $0.06 \pm 0.01$  mg/liter. Crawfish ponds with rice contained significantly more nitrite ( $\bar{x}=0.05$  mg/liter) than either naturally vegetated ponds ( $\bar{x}=0.01$  mg/liter) or the canals ( $\bar{x}=0.03$  mg/liter) (Table 2). Nitrite was negatively correlated with vegetation biomass ( $r=-0.89$ ;  $P<0.1$ ).

The nitrite concentrations found in the commercial crawfish ponds were similar to those reported in commercial catfish ponds (range: 0.0 to 0.03 mg/liter) with about 3,000 kg of catfish per hectare (Boyd et al. 1979). However, Brown and Boyd (1982) reported maximum nitrite of 3.5 mg/liter in commercial catfish ponds with up to 8,000 kg catfish per hectare. Zeman (1982) did not detect the presence of nitrite concentrations in experimental crawfish ponds.

The maximum nitrite concentration (0.20 mg/liter) in the five crawfish ponds was probably not high enough to adversely affect the crawfish. I found that nitrite concentrations of 1.0 mg/liter or less should have no negative effect on P. clarkii production (see page 93 of this thesis).

Nitrate. Nitrate ( $\text{NO}_3^-$ ) is produced from the nitrification of ammonia by chemoautotrophic bacteria (Alexander 1977). Nitrate is the most highly oxidized and usually the most abundant form of combined inorganic nitrogen in natural waters (Goldman and Horne 1983). Nitrate has little or no toxicity to aquatic organisms (Colt and Armstrong 1979).

Nitrate levels followed no consistent temporal pattern though considerable variation in concentrations occurred among the ponds (Figure 11). Nitrate ranged between 0.0 and 0.30 mg  $\text{NO}_3\text{-N}$ /liter ( $\bar{x} \pm \text{SE} = 0.10 \pm 0.02$ ) from November through May (Figure 11). No difference was

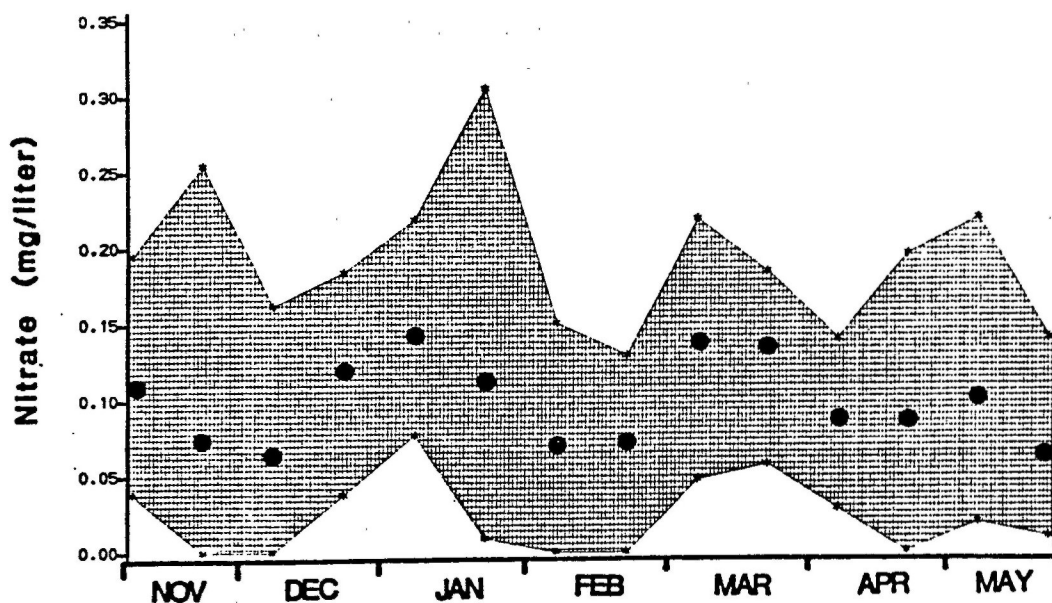


Figure 11. Temporal variation in nitrate ( $\text{NO}_3\text{-N}$ ) in five commercial crawfish ponds near Henderson, Louisiana. Dots are means and upper and lower solid lines are maximum and minimum concentrations in the ponds.

found between canal water and ponds with either rice or natural vegetation (Table 2). Furthermore, there was no correlation between nitrate concentration and vegetative biomass.

The nitrate concentrations in the crawfish ponds (range: 0.0 to 0.30 mg/liter) were similar in magnitude to those reported in fertilized fish ponds (range: 0.02 to 0.10 mg /liter) (Boyd 1976) and also in channel catfish ponds (range: 0.05 to 0.25 mg/liter) (Boyd 1974; Boyd et al. 1979).

The nitrate concentrations in the crawfish ponds were considerably below levels found to adversely affect aquatic animals. Nitrate levels of up to 400 mg/liter had no effect on the growth of channel catfish (Knepp and Arkin 1973) or juvenile penaeid shrimp (Wickins 1976).

Total nitrogen. The total nitrogen is a measure of both organic and inorganic nitrogen. Total nitrogen in the ponds ranged from 0.8 to 7.9 mg/liter ( $\bar{x} \pm SE = 3.7 \pm 0.6$  mg/liter) in November, and it increased to maximum concentrations in January with a range between 0.94 and 16.2 mg/liter ( $\bar{x} \pm SE = 11.9 \pm 0.5$  mg/liter) (Figure 12). Total nitrogen decreased four-fold by February with levels from 0.8 to 2.5 mg/liter ( $\bar{x} \pm SE = 2.9 \pm 0.4$  mg/liter) and it remained relatively constant through May.

Total nitrogen was significantly higher in the rice ponds ( $\bar{x}=5.4$  mg/liter) than in either the canal water ( $\bar{x}=4.4$  mg/liter) or the naturally vegetated ponds ( $\bar{x}=3.8$ ) ( $P<0.05$ ; Table 2). Total nitrogen and vegetative biomass were negatively correlated ( $r=-0.93$ ;  $P<0.1$ ).

The total nitrogen concentrations in the crawfish ponds were generally higher than the 0.9 mg/liter level reported by Chien (1980) in experimental crawfish ponds but similar in magnitude to those levels in

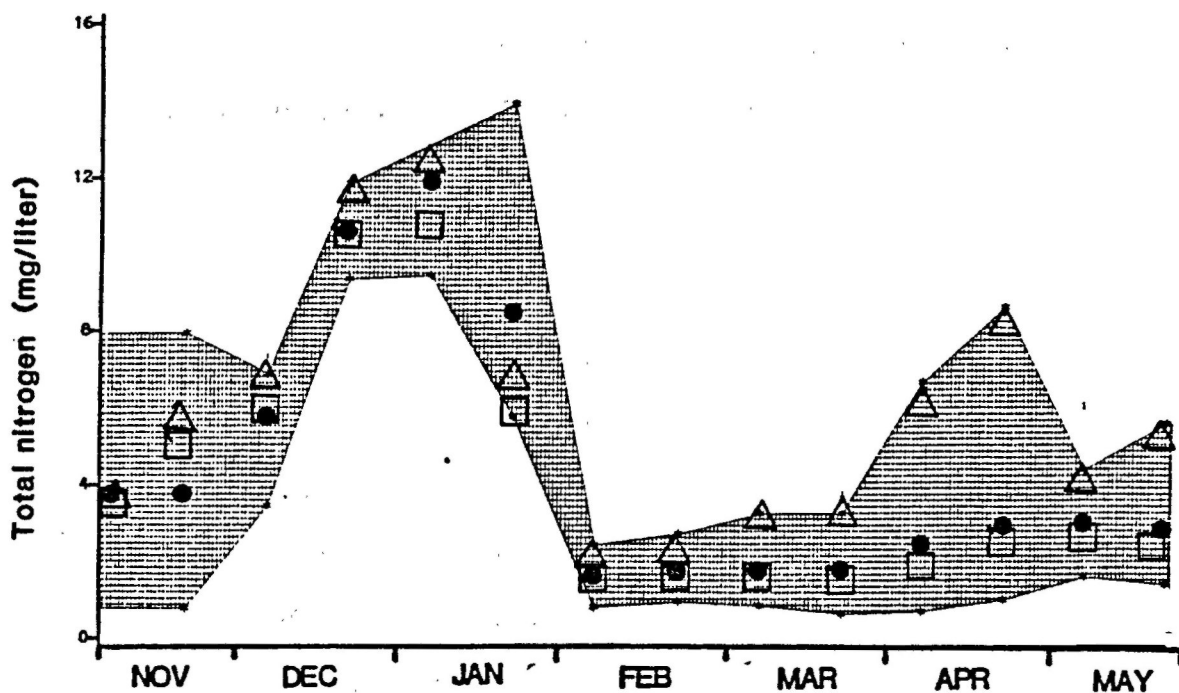


Figure 12. Temporal variation in total nitrogen in five commercial crawfish ponds near Henderson, Louisiana. Triangles and squares are means of rice and naturally vegetated ponds, respectively. Dots are means of all ponds combined and upper and lower solid lines are maximum and minimum concentrations for the ponds.

fertilized fish ponds in which total nitrogen concentrations of 3.1 mg/liter were measured (Hepher 1962). The large amount of total nitrogen during December and January ( $\bar{x}$ =10.3 mg/liter) was comparable to concentrations reported in sewage effluent (APHA et al. 1980) and the increase was probably related in some unexplained fashion to the decrease in biological activity and to large quantities of rainfall during this period.

### Phosphorus

Phosphorus is an important nutrient that regulates the productivity of natural waters (Boyd 1979, 1982). The principal sources of phosphorus in aquatic ecosystems include the watershed, inundated soil and vegetation, atmospheric precipitation, and that released in the decomposition of plants and animals (Boyd 1979). In ponds in which fish are fed prepared feeds, large amounts of phosphorus are released from uneaten feeds and in fish excreta. Phosphorus may be classified as orthophosphates (soluble inorganic phosphorus), soluble organic phosphorus, and particulate phosphorus (Boyd 1982).

Soluble inorganic phosphorus (SIP). Soluble inorganic phosphorus (SIP) or filtrable orthophosphate measures a fraction of the total phosphorus which can be taken up directly by bacteria, phytoplankton, and macrophytes. The SIP consists of  $\text{H}_2\text{PO}_4^-$ ,  $\text{HPO}_4^{2-}$ , and  $\text{PO}_4^{3-}$  and the concentrations of the three species of SIP are each dependent on pH.

The SIP exhibited greatest variation in November and December when concentrations ranged from 25 to 46  $\mu\text{g P/liter}$  ( $\bar{x} \pm \text{SE} = 32.0 \pm 1.0$   $\mu\text{g/liter}$ ) (Figure 13). The SIP decreased moderately through January



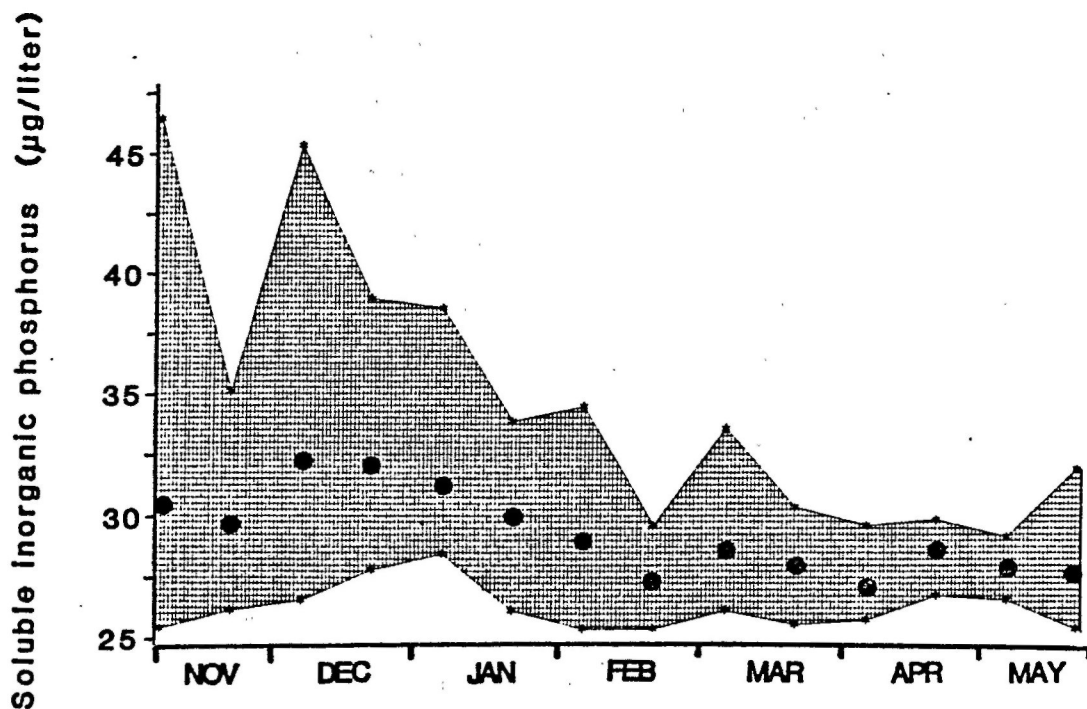


Figure 13. Temporal variation in soluble inorganic phosphorus (SIP) in five commercial crawfish ponds near Henderson, Louisiana. Dots are means and upper and lower solid lines are maximum and minimum concentrations in the ponds.

after which concentrations stabilized and ranged from 25 to 33  $\mu\text{g/liter}$  ( $\bar{x} \pm \text{SE} = 31.0 \pm 0.8 \mu\text{g/liter}$ ) through May. The SIP concentration in canal water ( $\bar{x}=29 \mu\text{g/liter}$ ) and naturally vegetated ponds ( $\bar{x}=29 \mu\text{g/liter}$ ) was significantly higher than that in the rice ponds ( $\bar{x}=28 \mu\text{g/liter}$ ) ( $P<0.05$ ; Table 2); however, the difference ( $1 \mu\text{g/liter}$ ) was negligible. The SIP significantly increased with an increase in vegetative biomass ( $r=0.90$ ;  $P<0.10$ )

Highest SIP concentrations were present 2 to 6 weeks after flooding the ponds and were associated principally with the decomposition and subsequent release of phosphorus from vegetation. The SIP declined in winter and spring from absorption of SIP by the sediments and SIP uptake by bacteria, phytoplankton, and aquatic macrophytes. Additional SIP losses could have resulted from dilution of pond waters by rainfall in December and January.

Soluble inorganic phosphorus concentrations seldom exceed 100  $\mu\text{g P/liter}$  even in highly eutrophic waters (Boyd 1979). The SIP levels found in the crawfish ponds (25 to 46  $\mu\text{g/liter}$ ) were greater than those reported from fertilized fish ponds ( $\bar{x}=20 \mu\text{g/liter}$ ) (Boyd 1976) and channel catfish production ponds ( $<10 \mu\text{g/liter}$ ) (Boyd 1974).

Total Phosphorus. The total phosphorus includes organic and inorganic phosphorus, both soluble and insoluble. The SIP concentration is generally less than 10% of the total phosphorus (Boyd 1982). For practical purposes, the difference between the concentration of total phosphorus and SIP may be used as an index of the phosphorus contained in plankton and detritus.

While the SIP declined slightly from November through May, the total phosphorus exhibited no consistent seasonal trend (Figure 14).

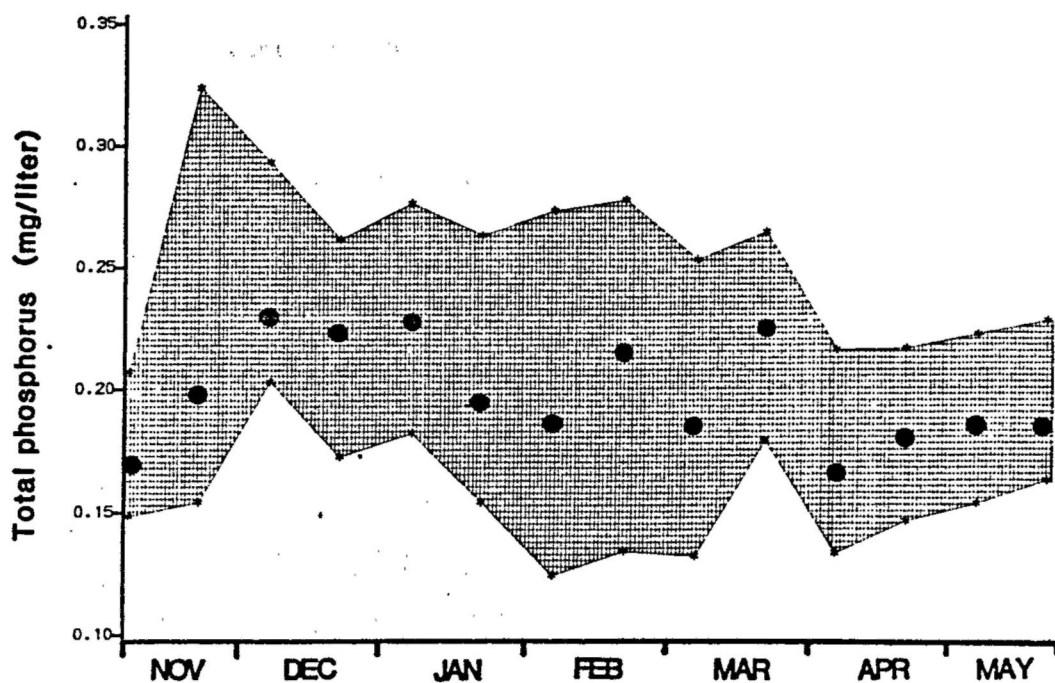


Figure 14. Temporal variation in total phosphorus in five commercial crawfish ponds near Henderson, Louisiana. Dots are means and upper and lower solid lines are maximum and minimum concentrations in the ponds.

Total phosphorus concentrations ranged from 0.12 to 0.32 mg/liter ( $\bar{x} \pm SE = 0.20 \pm 0.01$  mg/liter). The total phosphorus was significantly higher in the crawfish ponds ( $\bar{x}=0.20$  mg/liter) than in the canals ( $\bar{x}=0.18$  mg/liter) ( $P<0.05$ ; Table 2), but from a practical standpoint the 0.20 mg/liter difference was negligible. Total phosphorus concentration was not correlated with vegetative biomass.

The total phosphorus concentrations in the crawfish ponds (range: 0.12 to 0.32 mg/liter) were nearly 10 times greater than the SIP levels. Total phosphorus seldom exceeds 1 mg/liter in natural waters (Boyd 1979). The total phosphorus concentrations in the crawfish ponds were similar to those found in experimental crawfish ponds ( $\bar{x} = 0.1$  mg/liter) (Chien 1980) and also similar to those reported in fertilized fish ponds (0.15 to 0.22 mg/liter) (Boyd 1976) and in channel catfish production ponds (0.10 to 0.30 mg/liter) (Boyd 1974).

### Oxygen Demand

Chemical Oxygen Demand (COD). The oxygen required to chemically oxidize (at high temperature and highly acidic conditions) all organic matter in a water sample to carbon dioxide and water is the chemical oxygen demand (COD). The COD is often used as an index for organic matter concentration (e.g., plankton) in waters and it is also used to estimate rates of oxygen consumption by planktonic communities (Boyd 1973). The COD of water increases with increasing organic matter.

The COD in the five ponds increased from November through May. The COD varied significantly among the ponds, particularly in winter and spring (Figure 15). The COD in November ranged from 15 to 40 mg/liter ( $\bar{x} \pm SE = 25 \pm 2$  mg/liter). The COD increased significantly by

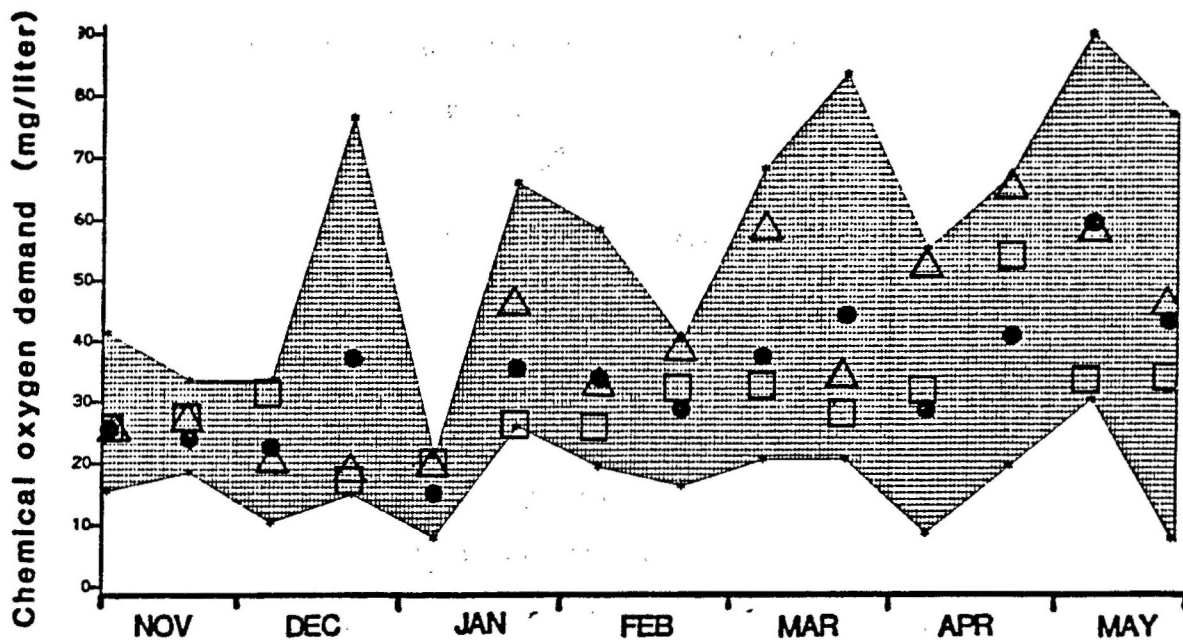


Figure 15. Temporal variation in chemical oxygen demand (COD) in five commercial crawfish ponds near Henderson, Louisiana. Triangles and squares are means of rice and naturally vegetated ponds, respectively. Dots are means of all ponds combined and upper and lower solid lines are maximum and minimum concentrations for the ponds.

mid-December when it ranged from 15 to 80 mg/liter ( $\bar{x} \pm \text{SE} = 50 \pm 6$  mg/liter). By May, the COD ranged between 25 and 85 mg/liter ( $\bar{x} \pm 1 \text{ SE} = 46 \pm 3$  mg/liter). The increase in COD resulted from an increase in organic matter from vegetative decomposition and increased phytoplankton abundance (see Chlorophyll a).

The COD in the ponds with rice ( $\bar{x} = 41$  mg/liter) was 25% higher than in naturally vegetated ponds ( $\bar{x}=31$  mg/liter) and both were significantly higher than the COD of canal water ( $\bar{x}=27$  mg/liter) ( $P<0.05$ ; Table 2). The COD decreased with an increase in vegetative biomass ( $r = -0.97$ ;  $P<0.1$ ), which appears to contradict the expected relationship. However, the principal source of COD in the ponds was plankton and not particulate detritus from decomposed vegetation. I determined from a relationship between phytoplankton abundance (as measured by chlorophyll a) and COD (Boyd 1979) that phytoplankton comprised more than 50% of the COD in the ponds from November to May. The COD decreased with increased vegetative biomass because the vascular macrophytes reduced phytoplankton production by reducing incident solar radiation (i.e., shading the ponds) and macrophytes competed with phytoplankton for nutrients. The COD was highest in the rice ponds because they had slightly more phytoplankton than naturally vegetated ponds.

The COD concentrations and temporal trends in the commercial crawfish ponds were comparable to those reported from experimental crawfish ponds (Day 1983), but 20-40 mg/liter less than COD concentrations measured in commercial catfish production ponds where fish were fed a maximum of 100 kg/hectare/day of pelleted rations (Boyd et al. 1979).

Biochemical oxygen demand. The biochemical oxygen demand (BOD) measures the amount of oxygen required by microorganisms to oxidize the organic matter in water. The standard BOD analysis is conducted for 5 days at 20°C. The BOD of pond waters results principally from the respiration of plankton and bacteria, and the magnitude of BOD depends principally on temperature, plankton density and concentration of organic matter (Boyd 1979). The BOD of pond waters may differ greatly, but in most ponds it is probably the most important oxygen sink (Boyd 1979).

The BOD is always less than the COD because microorganisms are unable to completely decompose all organic matter. Boyd (1973) demonstrated that BOD of pond waters was positively correlated with COD and the correlation was observed in this study (Figures 15 and 16). An increase in BOD paralleled an increase in COD. The BOD ranged from 0.1 to 7.5 mg O<sub>2</sub>/liter ( $\bar{x} \pm SE = 2.5 \pm 0.4$  mg/liter) in November and varied little through early February (range: 0.6–3.7 mg/liter) because colder water temperatures (5 to 15°C) limited biological activity. As water temperature increased (>15°C) in late February, the BOD also increased and by mid-April the BOD ranged from 3.8 to 8.0 mg O<sub>2</sub>/liter ( $\bar{x} \pm SE = 6.0 \pm 0.3$  mg/liter). No significant differences were found among the BOD's of canal water ( $\bar{x}=2.95$  mg/liter), ponds with rice ( $\bar{x}=3.39$  mg/liter), or natural vegetation ( $\bar{x}=3.05$  mg/liter) (Table 2). The BOD of pond waters and vegetative biomass were not significantly correlated.

### Turbidity

Turbidity is caused by the presence of suspended matter such as clay, silt, finely divided organic and inorganic matter, plankton and

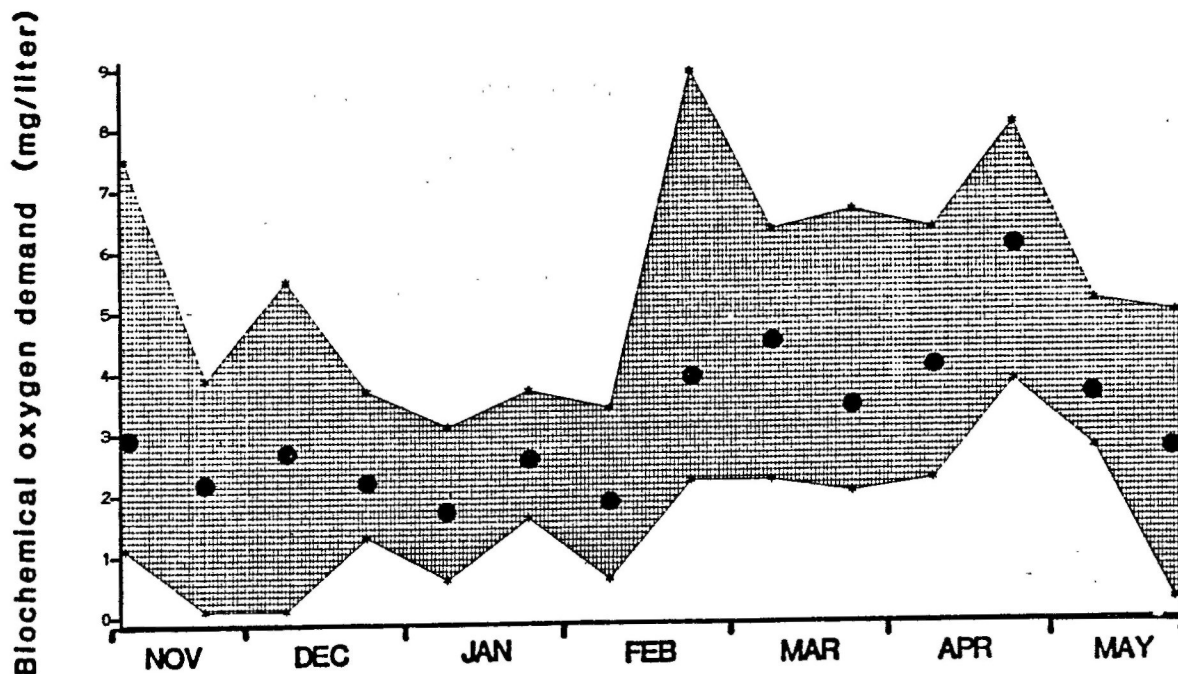


Figure 16. Temporal variation in biochemical oxygen demand (BOD) in five commercial crawfish ponds near Henderson, Louisiana. Dots are means and upper and lower solid lines are maximum and minimum concentrations in the ponds.



other microorganisms (Boyd 1982). Excess turbidity reduces light penetration and thereby reduces photosynthesis by primary producers (Boyd 1979).

The turbidity in the ponds gradually increased from November through May (Figure 17). The mean turbidity ranged from  $3.0 \pm 0.4$  NTU in November to  $44.0 \pm 7.0$  NTU in January. The turbidity increased substantially in February and by May the mean turbidity was  $190 \pm 30$  NTU. Rice ponds ( $\bar{x}=188$  NTU) had significantly greater turbidity than both canal water ( $\bar{x}=87$  NTU) and naturally vegetated ponds ( $\bar{x}=37$  NTU) ( $P<0.05$ ; Table 3). Turbidity decreased with an increase in vegetative biomass ( $r = -0.96$ ;  $P<0.1$ ).

The high variation in turbidity among the ponds was due to several factors. The high turbidity in rice ponds occurred subsequent to rice depletion by February. Water circulation from wind action and increased crawfish activity from increased water temperatures suspended pond sediments and resulted in high turbidity. The suspension of sediments made nutrients such as phosphorus available for increased phytoplankton production and the plankton further increased turbidity.

The ponds with volunteer vegetation were less turbid ( $\bar{x}=37$  NTU) because these ponds retained substantial quantities of aquatic and semi-aquatic macrophytes throughout the year. The macrophytes limited turbidity by reducing light penetration which limited phytoplankton production, and also by retarding wind and water circulation which tend not to suspend bottom sediments. In addition, humic acids released from decomposed vegetation tend to precipitate colloidal turbidity (Boyd 1979).

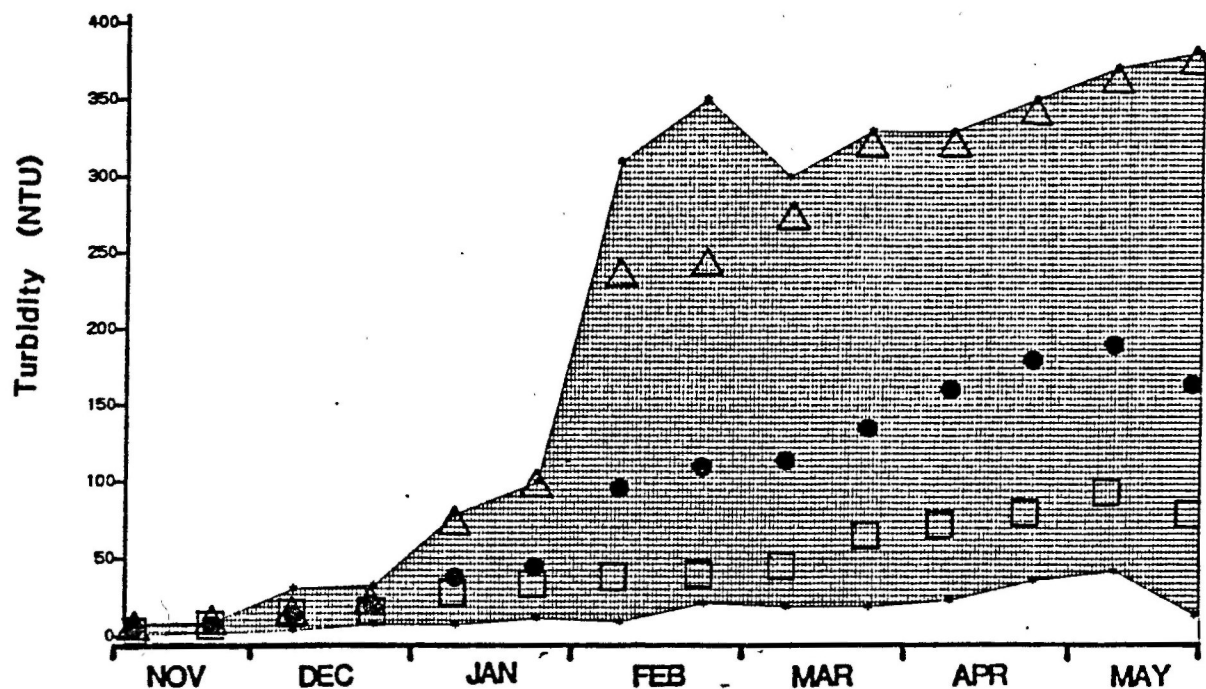


Figure 17. Temporal variation in turbidity in five commercial crawfish ponds near Henderson, Louisiana. Triangles and squares are means of rice and naturally vegetated ponds, respectively. Dots are means of all ponds combined and upper and lower solid lines are maximum and minimum concentrations for the ponds.

### Chlorophyll a

Chlorophyll a provides an estimate of phytoplankton abundance (Boyd 1979). Chlorophyll a increased from November ( $\bar{x} \pm SE = 10.0 \pm 0.0$   $\mu\text{g/liter}$ ) through early March ( $\bar{x} \pm SE = 31.4 \pm 5.1$   $\mu\text{g/liter}$ ) and ranged from 0 to 82  $\mu\text{g/liter}$  (Figure 18). Chlorophyll a decreased slightly from mid-March through May though large fluctuations in concentration were apparent (range: 50 to 90  $\mu\text{g/liter}$ ) . Chlorophyll a was 34% greater in canal water ( $\bar{x}=26$   $\mu\text{g/liter}$ ) than in pond waters ( $\bar{x}=17.0$   $\mu\text{g/liter}$ ), but there was no difference between the chlorophyll a concentration in rice ponds ( $\bar{x}=19$   $\mu\text{g/liter}$ ) and naturally vegetated ponds ( $\bar{x}=15$   $\mu\text{g/liter}$ ) (Table 2). Chlorophyll a was not statistically correlated with vegetative biomass.

Phytoplankton abundance is regulated by many factors, including pH, water temperature, light intensity and nutrient concentrations. Grazing by zooplankton, diseases, parasites, and release of algal toxins by other organisms also tend to limit phytoplankton populations (Boyd 1979). Phytoplankton production in crawfish ponds is limited by several factors, which include a reduction in incident solar radiation by standing macrophytes (Miltner 1980), removal of phosphorus and other essential nutrients by aquatic macrophytes (Boyd 1979), and the immobilization of ammonia and nitrates by bacteria during the decomposition of vascular macrophytes (Tusneem and Patrick 1971).

The low phytoplankton concentrations in the crawfish ponds during the fall reflected the predominance of a detritus-based ecosystem. However, phytoplankton became more predominant in winter and spring with a reduction in vascular macrophytes. The chlorophyll a levels found in the five crawfish ponds (range: 0 to 90  $\mu\text{g/liter}$ ) were similar to

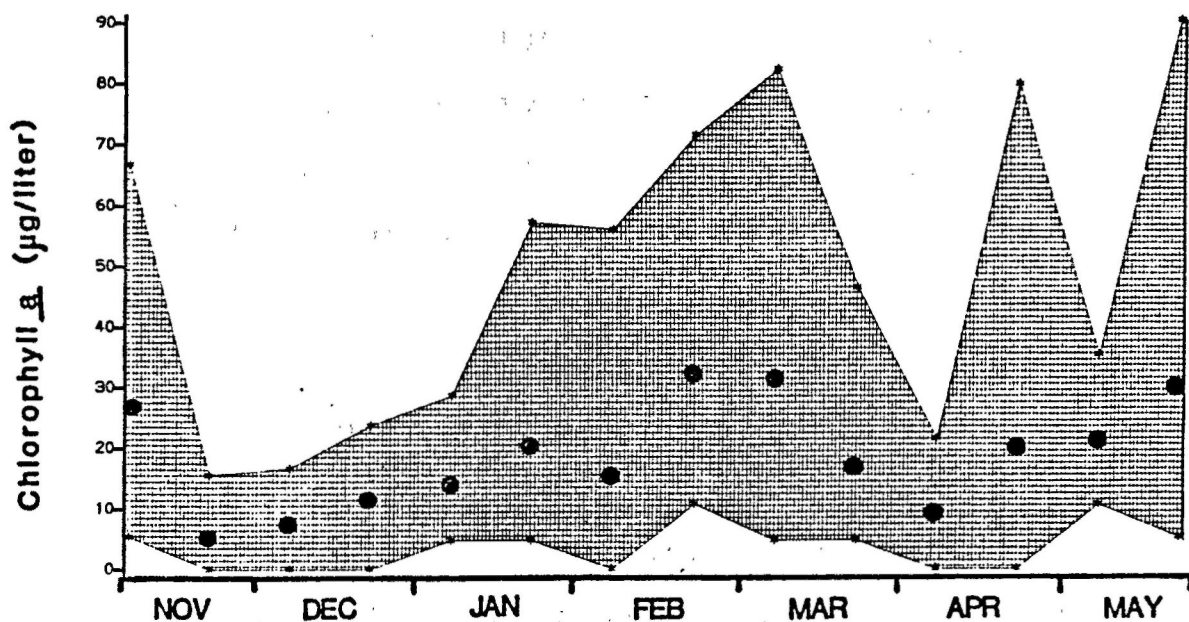


Figure 18. Temporal variation in chlorophyll *a* in five commercial crawfish ponds near Henderson, Louisiana. Dots are means and upper and lower solid lines are maximum and minimum concentrations in the ponds.

concentrations reported in unfertilized fish ponds (range: 9 to 116  $\mu\text{g/liter}$ ) (Hepher 1962) but generally lower than those reported in fertilized fish ponds (range: 20-212  $\mu\text{g/liter}$ ) (Hepher 1962; Boyd 1973). Ponds to which commercial pelleted rations are added have abundant phytoplankton because about 75% of the nutrients in the feed are excreted into the water (Boyd 1973). For example, chlorophyll a concentrations ranged from 20 to 250  $\mu\text{g/liter}$  in catfish ponds that received feed applications up to 100 kg of feed per hectare per day (Tucker and Boyd 1979; Brown and Boyd 1982).

#### Crawfish catch per unit effort

The average yield of crawfish from commercial crawfish ponds is about 600 kg per hectare per year, but it may vary from 50 to 4,000 kg per hectare, depending upon pond type and the level and intensity of management (Huner and Barr 1984). The frequency and intensity of crawfish harvest is influenced by weather, labor availability and the wholesale price of crawfish. The average crawfish catch should be 0.4 to 0.6 kg per trap per day during a 100- to 150-day harvesting season (Roberts 1983) for crawfish farming to be profitable. Typically, from 25 to 100 traps are set per hectare, and the catch is removed after 24 hours (Pfister and Romaine 1983).

The mean crawfish yield from the five commercial ponds was 787 kg per hectare which was 31% higher than the state-wide mean yield of 600 kg per hectare (Louisiana Cooperative Extension Service 1983). The yield ranged from 396 to 1,204 kg per hectare (Table 6) and the mean yield was 707 and 840 kg per hectare in rice ponds and naturally vegetated ponds, respectively. The number of harvest days ranged from

Table 6. Crawfish yield in five commerical crawfish ponds near Henderson, Louisiana.

Pond	Vegetative type	Number of harvest days	Yield per pond (kg)	Yield per hectare (kg)
R-1	Rice	115	18,586	882
R-2	Rice	92	12,259	531
B-1	Natural vegetation	105	17,603	1,204
A-1	Natural vegetation	74	7,476	396
A-2	Natural vegetation	99	18,649	919

74 to 115 days. The crawfish catch increased in the ponds from November through April and was followed by a sharp decline in May (Figure 19). The crawfish CPUE in November ranged from 0.01 to 0.23 kg/trap/day ( $\bar{x}=0.06$  kg/trap/day) and by April it was between 0.01 and 1.20 kg/trap/day ( $\bar{x}=0.35$  kg/trap/day). The CPUE in May ranged from 0.0 to 1.03 kg/trap/day ( $\bar{x}=0.21$  kg/trap/day). No difference in crawfish CPUE was found between rice ( $\bar{x}=0.19$  kg/trap/day) and naturally vegetated ponds ( $\bar{x}=0.22$  kg/trap/day).

Crawfish catch is reported to be influenced by water temperature and dissolved oxygen (Johnson 1983), underwater luminescence and lunar phase (Morrissey and Capilli 1981), and crawfish population structure (Momot and Romaine 1981). The crawfish CPUE increased with water temperature, total ammonia, COD, BOD, and turbidity ( $P<0.05$ ; Table 7). Conversely, the CPUE decreased with an increase in the DO, SIP, total alkalinity, total phosphorus, and total nitrogen ( $P<0.05$ ; Table 7). The CPUE was not correlated with pH, free  $\text{CO}_2$ ,  $\text{NO}_2^-$ ,  $\text{NO}_3^-$ , total hardness, conductivity, and chlorophyll a (Table 7). The relatively low correlations ( $r < \pm 0.50$ ) between specific water quality variables and crawfish catch may reflect a complex interaction between physical, chemical, and biological processes and CPUE. Any attempt to predict CPUE from changes in a water quality variable would be inaccurate.

The low crawfish CPUE from November through January indicates a relatively small population of broodstock and juvenile crawfish in the ponds that survived in burrows the preceeding summer (Avault et al. 1975). The CPUE increased from February through April because young-of-the-year crawfish had grown large enough to become susceptible to trapping (Huner and Barr 1984). The CPUE declined in May probably

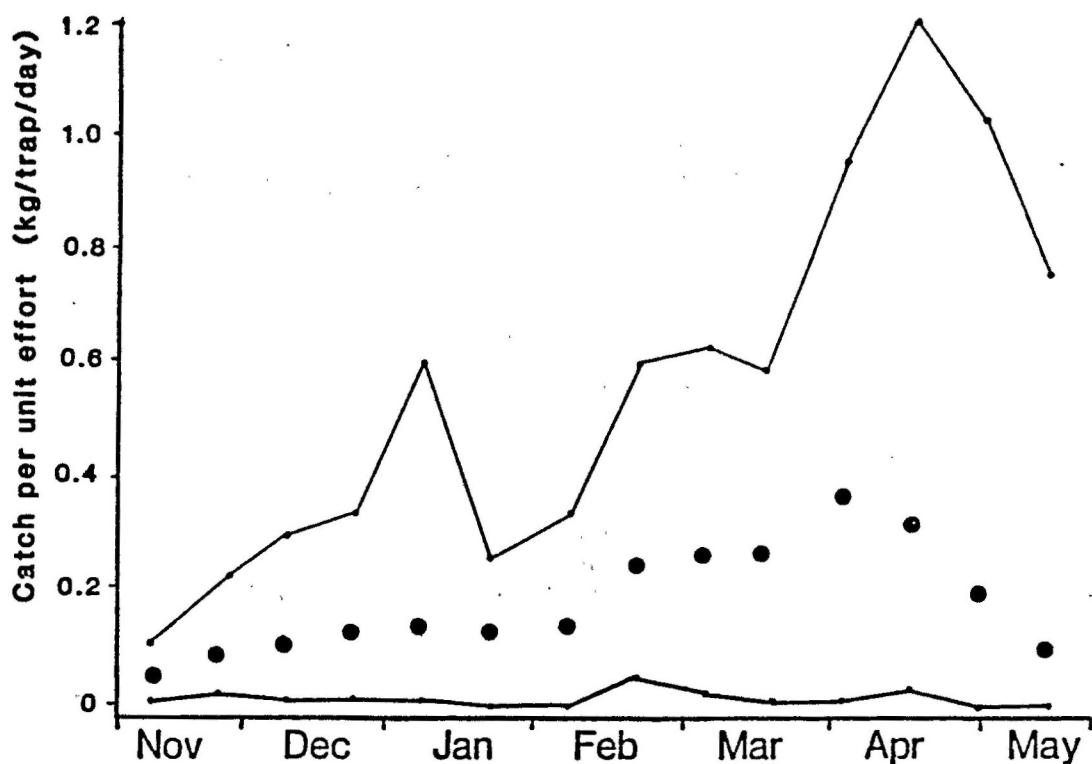


Figure 19. Temporal variation in catch per unit effort in five commercial crawfish ponds near Henderson, Louisiana. Solid dots are means and upper and lower solid lines are maximum and minimum concentrations in the ponds.



Table 7. Simple linear correlations of crawfish catch per unit effort (kg/trap/day) with water quality variables (N=107 to 116).

Water quality variable	Level of significance <sup>a</sup>	Correlation coefficient (r)
Temperature	**	0.26
DO	**	-0.34
pH	NS	0.13
Free CO <sub>2</sub>	NS	-0.11
Total alkalinity	**	-0.29
Total hardness	NS	0.07
Conductivity	NS	0.02
Total ammonia	**	0.35
Nitrite	NS	0.16
Nitrate	NS	0.16
Total nitrogen	**	-0.32
SIP	**	-0.24
Total phosphorus	*	0.20
COD	**	0.25
BOD	**	0.48
Turbidity	*	0.22
Chlorophyll <u>a</u>	NS	0.16

<sup>a</sup> \* = significant (P<0.05), \*\* = highly significant (P<0.01), NS = not significant (P>0.05).

from a decrease in crawfish number from continuous harvesting or many crawfish might have become stunted at unharvestable sizes. The increased crawfish CPUE with increased water temperature was a function of increased crawfish growth and activity stimulated by warmer temperatures (Johnson 1983; Huner and Barr 1984). Furthermore, warmer water temperatures and increased pond biota (e.g., crawfish, phytoplankton, bacteria, etc.) were responsible for increased concentrations of ammonia, turbidity, COD, and BOD. Therefore, it was not unexpected that CPUE would be positively correlated with these variables. The negative correlation of DO, SIP, total alkalinity, total phosphorus, and total nitrogen with crawfish CPUE appeared to be related to normal seasonal trends in these water quality variables and not to the direct effect of these variables on CPUE.

### Conclusions

1. Water quality was poorest in the crawfish ponds in November, 2-6 weeks after ponds were flooded, and in April and May when water temperatures exceeded 18°C. The most serious problem was low dissolved oxygen (< 2 mg/liter). Potential problems with high free CO<sub>2</sub> concentrations may occur within the first month after flooding when dissolved oxygen levels are generally low. Un-ionized ammonia (NH<sub>3</sub>) and nitrite (NO<sub>2</sub><sup>-</sup>) did not attain concentrations known to be toxic to P. clarkii. Total alkalinity, total hardness, and pH were maintained within levels known to be conducive to good crawfish production.

2. Water quality was poorest in naturally vegetated ponds because they had more vegetative biomass and persistence of emergent vegetation which reduced water circulation.
3. Crawfish catch was correlated with several water quality variables but, with the exception of water temperature, no single variable or group of variables appeared to directly affect crawfish catch. Crawfish catch may be affected by a complex interaction of water quality with other biological factors such as crawfish reproduction and population structure.
4. Crawfish ponds are very productive ecosystems, comparable to fertilized fish ponds and channel catfish production ponds, based on seasonal concentrations of phosphorus, nitrogen, chlorophyll a, chemical oxygen demand, and biochemical oxygen demand.

# ACUTE TOXICITY OF pH, AMMONIA, AND NITRITE

## Objectives

The objective of this study was to determine the median lethal toxicity (LC50 after 24, 48, 72, and 96 hours) of pH, un-ionized ammonia, and nitrite to juvenile red swamp crawfish, Procambarus clarkii.

## Materials and Methods

The acute toxicity of hydroxyl ( $\text{OH}^-$ ) and hydrogen ( $\text{H}^+$ ) ions, un-ionized ammonia ( $\text{NH}_3\text{-N}$ ), and nitrite ( $\text{NO}_2\text{-N}$ ) to juvenile P. clarki was determined from tests conducted according to standard procedures outlined in Methods for Acute Toxicity Tests with Fish, Macroinvertebrates, and Amphibians (USEPA 1975) and Standard Methods for the Examination of Water and Wastewater (APHA et al. 1980). All tests were conducted in a water chemistry laboratory at LSU.

Immature P. clarkii (25- to 35-mm total length) were collected from experimental crawfish ponds at Ben Hur Biological Research Area, LSU, Baton Rouge, Louisiana. The crawfish were acclimated for at least 48 hours in 150-liter fiberglass tanks filled with dechlorinated tap water. The water hardness was adjusted to 100 mg/liter ( $\text{CaCO}_3$  equivalent) with calcium chloride (de la Bretonne et al. 1969). Water temperature was maintained at 22 to 24°C during the holding period and the DO was sustained at 90-100% oxygen saturation by bubbling compressed air in each tank. The crawfish were not fed and mortality was less than 2% during the 48-hour pre-test period.

Two tests were conducted with each of the toxicants to estimate their acute toxicity to the crawfish. First, 24-hour range-finding tests were conducted to define the range of toxicant dilutions to be used in the definitive tests. Second, 24-, 48-, 72-, and 96-hour definitive tests were conducted to estimate the acute lethal toxicity of  $\text{OH}^-$ ,  $\text{H}^+$ ,  $\text{NH}_3\text{-N}$ , and  $\text{NO}_2\text{-N}$  to P. clarkii. The toxicant concentrations were prepared from dilutions of the following reagent-grade compounds: ammonia from ammonium chloride ( $\text{NH}_4\text{Cl}$ ), nitrite from sodium nitrite ( $\text{NaNO}_2$ ), hydrogen ion from sulfuric acid ( $\text{H}_2\text{SO}_4$ ), and hydroxyl ion from sodium hydroxide ( $\text{NaOH}$ ). The  $\text{Cl}^-$ ,  $\text{Na}^+$ , and  $\text{SO}_4^{2-}$  ions liberated in the preparation of the toxicants are relatively inert (Spotte 1979) and did not occur at concentrations considered toxic to freshwater crustaceans.

The toxicity tests were conducted in 10-liter circular plastic tanks fitted with disposable polyethylene liners. To prevent cannibalism, each crawfish was contained in a perforated (2-mm diameter holes) polyethylene bag (3-cm W x 8-cm L) which was suspended in the test solution. The perforated bags allowed for an exchange of toxicant between the bag and test solution. The tanks were washed with detergent and rinsed with 5% nitric acid, acetone and deionized water prior to use. Baton Rouge city water from subsurface artesian wells was used as dilution water in the tests. The chemical composition of the dilution water used in the toxicity tests appears in Table 16 (Appendix). The dilution water was passed through an activated charcoal filter to remove chlorine and organic compounds, and the water hardness was adjusted to 100 mg/liter ( $\text{CaCO}_3$  equivalent) with reagent-grade calcium chloride. Eight liters of dilution water were added to each tank and aerated to 90-100% oxygen saturation prior to the addition of toxicants. Each test was conducted at 24 to 26°C.

### Range-finding tests

The concentrations of hydroxyl and hydrogen ions, ammonia, and nitrite used in the range-finding tests were as follows:

Hydroxyl ion (moles/liter)	(pH)	Hydrogen ion (moles/liter)	(pH)	Ammonia mg N/liter	Nitrite mg N/liter
$10^{-1.0}$	12.5	$10^{-2.0}$	2.0	100	100
$10^{-1.5}$	12.0	$10^{-2.5}$	2.5	75	75
$10^{-2.5}$	11.5	$10^{-3.0}$	3.0	50	50
$10^{-3.0}$	11.0	$10^{-3.5}$	3.5	20	20
$10^{-3.5}$	10.5	$10^{-4.0}$	4.0	10	10
$10^{-4.0}$	10.0	$10^{-4.5}$	4.5	1	1
$10^{-4.5}$	9.5			0	0
$10^{-5.0}$	9.0				

Test concentrations were randomly assigned to the tanks (2 tanks per test concentration for each toxicant) in the range-finding tests. Four crawfish were randomly placed in each tank 30 minutes after the toxicants were introduced. Water temperature, DO, pH and crawfish mortality were recorded after 24 hours. The average total length and weight ( $\bar{x} \pm 1$  SD; N=30) of the crawfish were  $32 \pm 4$  mm and  $0.70 \pm 0.24$  g, respectively. The longest crawfish was no more than twice the length of the shortest crawfish, and the loading rate did not exceed 0.8 g of crawfish per liter of test solution (USEPA 1975). The criteria established for death of crawfish was the absence of body and gill movement and lack of reaction to gentle prodding.

### Definitive tests

The results of the range-finding tests provided an estimate of the concentrations of the four toxicants that were acutely toxic to P. clarkii. I selected a wide range of toxicant concentrations to use in

the definitive tests, some of which exceeded the range of concentrations used in the range-finding tests. The toxicant concentrations used insured that crawfish mortalities would range from 0 to 100% with several intermediate levels of mortality at 24, 48, 72, and 96 hours. A range in crawfish mortality from 0 to 100% is required to provide a good estimate of the median lethal concentration (LC50) with narrow (95%) confidence limits. The following toxicant concentrations of hydroxyl and hydrogen ions, ammonia, and nitrite were used in the definitive tests:

<u>Hydroxyl ion</u> <u>(moles/liter) (pH)</u>		<u>Hydrogen ion</u> <u>(moles/liter) (pH)</u>		<u>Ammonia</u> <u>mg N/liter</u>	<u>Nitrite</u> <u>mg N/liter</u>
$10^{-1.5}$	12.5	$10^{-2.0}$	2.0	14	20
$10^{-2.0}$	12.0	$10^{-2.5}$	2.5	12	16
$10^{-2.5}$	11.5	$10^{-3.0}$	3.0	10	12
$10^{-3.0}$	11.0	$10^{-3.5}$	3.5	9	10
$10^{-3.5}$	10.5	$10^{-4.0}$	4.0	7	8
$10^{-4.0}$	10.0	$10^{-4.5}$	4.5	6	6
$10^{-4.5}$	9.5	$10^{-5.0}$	5.0	4	4
$10^{-5.0}$	9.0	$10^{-7.0}$	7.0	3	1
$10^{-6.0}$	8.0			2	0
				1	
				0	

Ten crawfish were randomly assigned to each of two tanks (replicates) of each toxicant concentration. The crawfish were randomly placed in each tank 30 minutes after the toxicants were added.

The average total length and weight ( $\bar{x} \pm 1$  SD; N=60) of the crawfish were  $31 \pm 4$  mm and  $0.84 \pm 0.87$  g, respectively. The longest crawfish was no more than twice the length of the shortest crawfish, and the

loading rate did not exceed 0.8 g of crawfish/liter of test solution (USEPA 1975).

Water temperature, DO, and dead crawfish were monitored at 24-hour intervals in the four tests. The  $\text{OH}^-$  and  $\text{H}^+$  were measured at 24-hour intervals with a glass electrode and the  $\text{NH}_3\text{-N}$  and  $\text{NO}_2\text{-N}$  were measured with ion-specific electrodes (HNU Systems, INC.). Each electrode was coupled to a Fisher Accumet Model 750 microprocessor ion analyzer.

#### Data analysis

The mortality data from the definitive tests were analyzed by probit analysis (Finney 1971) to determine the median lethal toxicity (LC50) of hydroxyl and hydrogen ions, ammonia, and nitrite to juvenile P. clarkii at 24, 48, 72 and 96 hours. The empirically measured concentration of hydroxyl and hydrogen ions, ammonia, and nitrite at 24, 48, 72 and 96 hours were used in the analyses. The probit analysis was conducted with the PROBIT program of the Statistical Analysis System (SAS) package on an IBM 3083 computer.



## Results

### Range-finding tests

Water temperature was  $24^{\circ} \pm 1^{\circ}\text{C}$  during the 24-hour test periods. Dissolved oxygen concentrations exceeded 50% oxygen saturation in the four tests and no supplemental aeration was provided.

#### Hydroxyl ion ( $\text{OH}^-$ )

The  $\text{OH}^-$  at each test concentration declined nearly 5% over the 24-hour test period probably from the release of acidic metabolic waste products (e.g.,  $\text{CO}_2$  and  $\text{NH}_4^+$ ) by the crawfish. Total mortality of juvenile crawfish was observed after 24 hours at  $10^{-2.5}$  moles  $\text{OH}^-/\text{liter}$  (pH 11.5) (Table 17, Appendix). No crawfish mortality was observed during the 24-hour test at  $10^{-4.5}$  moles  $\text{OH}^-/\text{liter}$  (pH 9.5) or  $10^{-5.0}$  moles  $\text{OH}^-/\text{liter}$  (pH 9.00).

#### Hydrogen ion ( $\text{H}^+$ )

The hydrogen ion test concentrations remained stable during the 24-hour test period. Complete mortality of juvenile crawfish occurred after 24 hours at  $10^{-2.00}$  moles  $\text{H}^+/\text{liter}$  (pH 2.00) (Table 17, Appendix). No crawfish died during the 24-hour test at  $10^{-3.50}$  moles  $\text{H}^+/\text{liter}$  (pH 3.50) or less.

#### Ammonia ( $\text{NH}_3\text{-N}$ )

The pH was adjusted to 8.6 in each test container to obtain the proper concentration of un-ionized ammonia ( $\text{NH}_3\text{-N}$ ). Over the 24-hour test period the pH dropped to 8.4 at each ammonia concentration. Complete mortality of juvenile crawfish occurred at  $\text{NH}_3\text{-N}$  concentrations of 10 mg/liter and above in the 24-hour test (Table 18, Appendix). No crawfish died at  $\text{NH}_3\text{-N}$  concentrations of 1 mg/liter.

### Nitrite (NO<sub>2</sub>-N)

The pH ranged from 8.0 - 8.2 through the 24-hour test period. All juvenile crawfish died after 24 hours at NO<sub>2</sub>-N concentrations of 50 mg/liter (Table 18, Appendix) and above. No crawfish death occurred during the 24 hours at NO<sub>2</sub>-N concentrations of 10 mg/liter or less.

### Definitive tests

#### Hydroxyl ion (OH<sup>-</sup>)

The water temperature during the 96-hour test averaged  $24 \pm 1^{\circ}\text{C}$ . Dissolved oxygen (DO) declined an average of  $0.7 \pm 0.2$  mg/liter at each test concentration over 96 hours (Table 19, Appendix). The DO did not drop below 90% of oxygen saturation and no supplemental aeration was provided.

Hydroxyl ion concentrations declined an average of  $10^{-0.50}$  moles/liter (0.50 pH units) after 24 hours at each test concentration. Hydroxyl ion concentrations were re-established (except control) to their initial levels  $\pm 10^{-0.03}$  moles/liter (0.03 pH units) by the addition of 0.01 N NaOH (Table 19, Appendix).

The toxicity of OH<sup>-</sup> to juvenile P. clarkii appears in Table 8. The mortality of juvenile crawfish increased with exposure time. Absolute mortality (100%) was observed within 24 hours at OH<sup>-</sup> concentrations of  $10^{-2.00}$  moles/liter (pH 12.00) and greater. After 48 hours at  $10^{-3.00}$  moles OH<sup>-</sup>/liter (pH 11.00), 95% crawfish mortality was observed. Partial crawfish mortality (45 and 80%) occurred after 96 hours at OH<sup>-</sup> concentrations of  $10^{-5.00}$  and  $10^{-3.50}$  moles/liter (pH 10.50 and 10.00), respectively. No crawfish mortality occurred at OH<sup>-</sup> concentrations of  $10^{-4.99}$  moles/liter (pH 9.00) or less after 96 hours.

Table 8. Mortality of juvenile Procambarus clarkii at various hydroxyl ion ( $\text{OH}^-$ ) concentrations in 96-hour definitive test. The number of crawfish per test concentration was 20 ( $N=20$ ).

Hydroxyl ion (moles/liter)	pH	% crawfish mortality after			
		24 hours	48 hours	72 hours	96 hours
$10^{-1.50}$	12.50	100	100	100	100
$10^{-2.00}$	12.00	100	100	100	100
$10^{-2.50}$	11.50	95	95	100	100
$10^{-3.00}$	11.00	75	95	95	100
$10^{-3.50}$	10.50	50	55	75	80
$10^{-4.00}$	10.00	35	40	45	45
$10^{-4.50}$	9.50	0	5	5	5
$10^{-5.00}$	9.00	0	0	0	0
$10^{-6.00}$	8.00	0	0	0	0

The 24-, 48 and 72-hour median lethal concentrations (LC50) for hydroxyl ions ( $\text{OH}^-$ ) to juvenile P. clarkii were  $10^{-3.51}$ ,  $10^{-3.68}$  and  $10^{-3.29}$  moles/liter (pH 10.49, 10.32 and 10.71), respectively. The 96-hour LC50 was  $10^{-3.88}$  moles  $\text{OH}^-$ /liter (pH 10.12) (Table 9).

#### Hydrogen ion ( $\text{H}^+$ )

The water temperature during the 96-hour test averaged  $24.5 \pm 0.5$  °C. Dissolved oxygen (DO) declined no more than 1.3 mg/liter over the 96-hour test but oxygen consumption was greater in those tanks where crawfish mortality was less (Table 20, Appendix). The DO did not drop below 75% of oxygen saturation during the test and no supplemental aeration was provided.

Hydrogen ion concentration ( $\text{H}^+$ ) remained within  $10^{-0.07}$  moles/liter (0.07 pH units) of the initial test concentrations in each tank through the 96-hour test, and no  $\text{H}^+$  adjustment was necessary after the initial  $\text{H}^+$  levels were established (Table 20, Appendix).

The mortality of juvenile P. clarkii exposed to  $\text{H}^+$  is found in Table 10. Crawfish mortality increased with  $\text{H}^+$  concentration and death correspondingly increased with exposure time at  $\text{H}^+$  concentrations of  $10^{-3.00}$  moles/liter and greater. Total mortality (100%) was observed after 24 hours at  $\text{H}^+$  levels of  $10^{-2.00}$  moles/liter. After 48 hours 90% mortality occurred at  $10^{-2.50}$  moles  $\text{H}^+$ /liter while only 40% of the crawfish died at  $10^{-3.00}$  moles  $\text{H}^+$ /liter. No crawfish died at  $\text{H}^+$  concentrations of  $10^{-4.00}$  moles/liter or less.

The 24-, 48-, and 72-hour LC50's for hydrogen ion activity to crawfish were  $10^{-2.71}$ ,  $10^{-3.06}$  and  $10^{-3.19}$  moles/liter (pH 2.71, 3.06 and 3.19), respectively. The 96-hour LC50 of  $\text{H}^+$  to juvenile P. clarkii was  $10^{-3.21}$  moles/liter (pH 3.21) (Table 11).

Table 9. The median lethal toxicity (LC50) of hydroxyl ion ( $\text{OH}^-$ ) concentrations to juvenile Procambarus clarkii in 96-hour definitive test. The corresponding pH value appears in parentheses.

Exposure time	LC50	95% Confidence limits				
		Upper		Lower		
Hours	moles/liter	pH	moles/liter	pH	moles/liter	pH
24	$10^{-3.51}$	(10.49)	$10^{-3.33}$	(10.67)	$10^{-3.72}$	(10.28)
48	$10^{-3.68}$	(10.32)	$10^{-3.50}$	(10.50)	$10^{-3.87}$	(10.13)
72	$10^{-3.83}$	(10.17)	$10^{-3.67}$	(10.33)	$10^{-4.00}$	(10.00)
96	$10^{-3.88}$	(10.12)	$10^{-3.73}$	(10.27)	$10^{-4.04}$	(9.96)

Table 10. Mortality of juvenile Procambarus clarkii at various hydrogen ion concentrations in 96-hour definitive test. The number of crawfish per test concentration was 20 (N=20).

Hydrogen ion (moles/liter)	pH	% Crawfish mortality after			
		24 hours	48 hours	72 hours	96 hours
$10^{-2.00}$	2.00	100	100	100	100
$10^{-2.50}$	2.50	55	90	100	100
$10^{-3.00}$	3.00	15	40	55	60
$10^{-3.50}$	3.50	15	25	25	25
$10^{-4.00}$	4.00	0	0	0	0
$10^{-4.50}$	4.50	0	0	0	0
$10^{-5.00}$	5.00	0	0	0	0
$10^{-7.00}$	7.00	0	0	0	0

Table 11. The median lethal toxicity (LC50) of hydrogen ion ( $H^+$ ) concentrations to juvenile Procambarus clarkii in 96-hour definitive test.

Exposure time	LC50	95% Confidence limits				
		Upper		Lower		
Hours	moles/liter	pH	moles/liter	pH	moles/liter	pH
24	$10^{-2.71}$	(2.71)	$10^{-2.87}$	(2.87)	$10^{-2.52}$	(2.52)
48	$10^{-3.06}$	(3.06)	$10^{-2.90}$	(2.90)	$10^{-2.52}$	(2.52)
72	$10^{-3.19}$	(3.19)	$10^{-3.03}$	(3.03)	$10^{-3.34}$	(3.34)
96	$10^{-3.21}$	(3.21)	$10^{-3.06}$	(3.06)	$10^{-3.36}$	(3.36)

### Ammonia (NH<sub>3</sub>-N)

The water temperature averaged  $24.5 \pm 0.5^{\circ}\text{C}$  (Table 21, Appendix). Dissolved oxygen (DO) declined by  $0.4 \pm 0.2$  mg/liter during the test but did not fall below 75% oxygen saturation at any time. No supplemental aeration was provided.

The concentration of un-ionized ammonia (NH<sub>3</sub>-N) is significantly altered by changes in the pH and to a lesser extent temperature (Trussell 1972). Therefore, the un-ionized ammonia concentrations were maintained by keeping the pH and water temperature levels as constant as possible. However, because of a pH decline at all test concentrations, it was necessary to increase the pH to its initial level ( $8.60 \pm .04$ ) at each 24-hour interval by the addition of 0.01 N NaOH. The fluctuations in water temperature ( $\pm 0.5^{\circ}\text{C}$ ) had negligible influence on NH<sub>3</sub>-N concentration. The empirically determined concentrations of NH<sub>3</sub>-N in the 96-hour test are provided in Table 22 (Appendix).

The mortality of juvenile P. clarkii exposed to un-ionized ammonia is found in Table 12. The mortality of crawfish increased with increasing ammonia concentration and the death rate increased with exposure time. Absolute mortality occurred at and above 7 mg NH<sub>3</sub>-N/liter after 24 hour exposure. Crawfish mortality increased from 30% after 48 hours to 50% after 72 hours when exposed to 3 mg NH<sub>3</sub>-N/liter. No crawfish mortality occurred at or below 1 mg NH<sub>3</sub>-N/liter.

The 24-, 48- and 72-hour LC50's for NH<sub>3</sub>-N to juvenile P. clarkii were 4.02, 3.13 and 2.81 mg/liter, respectively. The 96-hour LC50 was 2.64 mg NH<sub>3</sub>-N/liter (Table 13).



Table 12. Mortality of juvenile Procambarus clarkii at various concentrations of un-ionized ammonia in 96-hour definitive test. The number of crawfish per test concentration was 20 (N=20).

Ammonia (mg N/liter)	% Crawfish mortality after			
	24 hours	48 hours	72 hours	96 hours
14.0	100	100	100	100
12.0	100	100	100	100
10.0	100	100	100	100
9.0	100	100	100	100
7.0	100	100	100	100
6.0	60	85	90	95
4.0	60	75	75	80
3.0	15	30	50	50
2.0	15	25	25	30
1.0	0	0	0	0
0.0	0	0	0	0

Table 13. The median lethal toxicity (LC50) of un-ionized ammonia to juvenile Procambarus clarkii in 96-hour definitive tests.

Exposure time	LC50	95% Confidence limits	
		Upper	Lower
<u>Hours</u>	<u>mg/liter</u>	<u>mg/liter</u>	<u>mg/liter</u>
24	4.02	4.68	3.46
48	3.13	3.62	2.65
72	2.81	3.26	2.35
96	2.64	3.05	2.21

Nitrite (NO<sub>2</sub>-N). The water temperature during the 96-hour test averaged  $25 \pm 1^{\circ}\text{C}$ . Dissolved oxygen decreased during the test but the decline was not proportional to toxicant concentration or crawfish mortality (Table 23, Appendix). The DO did not drop below 70% of oxygen saturation during the test and no supplemental aeration was provided. The pH ranged from 7.9 to 8.1 during the test (Table 23, Appendix).

The empirically determined concentrations of NO<sub>2</sub>-N in the 96-hour test are provided in Table 24 (Appendix). Nitrite levels declined less than 1% of initial concentrations in the 96-hour test. These decreases were considered negligible, so nitrite levels were not adjusted.

The mortality of juvenile P. clarkii exposed to nitrite (NO<sub>2</sub>-N) is presented in Table 14. Crawfish mortality increased with NO<sub>2</sub>-N concentrations as well as with exposure time. Absolute mortality (100%) was observed within 24 hours at 20 mg NO<sub>2</sub>-N/liter. Partial crawfish mortality (55%) occurred after 48 hours at 8 mg NO<sub>2</sub>-N/liter. Crawfish mortalities were 90 and 20% after 72 hours at 10 and 4 mg NO<sub>2</sub>-N/liter, respectively. No crawfish mortality occurred at or below 1 mg NO<sub>2</sub>-N/liter in the 96-hour test.

The 24-, 48- and 72-hour LC50's for nitrite to juvenile P. clarkii were 12.12, 6.79 and 6.12 mg NO<sub>2</sub>-N/liter, respectively (Table 15). The 96-hour LC50 of NO<sub>2</sub>-N was 5.94 mg/liter.

Table 14. Mortality of juvenile Procambarus clarkii at various concentrations of nitrite in 96-hour definitive tests. The number of crawfish per test concentration was 20 (N=20).

Nitrite (mg N/liter)	% Crawfish mortality after			
	24 hours	48 hours	72 hours	96 hours
20.0	100	100	100	100
16.0	55	100	100	100
12.0	65	95	100	100
10.0	55	85	90	95
8.0	0	55	70	75
6.0	0	30	35	40
4.0	0	15	20	20
1.0	0	0	0	0
0	0	0	0	0

Table 15. The median lethal toxicity (LC50) of nitrite (NO<sub>2</sub>-N) to juvenile Procambarus clarkii in 96-hour definitive tests.

Exposure time	LC50	95% Confidence limits	
		Upper	Lower
<u>Hours</u>	<u>mg/liter</u>	<u>mg/liter</u>	<u>mg/liter</u>
24	12.12	10.70	13.55
48	6.79	5.94	7.64
72	6.12	5.31	6.90
96	5.94	5.17	6.67

## Discussion

### pH

Waters with a pH of 6.5 to 9.0 at daybreak are generally recognized most conducive to fish production (Boyd 1979, 1982). A pH that is constantly less than 4.5 or greater than 10 is considered detrimental to fish populations, but a pH exceeding these limits rarely occurs in non-polluted waters (Royce 1972). Alabaster and Lloyd (1982) reviewed current literature on the effects of extreme pH on many fish species and reported that most finfish are adversely affected after extended exposure to pH below 4.5 and above 10.0. Salmonids appear to be more sensitive to extreme pH than most other fish species. Alabaster and Lloyd (1982) reported that there is no specific pH range of natural waters in which a fishery is unharmed and outside which it is damaged, but rather a gradual deterioration in fish production occurs as the pH of the water deviates from the "normal" range. Nonetheless, long-term acidification of lakes by acid precipitation is having disastrous effects on fish and crawfish production in many areas of North America and Europe (Boyd 1982).

The toxicity of hydrogen ion ( $H^+$ ) to fish has been attributed to disruption of gill epithelium, production of mucous on gills, inability to osmoregulate, and acidosis of the blood. High levels of hydroxyl ion destroy the gill and skin epithelium (Alabaster and Lloyd 1982).

Previous research indicated that the ability of crawfish to tolerate acidic (i.e., high  $H^+$ ) water may vary considerably among species (Newcombe 1975; Jay and Holdich 1977; Baker 1979; McMahon and Morgan 1981). Limited research has been conducted on the effects of

highly alkaline water (i.e., high  $\text{OH}^-$ ) on crawfish (Jay and Holdich 1977). The acute toxicity tests with hydrogen and hydroxyl ions in this study demonstrated that juvenile P. clarkii can tolerate waters with a wide range in pH. No crawfish mortality occurred at pH 4.1 to 9.0 which would indicate that P. clarkii are more acid tolerant than many finfishes (Alabaster and Lloyd 1982).

McMahon and Morgan (1981) reported the 96-hour LC50 of sulfuric acid ( $\text{H}_2\text{SO}_4$ ) to adult P. clarkii was  $10^{-2.8}$  moles  $\text{H}^+$ /liter (pH 2.8). I found juvenile P. clarkii to be slightly less tolerant to acidic waters (96-hour LC50 = pH 3.2); this finding is consistent with the general hypothesis that juvenile or immature stages of aquatic animals are less tolerant to toxic compounds than are adults (Jay and Holdich 1977). McMahon and Morgan (1981) also reported that intermolt adult Orconectes rusticus, a northern cambarid crawfish, had a 96-hour LC50 to  $\text{H}_2\text{SO}_4$  of  $10^{-2.5}$  moles  $\text{H}^+$ /liter (pH 2.5), 0.3 to 0.7 pH units lower than that for P. clarkii, a southern cambarid species. Baker (1979) reported newly hatched O. rusticus had a 96-hour LC50 of  $10^{-4.0}$  moles  $\text{H}^+$ /liter (pH 4.0).

Jay and Holdich (1977) evaluated acid and alkaline tolerance of the crawfish Austropotamobius pallipes (family Astacidae). Adult A. pallipes survived 4.5 hours at  $10^{-1.5}$  moles  $\text{H}^+$ /liter (pH 1.5), 7 days at  $10^{-5.0}$  moles  $\text{H}^+$ /liter (pH 5.0), and indefinitely from  $10^{-7.0}$  to  $10^{-8.0}$  moles  $\text{H}^+$ /liter (pH 7.0 to 8.0). The species survived 15.3 hours at  $10^{-1.5}$  moles  $\text{OH}^-$ /liter (pH 12.5). A. pallipes inhabit natural waters of narrow and stable pH (6.8 to 8.2). The Tasmanian crawfish Parastacoides tasmanicus (family Parastacidae) inhabits naturally acid waters of about  $10^{4.5}$  moles  $\text{H}^+$ /liter (pH 4.5) and this species reportedly can survive

indefinitely in waters with  $10^{-2.5}$  moles  $H^+$ /liter (pH 2.5) (Newcombe 1975).

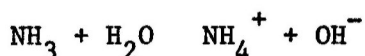
Physiological responses of Procambarus clarkii and O. rusticus to sublethal exposure to acid waters (4 days at ambient pH of 3.8) were studied by McMahon and Morgan (1981). They observed that both species developed severe hemolymph acidosis, so they hypothesized that the ability of crawfish to tolerate acid waters may be due in part to the chitinous, calcified exoskeleton. The exoskeleton extends as a thin chitinous layer over the gills and may impede the influx of  $H^+$  across the gill-water interface in addition to serving as a buffer to  $H^+$  influx. Crawfish death from acid waters is probably caused by an imbalance in body-salt regulation (Jay and Holdich 1977).

The pH of water in experimental crawfish ponds in Louisiana and Texas has been reported to range from 5.5 to 7.7 (Zeman 1982; Day 1983). I found a similar pH range in commercial crawfish ponds. These pH levels were in the range considered optimal for finfish production (Boyd 1979) and were within the tolerance levels established in the toxicity tests. The pH of pond waters in properly constructed and well managed crawfish ponds would be adequate provided the total alkalinity is maintained between 20 to 200 mg/liter as  $CaCO_3$  equivalent. The pH of acid water can be increased by liming with calcite ( $CaCO_3$ ) or dolomite ( $CaMg(CO_3)_2$ ), and the pH of highly alkaline waters can be lowered by the addition of alum ( $Al_2(SO_4)_3$ ) or gypsum ( $CaSO_4$ ) (Boyd 1979, 1982). Until long-term studies on the sublethal effects of acidic and alkaline waters on crawfish production are conducted, a pH between 5 and 9 should be considered adequate for culture of P. clarkii, with pH 6.5 to 8.5 optimal.



## Ammonia

The principal nitrogenous compound excreted by aquatic animals is ammonia (Campbell 1973). Ammonia may attain concentrations that are deleterious to fish and it can reduce fish production in intensive culture systems (Boyd 1979). Ammonia toxicity is attributed primarily to the un-ionized form ( $\text{NH}_3$ ), while the ionized form ( $\text{NH}_4^+$ ) is non-toxic (Robinette 1976). The concentrations of ionized and un-ionized ammonia are dependent on pH and to a lesser extent on temperature (Trussell 1972). This relationship is depicted as follows:



As pH (i.e.,  $\text{OH}^-$  concentration) and temperature increase, the reaction is shifted to the left to maintain equilibrium and the percentage of toxic un-ionized ammonia increases.

The effects of ammonia on finfish have been studied extensively; however, the effects of ammonia on crustaceans is little known. The European Inland Fisheries Advisory Commission (1973) stated that toxic concentrations of ammonia for short-term exposure are between 0.6 and 2 mg/liter of  $\text{NH}_3\text{-N}$  for most fish species. The acute toxicity of un-ionized ammonia (96-hour  $\text{LC}_{50}$ ) for finfishes ranges from 0.4 to 3.1 mg  $\text{NH}_3\text{-N}$ /liter (Ball 1967; Colt and Tchobanoglous 1976). Sublethal exposure of ammonia to fishes can damage gill and kidney tissues (Burrows 1964; Smart 1978), reduce growth (Burrows 1964; Colt and Tchobanoglous 1978), increase respiration (Smart 1978), and reduce oxygen carrying capacity of hemoglobin (Sousa and Meade 1977). The mechanisms of ammonia toxicity have been reviewed by Tomasso et al. (1980).

The acute toxicity (96-hour LC50) of un-ionized ammonia to crustaceans is reported to range from 0.40 to 2.60 mg  $\text{NH}_3\text{-N/liter}$  (Wickens 1976; Armstrong et al. 1978; Evans 1979). The ability of juvenile P. clarkii to tolerate un-ionized ammonia (96-hour LC50, 2.65 mg  $\text{NH}_3\text{-N/liter}$ ) is greater than that of some marine and freshwater shrimp (Wickens 1976; Armstrong et al. 1978) but similar to tolerance by adult Orconectes nais (Evans 1979). Evans reported a 96-hour LC50 of 2.60 mg  $\text{NH}_3\text{-N/liter}$  (95% confidence interval, 1.78 to 3.80 mg  $\text{NH}_3\text{-N/liter}$ ) in studies conducted in a continuous-flow bioassay system.

Johnson (1982) conducted 24-hour exposure tests to estimate the ammonia tolerance of Procambarus acutus acutus. He reported that adults survived 24 hours at 300 mg/liter of total ammonia (4.9 mg  $\text{NH}_3\text{-N/liter}$  at 26°C and pH 7.5), but all P. a. acutus hatchlings died at total ammonia concentrations of 124 mg/liter (2.0 mg  $\text{NH}_3\text{-N/liter}$ ).

The tolerance of juvenile P. clarkii to un-ionized ammonia is similar to that described for channel catfish. Colt and Tchobanoglous (1976) reported the 96-hour LC50 of un-ionized ammonia to channel catfish at 22, 26 and 30°C to be 1.97, 2.38, and 3.12 mg  $\text{NH}_3\text{-N}$ , respectively. Robinette (1976) found that fingerling channel catfish exhibited no growth when exposed to 0.12 and 0.13 mg  $\text{NH}_3\text{-N/liter}$  but concentrations below 0.06 mg  $\text{NH}_3\text{-N/liter}$  had no effect on growth.

The un-ionized ammonia concentrations found toxic to crawfish are substantially greater than the concentrations reported to occur in crawfish ponds. I found a maximum total ammonia (TA-N) level of 0.9 mg/liter (at pH 7.2 and 22°C) in five commercial crawfish ponds. This corresponds to an un-ionized ammonia level of 0.007 mg  $\text{NH}_3\text{-N/liter}$ , more than 400 times lower than the 96-hour LC50 of 2.65 mg  $\text{NH}_3\text{-N/liter}$  and

100 times lower than the concentration ( $0.76 \text{ mg NH}_3\text{-N/liter}$ ) at which no P. clarkii death occurred.

The USEPA (1975) recommends that concentrations of nonpersistent or noncumulative toxicants (e.g., ammonia) should not exceed 0.1 of the 96-hour LC50 or  $0.265 \text{ mg NH}_3\text{-N/liter}$  ( $2.65 \text{ mg/liter}$  96-hour LC50  $\times$  0.1) for P. clarkii. Ammonia concentrations from 0.0 to  $0.076 \text{ mg NH}_3\text{-N/liter}$  have been reported in experimental crawfish ponds of less than 1 hectare (Zeman 1982). Johnson (1980) reported  $\text{NH}_3\text{-N}$  as high as  $1.24 \text{ mg/liter}$  in experimental crawfish ponds in which large quantities ( $143 \text{ kg/hectare}$ ) of nitrogen-rich poultry manure was added biweekly. I believe that  $\text{NH}_3\text{-N}$  levels below  $0.06 \text{ mg/liter}$  should have no effect on the growth and health of P. clarkii based principally on my results and those of Robinette (1976) for channel catfish. Moreover, ammonia levels above  $0.007 \text{ mg NH}_3\text{-N/liter}$  have not been reported in commercial crawfish ponds. Un-ionized ammonia concentrations in commercial crawfish ponds will be minimal provided the pH does not exceed 8.5 for an extended period.

The results of my toxicity test and of other studies with crawfish indicate that ammonia probably poses little threat to crawfish provided farmers follow present recommendations for water management. However, long-term studies on the effects of sub-lethal concentrations of un-ionized ammonia to P. clarkii must be conducted to accurately assess the impact of ammonia on crawfish in ponds.

#### Nitrite

Nitrite ( $\text{NO}_2^-$ ) is an intermediate compound formed during nitrification of ammonia. Nitrite accumulates when nitrification is

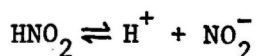
inhibited by nitrous acid ( $\text{HNO}_2$ ) and un-ionized ammonia (Anthonisen et al. 1976). Nitrite can attain levels toxic to fish or stress fish to such a degree that decreased growth and predisposition to disease occur.

Extensive literature is available on the effects of nitrite on finfishes; however, large discrepancies in toxicity levels are reported. Many of these discrepancies are related to differences in water chemistry (e.g., chloride and pH) which affect the toxicity of nitrite, as well as the specific tolerance of fish species to nitrite.

In general, warmwater fishes are more tolerant of nitrite than are coldwater fishes. Nitrite toxicity levels (96-hour  $\text{LC}_{50}$ ) for warmwater fishes range from 7.5 to 13.4 mg  $\text{NO}_2\text{-N/liter}$  (Konikoff 1975; Colt and Tchobanoglous 1976) while the toxicity levels of nitrite to coldwater fishes range between 0.2 and 0.9 mg  $\text{NO}_2\text{-N/liter}$  (Westin 1974, Russo and Thurston 1977). Colt et al. (1981) reported a 20% reduction in growth in juvenile channel catfish at 1.60 mg  $\text{NO}_2\text{-N/liter}$  with no effects observed below this level.

Nitrite is toxic to finfishes because it converts normal hemoglobin to methemoglobin which impedes efficient respiration (Russo et al. 1974; Smith and Williams 1974; Huey and Beitinger 1980; Huey et al. 1980). Methemoglobin is an oxidized form of hemoglobin and is incapable of binding with oxygen (Bodansky 1951). Formation of methemoglobin is reduced by chloride (Perrone and Meade 1977; Russo and Thurston 1977; Huey et al. 1980) or by calcium (Crawford and Allen 1977; Wedemeyer and Yasutake 1978).

Nitrite exists in a pH dependent equilibrium with nitrous acid as follows:



Nitrite toxicity increases as pH (i.e.,  $H^+$  concentration) decreases which would indicate that toxicity is caused at least in part by nitrous acid. Nitrous acid is a strong oxidizing agent capable of forming methemoglobin (Wedemeyer and Yasutake 1978; Meade and Perrone 1980).

Although much research has been conducted on toxic effects of nitrite to finfishes, a paucity of information is available on nitrite toxicity to crawfish and other crustaceans. Finfish hemoglobin contains iron for oxygen binding, whereas the oxygen-carrying pigment in crawfish is hemocyanin which contains copper. A similar reaction probably occurs with the copper in hemocyanin as with the iron of hemoglobin but this remains to be confirmed (Colt and Armstrong 1979). Felsenfield and Printz (1959) were able to produce methemocyanin in vivo in the horseshoe crab (Limulus polyphemus) and the marine whelk (Busycon canaliculatum) with hydrogen peroxide ( $H_2O_2$ ) but no recent work has been reported.

Previous studies of the effects of nitrite on warm-water crustaceans found acute toxicities (96-hour LC50) that ranged from 1.9 to 4.7 mg  $NO_2$ -N/liter (Wickens 1976; Beitinger and Huey 1981). Armstrong et al. (1976) reported a 96-hour LC50 to Machrobrachium rosenbergii of 4.7 mg  $NO_2$ -N/liter and reported a 35% reduction in the growth of M. rosenbergii larvae exposed to 1.8 mg  $NO_2$ -N/liter for 50 days. Wickens (1976) reported that 6.2 mg  $NO_2$ -N/liter caused a 50% reduction in the growth of juvenile marine shrimp, Penaeus spp.

The 24-, 48-, 72- and 96-hour LC50's of nitrite to juvenile Procambarus clarkii found in this study (i.e., 12.1, 6.8, 6.1 and 5.9 mg  $NO_2$ -N/liter) were slightly higher than that reported by Beitinger and Huey (1981) for adult P. similans. They reported the nitrite LC50's at

24, 48, 72 and 96 hours to be 9.6, 4.2, 2.6 and 1.9 mg NO<sub>2</sub>-N/liter, respectively. The difference in nitrite toxicity between the two procambarid species may be due to their specific tolerance of nitrite, differences in water chemistry, and also to criterion that they used for determining lethality, that is, loss of equilibrium instead of lack of response to prodding with a glass rod. Beitinger and Huey (1981) found that an increase in chloride to 300 mg/liter reduced nitrite toxicity to crawfish; however, a decrease in pH from 7.0 to 5.6 reduced the protective action of chloride ions and increased crawfish mortality by 50% over 48 hours.

The maximum concentration of nitrite measured in the five commercial crawfish ponds (0.20 mg NO<sub>2</sub>-N/liter) was nearly 30 times lower than the 96-hour LC50 (5.95 mg NO<sub>2</sub>-N/liter) to juvenile P. clarkii and 2 times lower than the concentration (0.60 mg NO<sub>2</sub>-N/liter) that the USEPA (1975) recommends for toxicants that are nonpersistent or have noncumulative effects (96-hour LC50, 5.95 mg/liter x 0.1 = 0.595 mg/liter).

The P. clarkii appear to be as tolerant of nitrite as channel catfish and more tolerant than the freshwater Malaysian prawn. Long-term studies are needed to assess the effects of sub-lethal concentrations of nitrite on growth and survival of P. clarkii. However, the findings of my studies and others indicate that non-persistent nitrite concentrations of 1.0 mg NO<sub>2</sub>-N/liter or less would have little or no effects on the growth and health of P. clarkii.

### Conclusions

1. A pH range between 5 and 9 appears adequate for culture of Procambarus clarkii, with pH 6.5 to 8.5 optimal.
2. Ammonia concentrations below 0.06 mg  $\text{NH}_3\text{-N}$ /liter should have no effect on the growth and health of P. clarkii provided the pH does not exceed 8.5 for an extended period.
3. Nitrite concentrations of 1.0 mg  $\text{NO}_2\text{-N}$ /liter or less would probably have little or no effect on the growth and health of P. clarkii.

## SUMMARY AND CONCLUSIONS

Water quality was monitored in five southern Louisiana commercial crawfish (Procambarus clarkii and P. acutus acutus) ponds from November 1982 through May 1983. Two ponds were planted with rice (Oryza sativa) and three ponds contained volunteer terrestrial and semi-aquatic vegetation as crawfish forage. The following water quality variables were measured: temperature, dissolved oxygen (DO), pH, free carbon dioxide ( $\text{CO}_2$ ), total ammonia, nitrite, nitrate, total nitrogen, soluble inorganic phosphorus, total phosphorus, chemical oxygen demand (COD), and biochemical oxygen demand (BOD). Additional analyses included total hardness, total alkalinity, conductivity, chlorophyll a, and turbidity. Laboratory studies were also conducted to determine the median lethal toxicity (LC50) of hydroxyl and hydrogen ions (i.e., pH), ammonia, and nitrite to juvenile P. clarkii.

Water quality in the ponds was poorest in November, April, and May when water temperature exceeded  $18^\circ\text{C}$ . However, only DO attained concentrations ( $<1.0$  mg/liter) considered acutely toxic to crawfish. Free  $\text{CO}_2$  concentrations in November (12.0 mg/liter) exceeded levels that could have stressed crawfish when the DO was low ( $<2.0$  mg/liter). Maximum ammonia concentrations (0.007 mg  $\text{NH}_3\text{-N}$ /liter) in the ponds were nearly 400 times lower than concentrations observed toxic to juvenile P. clarkii in the toxicity test (96-hour LC50 = 2.65 mg  $\text{NH}_3\text{-N}$ /liter). Ammonia levels below 0.06 mg/liter should have no effect on P. clarkii production provided the pH does not exceed 8.5 for an extended period. Maximum nitrite concentrations in the ponds (0.20 mg  $\text{NO}_2\text{-N}$ /liter) were 30 times less than levels found toxic to crawfish in the laboratory test (96-hour LC50 = 5.95 mg/liter). Nitrite concentrations of 1.0 or less



in commercial crawfish ponds should not be deleterious to the production of P. clarkii.

Total alkalinity, total hardness, and pH in the ponds were maintained at levels known conducive for good crawfish production. The acute toxicity of hydrogen (96-hour LC50 = pH 3.21) and hydroxyl ions (96-hour LC50 = pH 10.12) to P. clarkii in the laboratory studies indicated that a pH range between 6.5 and 8.5 should be optimum for crawfish production.

Water quality was poorest in the naturally vegetated crawfish ponds because they contained 29% more vegetative biomass than did ponds planted with rice. Additionally, natural vegetation was more persistent (i.e. decomposed slowly) and this reduced water circulation and negatively impacted water quality. Water temperature, pH, free CO<sub>2</sub>, nitrate, total phosphorus, biochemical oxygen demand (BOD), chlorophyll a, total hardness, and total alkalinity did not significantly differ between rice ponds and ponds with volunteer vegetation. Dissolved oxygen, nitrite, total nitrogen, and COD were highest in rice ponds ( $P < 0.05$ ). Soluble inorganic phosphorus and conductivity were highest in ponds with volunteer vegetation ( $P < 0.05$ ). Free CO<sub>2</sub>, SIP, conductivity, and total alkalinity levels increased with an increase in macrophytic biomass ( $P < 0.01$ ). However, nitrite, total nitrogen, COD, BOD, turbidity, temperature, and DO decreased with an increase in vegetative biomass ( $P < 0.1$ ). Total ammonia, pH, nitrate, total phosphorus, and chlorophyll a were not correlated with vegetative biomass.

Crawfish catch (kg crawfish/trap/day) was positively correlated with total ammonia, BOD, COD, and water temperature, and negatively correlated with total nitrogen, total phosphorus, SIP, total alkalinity,

and DO ( $P < 0.05$ ). With the exception of water temperature, no single water quality variable or combination of variables directly affected crawfish catch. Crawfish catch is probably most influenced by a set of complex interactions of water quality and biological factors such as crawfish population density and structure.

Crawfish ponds are highly productive ecosystems, comparable to fertilized fish ponds and channel catfish production ponds, based on seasonal concentrations of phosphorus, nitrogen, chlorophyll a, COD, and BOD.

This study indicates that, if DO in crawfish ponds is maintained at or above 2.0 mg/liter by water management techniques such as aeration, recirculation, or flushing, then other potentially toxic water quality variables (ammonia and nitrite, for example) should present little or no problem in crawfish culture. However, long-term studies are needed to determine the effects of sub-lethal concentrations of ammonia and nitrite on crawfish. Additional research should be conducted to determine the effects of types and quantities of vegetation on water quality in crawfish ponds.

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## APPENDIX

Table 16. Chemical composition of Baton Rouge city (dilution) water.

Ions	Concentration (mg/liter)
$\text{Na}^+$	89.7
$\text{K}^+$	0.0
$\text{Fe}^{2+}$	0.16
$\text{F}^-$	0.26
$\text{Cl}^-$	7.9
$\text{HCO}_3^-$	140.0 as $\text{CaCO}_3$
$\text{CO}_3^{2-}$	47.1 as $\text{CaCO}_3$
$\text{Mn}^{2+}$	0.0
$\text{Cl}_2$	2.0 <sup>a</sup>
$\text{H}^+$	$2.51 \times 10^{-9}$ (pH = 8.6)
$\text{Mg}^{2+}$ and $\text{Ca}^{2+}$	0.0 <sup>b</sup>

<sup>a</sup> Removed in activated charcoal filter prior to use.

<sup>b</sup> Total hardness adjusted to 100 mg/liter as  $\text{CaCO}_3$  equivalent prior to use.



Table 17. Mortality of juvenile Procambarus clarkii at different hydroxyl and hydrogen ion concentrations in the 24-hour range-finding tests. The number of crawfish at each test concentration was eight (N=8).

Hydroxyl ion (moles/liter)	pH	% Dead after 24 hours
$10^{-1.5}$	12.50	100
$10^{-2.0}$	12.00	100
$10^{-2.5}$	11.50	100
$10^{-3.0}$	11.00	75
$10^{-3.5}$	10.50	25
$10^{-4.5}$	10.00	25
$10^{-4.5}$	9.50	0
$10^{-5.0}$	9.00	0

Hydrogen ion (moles/liter)	pH	% Dead after 24 hours.
$10^{-2.0}$	2.00	100
$10^{-2.5}$	2.50	50
$10^{-3.0}$	3.00	25
$10^{-3.5}$	3.50	0
$10^{-4.0}$	4.00	0
$10^{-4.5}$	4.50	0

Table 18. Mortality of juvenile Procambarus clarkii at different nitrite and un-ionized ammonia concentrations in the 24-hour range-finding tests. The number of crawfish at each test concentration was eight (N=8).

Ammonia mg N/liter	% Dead after 24 hours
100	100
75	100
50	100
20	100
10	100
1	0
0	0

Nitrite mg N/liter	% Dead after 24 hours
100	100
75	100
50	100
20	75
10	0
1	0
0	0

Table 19. Water quality at various hydroxyl ion concentrations in 96-hour definitive test.

Hydroxyl ion (moles/liter)	pH	Dissolved oxygen (mg/liter)					pH					Temperature (C)				
		0 <sup>a</sup>	24	48	72	96	0	24	48	72	96	0	24	48	72	96
10 <sup>-1.50</sup>	12.50	8.2	8.0	-- <sup>b</sup>	--	--	12.50	12.60	--	--	--	24	25	--	--	--
10 <sup>-2.00</sup>	12.00	8.1	7.8	--	--	--	12.00	12.00	--	--	--	24	24	--	--	--
10 <sup>-2.50</sup>	11.50	8.3	7.9	7.8	7.6	7.6	11.50	11.50	11.50	11.52	11.50	25	25	25	25	25
10 <sup>-3.00</sup>	11.00	8.3	8.0	8.0	7.6	7.6	11.00	11.00	11.00	11.00	11.00	24	24	25	25	25
10 <sup>-3.50</sup>	10.50	8.3	8.1	8.0	7.8	7.6	10.50	10.53	10.52	10.52	10.50	24	23	24	24	24
10 <sup>-4.00</sup>	10.00	8.3	8.0	7.9	7.6	7.6	10.00	10.00	10.03	10.03	10.00	25	24	24	24	24
10 <sup>-4.50</sup>	9.50	8.2	7.8	7.8	7.6	7.6	9.50	9.50	9.52	9.51	9.50	24	25	25	25	25
10 <sup>-5.00</sup>	9.00	8.2	7.8	7.4	7.4	7.3	9.00	9.01	9.03	9.01	9.00	24	24	24	24	24
10 <sup>-6.00</sup>	8.00	8.2	8.0	7.6	7.6	7.4	8.00	8.33	7.95	7.88	7.85	24	25	25	25	25

<sup>a</sup> Test period in hours.

<sup>b</sup> Measurements discontinued after 100% mortality.

Table 20. Water quality at various hydrogen ion concentrations in 96-hour definitive test.

Hydrogen ion (moles/liter)	pH	Dissolved oxygen (mg/liter)					pH					Temperature (C)				
		0 <sup>a</sup>	24	48	72	96	0	24	48	72	96	0	24	48	72	96
10 <sup>-2.00</sup>	2.00	8.4	8.5	-- <sup>b</sup>	--	--	2.07	2.07	--	--	--	24	25	--	--	--
10 <sup>-2.50</sup>	2.50	8.0	7.8	7.6	7.6	7.6	2.50	2.53	2.54	2.54	2.55	24	25	24	24	24
10 <sup>-3.00</sup>	3.00	8.1	8.0	8.0	7.9	7.8	3.00	3.01	3.01	3.02	3.02	24	25	25	24	24
10 <sup>-3.50</sup>	3.50	8.2	7.7	7.6	7.6	7.6	3.50	3.50	3.52	3.53	3.54	24	24	24	24	24
10 <sup>-4.00</sup>	4.00	7.8	7.0	6.7	6.6	6.5	4.00	4.05	4.05	4.06	4.07	24	24	24	25	24
10 <sup>-4.50</sup>	4.50	7.7	6.4	6.4	6.4	6.2	4.00	4.51	4.52	4.53	4.53	24	25	24	25	24
10 <sup>-5.00</sup>	5.00	7.8	6.5	6.4	6.4	6.4	5.00	5.07	5.09	5.10	5.11	24	24	24	25	25
10 <sup>-7.00</sup>	7.00	7.7	6.8	6.8	6.8	6.7	7.00	7.01	7.05	7.06	7.06	24	25	24	25	25

<sup>a</sup> Test period in hours.

<sup>b</sup> Measurements discontinued after 100% mortality.

Table 21. Water quality at various concentrations of un-ionized ammonia in 96-hour definitive test.

Ammonia (mg N/liter)	Dissolved oxygen (mg/liter)					pH					Temperature (C)				
	0 <sup>a</sup>	24	48	72	96	0	24	48	72	96	0	24	48	72	96
14.0	7.5	-- <sup>b</sup>	--	--	88	8.62	--	--	--	--	24	--	--	--	--
12.0	7.2	*	*	*	*	8.58	*	--	--	--	24	--	--	--	--
10.0	7.3	7.2	*	*	*	8.58	8.60	--	--	--	24	24	--	--	--
9.0	7.0	6.9	*	*	*	8.58	9.63	--	--	--	24	24	--	--	--
7.0	7.0	6.8	*	*	*	8.58	8.62	--	--	--	24	24	--	--	--
6.0	7.3	7.3	7.3	7.2	7.2	8.59	8.60	8.61	8.64	8.37	24	24	25	24	24
4.0	7.2	7.1	7.1	7.1	7.0	8.58	8.63	8.60	8.59	8.38	24	24	25	24	24
3.0	6.7	6.7	6.7	6.5	6.4	8.58	8.62	8.62	8.61	8.38	24	24	25	24	24
2.0	6.2	6.1	6.1	5.8	5.8	8.60	8.64	8.60	8.62	8.33	24	24	25	24	24
1.0	6.7	6.3	6.3	6.1	6.1	8.63	8.64	8.62	8.60	8.33	24	24	25	24	24
0.0	7.4	7.3	7.3	7.2	7.1	8.60	8.57	8.57	8.59	8.57	24	24	25	24	24

<sup>a</sup> Test period in hours.

<sup>b</sup> Measurements discontinued after 100% mortality.

Table 22. Concentration of un-ionized ammonia in test containers with juvenile Procambarus clarkii in 96-hour definitive test.

Ammonia (mg N/liter)	Test period				
	0	24	48	72	96
	Measured concentration (mg/liter)				
14	13.39	-- <sup>a</sup>	--	--	--
12	12.20	--	--	--	--
10	10.84	10.40	--	--	--
9	9.26	8.81	--	--	--
7	7.17	6.93	--	--	--
6	5.96	5.87	5.87	5.48	5.42
4	4.38	4.26	4.20	4.04	3.98
3	2.79	2.77	2.59	2.57	2.55
2	2.05	2.04	1.98	1.97	1.96
1	0.79	0.77	0.76	0.75	0.75
0	0.09	0.09	0.09	0.09	0.09

<sup>a</sup> Measurements discontinued after 100% mortality.

Table 23. Water quality at various concentrations of nitrite in 96-hour definitive test.

Nitrite (mg N/liter)	Dissolved oxygen (mg/liter)					pH					Temperature (C)				
	0 <sup>a</sup>	24	48	72	96	0	24	48	72	96	0	24	48	72	96
20.0	8.2	7.0	-- <sup>b</sup>	--	--	8.0	8.0	--	--	--	25	25	--	--	--
16.0	8.3	6.5	--	--	--	8.0	8.0	--	--	--	25	25	--	--	--
12.0	8.0	7.8	7.7	7.1	6.9	8.0	8.0	8.0	7.9	8.0	25	25	25	25	26
10.0	8.3	6.8	6.5	6.4	6.2	7.9	8.0	8.1	8.0	8.1	25	25	25	24	25
8.0	8.0	7.6	7.3	7.1	7.0	8.0	8.0	8.0	8.0	8.0	25	25	25	24	25
6.0	8.1	7.1	7.0	6.8	6.8	8.1	8.0	8.1	8.0	8.1	25	25	25	24	25
4.0	8.1	7.3	6.5	6.3	6.3	8.0	8.1	8.0	7.9	8.0	25	25	25	25	26
1.0	8.1	6.3	6.0	5.9	5.8	8.0	8.1	8.0	8.0	8.0	25	25	25	25	25
0.0	8.2	7.9	7.2	7.0	6.8	8.1	8.0	8.0	7.9	8.1	25	25	25	25	25

<sup>a</sup> Test period in hours.

<sup>b</sup> Measurements discontinued after 100% mortality.

Table 24. Concentration of nitrite in test containers with juvenile Procambarus clarkii in 96-hour definitive test.

Nitrite (mg N/liter)	Test period				
	0 Hours	24 Hours	48 Hours	72 Hours	96 Hours
Measured concentrations (mg/liter)					
20	21.84	21.82	--	--	--
16	16.50	16.47	16.45	--	--
12	12.80	12.80	12.75	12.74	--
10	10.35	10.32	10.27	10.27	10.25
8	7.60	7.58	7.54	7.53	7.52
6	5.87	5.87	5.87	5.86	5.84
4	3.94	3.94	3.95	3.94	3.94
1	1.40	1.41	1.41	1.40	1.40
0	0.00	0.00	0.00	0.00	0.00

<sup>a</sup> Measurements discontinued after 100% mortality.



## VITA

Thomas Michael Hymel was born September 3, 1955, in Mobile, Alabama. He graduated from Biloxi High School, Biloxi, Mississippi, in 1973. He attended Sumter Area Technical College, Sumter, South Carolina, and Mississippi Gulf Coast Junior College, Gulfport, and received a diploma in air conditioning, heating, and refrigeration in May 1975. He also attended the University of South Carolina, Sumter, and Clemson University, Clemson, South Carolina and graduated with a Bachelor of Science degree in Zoology in December 1981.

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# EXAMINATION AND THESIS REPORT

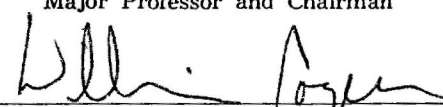
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Major Field: Fisheries

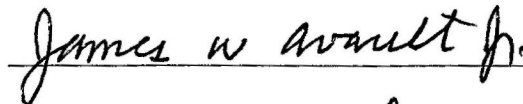
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Toxicity of Selected Water Quality Variables to  
Procambarus clarkii

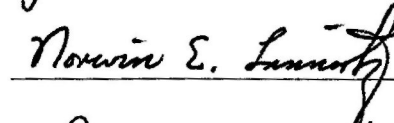
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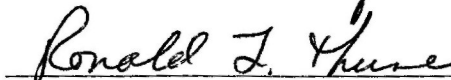
  
Major Professor and Chairman

  
Dean of the Graduate School

## EXAMINING COMMITTEE:







Date of Examination:

