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## **Bioenergetics of Red Swamp Crawfish (*Procambarus Clarkii*) and White River Crawfish (*Procambarus Acutus Acutus*) in Cultivated, Noncultivated and Wooded Ponds in South Louisiana.**

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**Bioenergetics of red swamp crawfish (*Procambarus clarkii*) and  
white river crawfish (*Procambarus acutus acutus*) in cultivated,  
noncultivated and wooded ponds in south Louisiana**

**Sanguanruang, Mattana, Ph.D.**

**The Louisiana State University and Agricultural and Mechanical Col., 1988**

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BIOENERGETICS OF RED SWAMP CRAWFISH (PROCAMBARUS CLARKII)  
AND WHITE RIVER CRAWFISH (PROCAMBARUS ACUTUS ACUTUS)  
IN CULTIVATED, NONCULTIVATED AND WOODED PONDS  
IN SOUTH LOUISIANA

A Dissertation

Submitted to the Graduate Faculty of the  
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Doctor of Philosophy

in

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by

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## ABSTRACT

Stomach content analysis and stable carbon isotope ratios were used to determine diet and food assimilation of red swamp crawfish (Procambarus clarkii) and white river crawfish (Procambarus acutus acutus) in three types of commercial ponds. Crawfish stomachs contained largely macrophytes, detritus and leaf litter, and to a lesser extent aquatic insects and zooplankton. Food habits of P. clarkii and P. acutus acutus were similar, but P. acutus acutus consumed more animal material than did P. clarkii. Food habits were similar among different sizes of P. clarkii and P. acutus acutus.

Seasonal stable carbon isotope ratios ( $\delta^{13}\text{C}$ ) of P. clarkii and P. acutus acutus were similar. Crawfish in the open pond had higher  $\delta^{13}\text{C}$  ( $\bar{X} = -18.1$ ,  $P < 0.01$ ) than those in the rice ( $\bar{X} = -23.5$ ) and wooded pond ( $\bar{X} = -26.7$ ), which indicated different major food sources for crawfish in each pond. Crawfish  $\delta^{13}\text{C}$  values changed from November through May ( $P < 0.05$ ). Temporal change in crawfish  $\delta^{13}\text{C}$  paralleled change in  $\delta^{13}\text{C}$  values of periphyton and the increased consumption of animal material by crawfish in spring. Macrophytes, detritus, periphyton and leaf litter collectively contributed 90% to crawfish growth in November, 85% in January, 61% in March and 56% in May, while insects and zooplankton contributed 6% to crawfish growth in November, 12% in January, 23% in March and 28% in May.

Food consumption, digestibility coefficients, growth and energy budget of juvenile P. clarkii and P. acutus acutus were determined in the laboratory. Crawfish were fed five natural diets (rice, Oryza sativa; alligatorweed, Alternanthera philoxeroides; 120-day-old rice

detritus; filamentous algae, Spirogyra spp.; earthworm, Lumbricus terrestris) and two formulated diets (Purina Jumbo crawfish bait and Zeigler shrimp ration). Mean daily food consumption rate ranged from 1.6% (rice detritus) to 5.8% (earthworm). Apparent dry matter digestibility (ADMD, 60-85%) and apparent energy digestibility (AED, 63-88%) coefficients for the seven diets were similar for both crawfish species. Crawfish fed formulated diets converted 24-33% of ingested energy ( $K_1$ ) and 27-38% of digested energy ( $K_2$ ) to growth while crawfish fed earthworm and rice exhibited little growth ( $K_1 = 1\%$  and  $K_2 = 2\%$ ). Crawfish fed algae, alligatorweed and rice detritus catabolized tissue to meet energy needs.



## INTRODUCTION

Production of red swamp crawfish, Procambarus clarkii, and white river crawfish, Procambarus acutus acutus, is a major aquaculture industry in Louisiana. In 1986-1987 about 50,000 ha of procambarid crawfish were cultivated in Louisiana with an estimated dock-side value of \$50 million (Louisiana Cooperative Extension Service 1987). Procambarid crawfish are also commercially cultivated in Arkansas, Florida, Georgia, Mississippi, North Carolina, South Carolina and Texas.

Procambarid crawfish culture as currently practiced in the U.S. utilizes an extensive management regime. Hatcheries are not used to produce juveniles for stocking ponds; rather self-sustaining populations of crawfish are established after ponds are stocked with 50-75 kg/ha of brood crawfish (Avault and Huner 1985). Crawfish are normally not fed formulated rations. Cultivated vegetation such as rice (Oryza sativa<sup>1</sup>), millets (Echinochloa spp.), sorghums (Sorghum spp.) and grasses (Graminae) (Chien 1978; Chien and Avault 1980; Avault et al. 1983; Johnson et al. 1983; Brunson 1987; Brunson and Taylor 1987), and "natural" vegetation such as smartweed (Polygonum spp.), alligatorweed (Alternanthera philoxeroides), water primrose (Ludwigia spp.), delta duck potato (Sagittaria graminea platyphylla) and other volunteer terrestrial and aquatic plants (Clark et al. 1975; Rivas et al. 1979; Johnson et al. 1983; Garces and Avault 1985; Avault and Huner 1985) are grown as food for crawfish in summer when ponds are dry and

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<sup>1</sup> Scientific nomenclature for plants follows Radford et al. (1973).

the crawfish are in burrows (Huner and Barr 1984). Vegetation established in crawfish ponds is the base of a detrital food web that serves as an energy source for crawfish (Goyert and Avault 1977; Avault et al. 1983).

Crawfish are polytrophic benthic omnivores (Momot et al. 1978; Lorman and Magnuson 1978; Momot 1984) that consume a diversity of plant and animal materials including detritus<sup>2</sup> (Huner and Barr 1984; Goddard 1988). Detritus with a carbon:nitrogen ratio of  $\leq 17:1$  is an important food for crawfish (Mason 1975; Momot et al. 1978; Morrissy 1979; Reynolds 1979; Avault et al. 1983; Mills and McCloud 1983). Plant material found in the natural diet of crawfish is primarily decomposed leaves and roots of aquatic and terrestrial vegetation (Tack 1941; Abrahamsson 1966; Prins 1968; Mason 1975; Momot et al. 1978; Hessen and Skurdal 1984; Westman et al. 1986; Huner and Naqvi 1986). The most common animals consumed by crawfish are aquatic insect larvae, mollusks, oligochaetes, small crustaceans and crawfish (Tack 1941; Vannote 1963; Abrahamsson 1966; Moriarty 1973; Mason 1975; Momot et al. 1978; Reynolds 1979; Capelli 1980; Hessen and Skurdal 1984; Westman et al. 1986; Huner and Naqvi 1986).

Little information is available on the relative proportion of plant and animal material in the diets of P. clarkii and P. acutus acutus. Generally, living plants and detritus are the most abundant component of the diet, whereas animal matter contributes a small percentage of the volume of food consumed (Huner and Naqvi 1986).

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<sup>2</sup> Detritus consists of decomposed plant and animal fragments and associated micro-organisms, i.e., bacteria, fungi, algae and protozoans (Goddard 1988).

Although the majority of the P. clarkii and P. acutus acutus diet is of plant origin, animal matter (principally benthic organisms<sup>3</sup>) and periphyton<sup>4</sup> have been suggested as important foods for crawfish (Chien 1980; Chien and Avault 1985).

Numerous studies have been conducted on the feeding habits and food preferences of other crawfish species using stomach content analysis (Tack 1941; Prins 1968; Mason 1975; Capelli 1980; Hessen and Skurdal 1984; Westman et al. 1986). However, diet assessment of crawfish using stomach content analysis is difficult because the food is ground into small fragments and unidentifiable after it passes through the gastric mill (Huxley 1974; Huner and Barr 1984; Holdich and Reeve 1988). Additionally, stomach content analysis neither identifies which materials in the gut are metabolized and assimilated by crawfish nor quantifies the contribution of those foods to growth of crawfish.

Researchers have used stable carbon isotopes as a naturally occurring tracer to aid in identifying foods metabolized by aquatic animals. Haines (1976) and Haines and Montague (1979) successfully used the stable carbon isotope method to identify cordgrass (Spartina alterniflora) as an important component of the foods of fiddler crab (Uca pugnax). Rau (1980) used stable carbon isotopes to determine if aquatic insects fed on plankton, periphyton, or allochthonous terrestrial plant litter (conifer tree detritus). Schroeder (1983a,

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<sup>3</sup> Benthic organisms associated with the detritus-based ecosystem include small crustaceans, worms, mollusks and insects.

<sup>4</sup> Periphyton refers to organisms, both flora and fauna, attached to submerged stems or leaves of green aquatic and semi-aquatic plants (Cole 1979).

1983b, 1983c) employed the stable carbon isotope techniques to determine sources of fish and prawn nutrition in ponds containing Tilapia aurea, Hypophthalmichthys molitrix, Cyprinus carpio, Ctenopharyngodon idella and Macrobrachium rosenbergii. Anderson et al. (1987) used stable carbon isotope ratios to estimate the relative contribution of natural foods and feed rations to the growth of Penaeus vannamei. Lilyestrom et al. (1987) used stable carbon isotope ratios to identify the foods of Malaysian prawn (Macrobrachium rosenbergii) and channel catfish (Ictalurus punctatus) in a polyculture system.

Carbon isotopic measurements ( $^{13}\text{C}/^{12}\text{C}$  ratio expressed as  $\delta^{13}\text{C}$ ), in contrast to stomach content analysis, can be used to determine which foods are converted to tissue. The determination is based on isotopic similarity of an animal to its diet. Animal tissue has a higher  $\delta^{13}\text{C}$  value than the diet by up to two parts per mil because of the preferential loss of  $^{12}\text{CO}_2$  in respiration (DeNiro and Epstein 1978; Fry et al. 1978; Fry and Arnold 1982). The  $\delta^{13}\text{C}$  of crawfish and the  $\delta^{13}\text{C}$  of available foods must be determined to obtain a quantitative measurement of the relative contribution of each food or food group to the diet of crawfish. Because of overlapping  $\delta^{13}\text{C}$  values in some potential foods, the relationship between crawfish tissue and diet is difficult to interpret, therefore direct knowledge of crawfish feeding habits (stomach content analysis) is necessary for correct interpretation of isotope data.

Although much research has been conducted on evaluating planted forages for P. clarkii and P. acutus acutus (Clark et al. 1975; Chien 1978; Rivas et al. 1979; Chien and Avault 1980; Avault et al. 1983;

Johnson et al. 1983; Avault and Huner 1985; Garces and Avault 1985; Brunson 1987; Brunson and Taylor 1987; Brunson and Griffin 1988), these studies concentrated on crawfish growth and yield trials. There is limited information available on the consumption, digestibility, and utilization of foods commonly cultivated for P. clarkii and P. acutus acutus. A few investigators have examined digestibility coefficients for foods and feed ingredients fed to P. clarkii and P. acutus acutus. Wiernicki (1984) reported that the apparent dry matter digestibility of elodea (Egera densa) fed to P. clarkii (20-90 mm total length, TL) ranged from 20 to 45% depending on crawfish size. Brown et al. (1986) reported that digestibility coefficients for alpha-soy protein, shrimp meal, wheat gluten, fish meal, casein, chitin, soybean meal, wheat bran and rice bran fed to adult P. clarkii ranged from 25 to 89%.

Digestibility coefficients and energy budgets for other commercially cultivated or harvested crawfish species have been determined, including Cherax destructor (Woodland 1969), Pacifastacus leniusculus (Moshiri and Goldman 1969; Mason 1975), Orconectes limosus (Kossakowski 1975), Astacus leptodactylus (Tcherkashina 1977) and Orconectes virilis (Jones and Momot 1983).

Procambarid crawfish culture depends on cultivated forages and natural foods containing an appropriate balance of nutrients and energy for optimum growth. Determination of digestibility coefficients for foods commonly present in commercial crawfish ponds and quantification of energy budgets for P. clarkii and P. acutus acutus is necessary for understanding the role of these cultivated forages and other natural foods in crawfish production.

DIET AND FOOD ASSIMILATION OF RED SWAMP CRAWFISH AND WHITE RIVER  
CRAWFISH IN COMMERCIAL PONDS AS DETERMINED BY STOMACH  
CONTENT ANALYSIS AND STABLE CARBON ISOTOPE RATIOS

**Objectives**

Objectives of this study were to determine the diet and food assimilation of P. clarkii and P. acutus acutus in three types of commercial ponds using stomach content analysis and stable carbon isotope analysis. Stomach content analysis was used to complement interpretation of isotope data by documenting the presence or absence of foods in crawfish stomachs.

**Materials and Methods**

Study Area

Food habit studies of P. clarkii and P. acutus acutus were conducted in three types of commercial crawfish ponds - rice, open and wooded - at the Indigo Island Crawfish and Migratory Waterfowl Experimental Station, 40 km south of Baton Rouge in Iberville Parish, south-central Louisiana (Figure 1). The island is owned and operated by William's Incorporated.

In the "rice" pond (4.5 ha), rice was the predominant forage for crawfish. Predominant vegetation in the "open" pond (10.5 ha) was a mixture of terrestrial grasses and semi-aquatic flora. The plant species most common in this impoundment were fall panicum (Panicum dichotomiflorum), flatsedge (Cyperus odoratus), millet (Echinochloa colonum), millet fimbristylis (Fimbristylis miliacea), sprangletop

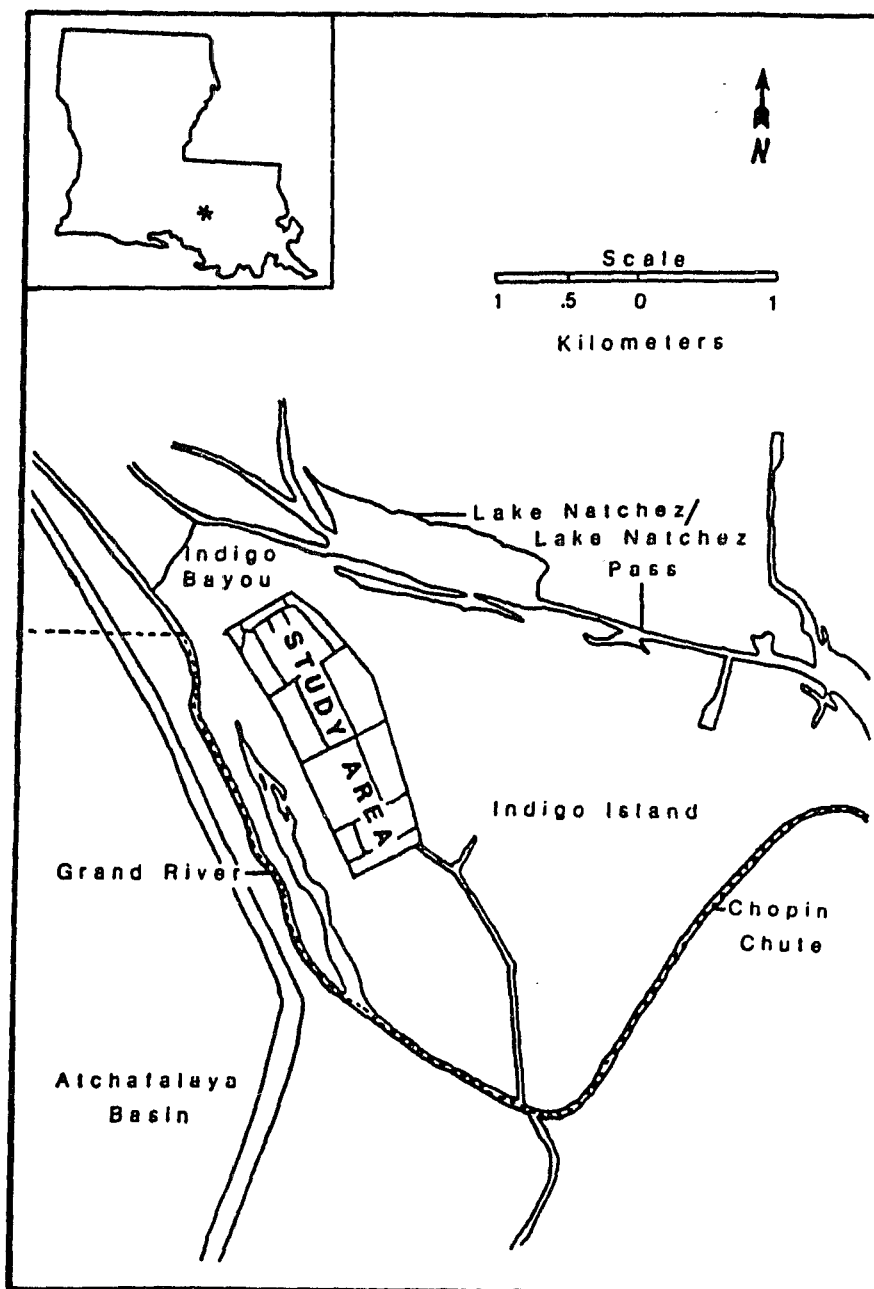


Figure 1. Location of three types of commercial crawfish ponds used in crawfish diet and food assimilation study, Indigo Island Crawfish and Migratory Waterfowl Experimental Station, Iberville Parish, Louisiana.

(Leptochloa filiformis), swamp smartweed (Polygonum punctatum), umbrella-sedge (Cyperus iria) and Walter's millet (Echinochloa walteri) (Takagi 1983; Martin 1985). The "wooded" pond had standing trees and understory vegetation. Major tree species were red maple (Acer rubrum), ash (Fraxinus pennsylvanica) and black willow (Salix nigra) (Harrison 1984). Understory vegetation was composed of floating aquatic plants (water hyacinth Eichhornia crassipes, duckweed Lemna spp. and water fern Azolla carolinensis), and emergent terrestrial and aquatic plants (fall panicum, alligatorweed and smartweed) (Harrison 1984).

Ponds were managed to provide wintering ground for waterfowl and for crawfish production, since 1979 (Nassar 1982). Ponds are drained in May to stimulate burrowing of crawfish and they remain dry through October (de la Bretonne and Avault 1977). Beginning in July 1985 the rice pond was thoroughly disked to eliminate undesirable plant species, and then flooded with about 2.5 cm of water. In mid-July 1985 domestic rice seed (cultivar 'Mars') was aerially seeded in the rice pond at a rate of 150 kg/ha. In July 1985, the open pond was disked and natural vegetation was allowed to reestablish. Fertilizers, pesticides and herbicides were not used in the rice or open pond but shallow flooding was employed to control undesirable plant species in the rice pond. Vegetation provided seeds for wintering waterfowl and forage for crawfish (Nassar 1982). The wooded pond remained dry until October and natural vegetation was allowed to grow. The three ponds were filled in October to an average depth of 0.6 m with water from a canal connected to the Grand River (Figure 1). Ponds were occasionally flushed with



canal water to maintain adequate dissolved oxygen concentrations throughout October-May production season.

Crawfish were commercially harvested with two-funnel, 1.9 cm hexagonal mesh traps from January through May. Approximately 25 traps/ha were used in each pond, but fishing efforts were highly variable among ponds. Pond surface area, flooding and draining dates, trapping effort and crawfish yield from the three ponds during 1985-1986 are detailed in Table 1.

#### Stomach Content Analysis of Crawfish

Crawfish were collected with a 5-mm mesh dip net (Lutz 1983) and a 6-mm mesh, 4.5-m long seine (Momot and Romaine 1983) on 20 November 1985, 22 January, 25 March and 13 May 1986. On each sampling date, 5-10 dip net sweeps or 2-4 seine hauls were made at random locations in each pond, 3-5 m out from the perimeter levee, to obtain about 20 P. clarkii and 20 P. acutus acutus of  $\geq 35$  mm total length (TL, tip of rostrum to end of telson). Crawfish were chilled in ice to prevent regurgitation of stomach contents, killed and preserved in 10% formalin within one hour after collection (Clary 1985). After two weeks, crawfish were transferred into 80% ethyl alcohol until further analysis (Pennak 1978).

In the laboratory, cardiac and pyloric stomachs (Figure 2) were excised from crawfish, and weighed to the nearest 0.01 g on a digital balance (Mettler Model PE1600). The stomach contents were removed by rinsing with 25 ml of 5% formalin. The weight of material consumed by crawfish was determined as the weight difference between the rinsed and

Table 1. Pond surface area, flooding and draining dates, trapping effort and crawfish yield for rice, open and wooded ponds, Indigo Island Crawfish and Migratory Waterfowl Experimental Station, Iberville Parish, Louisiana.

Pond	Surface Area (ha)	Flooding Date	Draining Date	Trapping Effort (man-day)	Crawfish Yield (kg/ha)
Rice	4.5	17 Oct 85	23 May 86	33	534
Open	10.5	25 Oct 85	26 May 86	34	244
Wooded	65.0	16 Oct 85	15 May 86	67	81

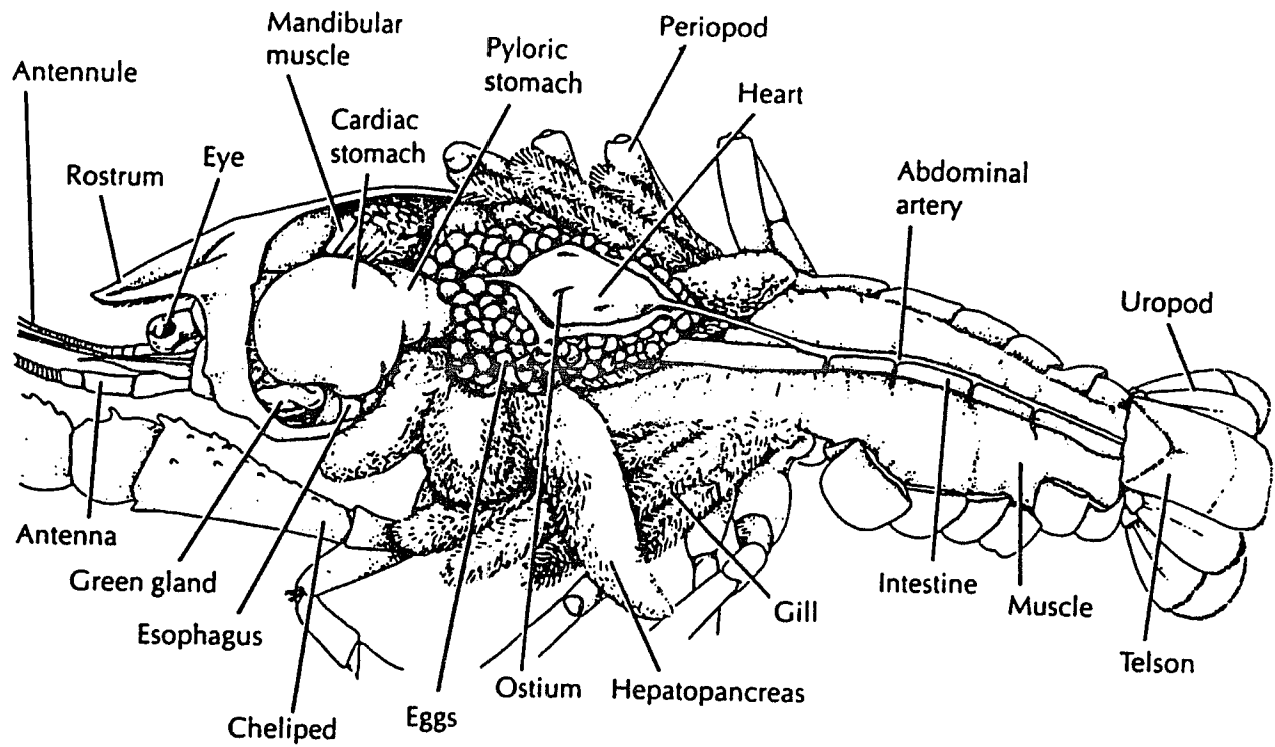


Figure 2. Diagram of crawfish internal anatomy (adapted from Pierce and Maugel 1987).

stomach weights. Stomach contents were stained with 0.5-1 ml of 0.03 M Eosin B (Williams 1974), an acid xanthene dye (Lillie 1977), in a petri dish. Eosin B was used to separate animal material from plant material. Eosin B stains animal material red but does not stain living or dead plant material (Williams 1974). After 24 hours, stained samples were rinsed thoroughly with 5% formalin, and materials were identified with the aid of a stereoscopic microscope (Zeiss Model 475052) and compound microscope (Zeiss Model 470916) (Capelli 1980). All materials were qualitatively identified to the closest taxonomic group or category using the taxonomic keys of Needham and Needham (1962); Edmondson (1963); Pennak (1978); Prescott 1978; McCafferty (1981); Balcer et al. (1984); Merritt and Cummins (1984) and Pentecost (1984).

A quantitative assessment of materials consumed by crawfish was determined using two methods. The method of frequency of occurrence (Jones 1950) determines the frequency of each "food" group in all crawfish analyzed and provides information on how often each particular "food" was found in crawfish stomachs. The points method (Hynes 1950) utilized a procedure in which each stomach was awarded a point according to its degree of fullness (0 to 1.0 as determined by visual observation, 0 = empty, 1.0 = 100% fullness), and each "food" found in the stomach was awarded a point according to the proportion of the total volume of the stomach that it occupied (0 to 1.0 as determined by visual observation). The total score (0 to 100) of each "food" group consumed by crawfish was calculated by multiplying the point score of a particular food group by the point score of degree of fullness for the

same stomach, and multiplied by 100.

### Stable Carbon Isotope Analysis

#### Sample Collection

Crawfish and potential food organisms needed for stable carbon isotope analysis were collected in the three ponds on the same dates that crawfish were collected for stomach content analysis. The abdominal muscle tissue of three juvenile (35-75 mm TL) and three adult crawfish (> 75 mm TL) of each species from each sampling date was removed for carbon isotope analysis (Schroeder 1983a, 1983b, 1983c).

Zooplankton was collected with a 10-cm diameter translucent plastic tube placed vertically through the water column from water surface to pond bottom (about 50-60 cm depth). The bottom of the tube was capped while still in the water, the tube was lifted and the contents poured sequentially through a 125- $\mu$  mesh metal sieve (U.S. Standard No. 120) for macro-zooplankton and 79- $\mu$  mesh Nitex sieve for micro-zooplankton (Huner and Nagvi 1986; Lilyestrom et al. 1987). Zooplankton was washed into 10 ml glass vials with distilled water. The procedure was repeated until an estimated 3-5 mg (dry weight) of macro- and micro-zooplankton had been collected for carbon isotope analysis.

Filamentous algae, macrophytes, detritus and leaf litter (wooded pond only) were collected by hand. Leaf litter was defined as decomposed tree leaves. Periphyton attached to the external submerged surfaces of living macrophytes was removed by gentle scraping with a spatula. Sediment was collected from 3 to 5 locations in each pond

with a 7.5 cm diameter polyvinyl chloride (PVC) pipe pushed 1 cm in the pond bottom. Macro-benthos was washed and separated from pond sediment in a 600  $\mu$  metal sieve (U.S. Standard No. 30) (Huner and Naqvi 1986). Macro-invertebrates and vertebrates were collected with a 1-mm mesh sweep net (Huner and Naqvi 1986). Natural crawfish baits, such as menhaden (Brevoortia patronus), mullet (Mugil cephalus) and gizzard shad (Dorosoma cepedianum), were collected from baited crawfish traps.

### Isotope Analysis

All samples of aquatic biota and sediment were dried at 90°C for 48 hours in a gravity convection oven and stored in glass vials. Prior to  $\delta^{13}\text{C}$  analysis, each sample was homogenized to a particle size of 1 mm with a mortar and pestle. Samples were immersed for 1-12 hours in 0.3 N HCl to remove inorganic carbon ( $\text{CaCO}_3$ ), then centrifuged at 6,000 rpm and rinsed five times with distilled water before drying at 90°C (Chmura et al. 1987; Lilyestrom et al. 1987).

A 3-7 mg aliquot of each sample was flame sealed in a separate 10 to 15-cm long by 6-mm diameter evacuated quartz tube with 50-80 mg of pelletized copper oxide ( $\text{CuO}$ ) powder as oxidizer and a 2-cm long, 4- $\mu$  diameter silver wire as catalyst (Sofer 1980; Chmura et al. 1987). Tubes were placed in a muffle furnace at 900°C for a minimum of nine hours to combust organic matter to gases (Sofer 1980), then cooled to room temperature. Carbon dioxide gas was separated from other sample components through cryogenic distillation and the  $^{13}\text{CO}_2/^{12}\text{CO}_2$  ratio was determined with a Nier-type automated triple collector mass spectrometer in the Stable Isotope Laboratory, Department of Geology

and Geophysics, Louisiana State University (Aharon and Chappell 1986). Completeness of combustion and accuracy of mass spectrometry were determined by analysis of an international standard, poly-ethylene foil (PEF 1), relative to a Peedee Belemnite (PDB) standard. Peedee Belemnite standard is a carbonate of the fossil skeleton of Belemnitella americana from the Peedee formation of South Carolina, which is crushed and ground prior to treatment with acid (Craig 1957). The measured value of PEF 1 was -31.8 compared to the accepted value of  $-31.6 \pm 0.2$  parts per mil (Gerstenberger 1982). Results were reported in delta ( $\delta$ ) notation in part per mil relative to the international PDB standard (Craig 1957).

$$\delta^{13}\text{C} = \left[ \frac{^{13}\text{C}/^{12}\text{C sample}}{^{13}\text{C}/^{12}\text{C standard}} - 1 \right] * 1,000 \quad (1)$$

#### Predictive Value

The use of  $\delta^{13}\text{C}$  to determine the contribution of each food to the growth of an animal is based on the isotopic similarity of the animal body and the diet (Schroeder 1983a, 1983b, 1983c; Anderson et al. 1987; Lilyestrom et al. 1987). In a complex system where two or more foods of different  $\delta^{13}\text{C}$  values are consumed by an animal, the carbon isotopic value of the animal body cannot be used directly to determine how much each food contributed to tissue elaboration. Additional information, such as the digestibility coefficient and the amount of each food consumed, are needed. In this study, percentages of identifiable foods in crawfish stomachs were obtained using the points

method, and digestibility coefficients (energy ratio of digested food to consumed food) for macrophytes, algae and worms were obtained from the digestibility study. Digestibility coefficients for other identifiable foods were estimated from data for similar species (Table 2). The  $\delta^{13}\text{C}$  value of each unidentifiable food group found in the stomach was estimated as an average of potential foods available in the pond on that sampling date. For example,  $\delta^{13}\text{C}$  value of vegetative material in the rice pond in November was an average of  $\delta^{13}\text{C}$  values of green rice (-25.4), rice detritus (-15.1) and periphyton (-24.9). Although periphyton could not be directly identified from stomach contents, periphyton  $\delta^{13}\text{C}$  values were always used as part of vegetative material  $\delta^{13}\text{C}$  values in equation 2 to estimate the  $\delta^{13}\text{C}$  of crawfish. The same method was applied to calculate  $\delta^{13}\text{C}$  values of unidentified algae and insects.

Predicted  $\delta^{13}\text{C}$  value for each crawfish was obtained from a simple mixed model, based on the assumption that sources of crawfish carbon are in direct proportion to the amount of each food assimilated:

$$\delta^{13}\text{C}_{\text{crawfish}} = \sum_{i=1}^n \left[ \frac{(P_i * D_i)}{\sum_{i=1}^n (P_i * D_i)} * \delta^{13}\text{C}_i \right] \quad (2)$$

where

$i$  = index for each food found in a crawfish's stomach;

$n$  = numbers of foods found in a crawfish's stomach;



Table 2. Estimated digestibility coefficients ( $D_i$ ) for foods consumed by Procambarus clarkii and Procambarus acutus acutus in three types of commercial ponds, Indigo Island Crawfish and Migratory Waterfowl Experimental Station, Iberville Parish, Louisiana, November 1985 through May 1986.

Food	$D_i$	Reference
Macrophytes <sup>1</sup>	0.6 <sup>2</sup>	
Algae	0.8 <sup>2</sup>	
Plant seeds	0.3 <sup>3</sup>	
Zooplankton	0.8	Condrey et al. (1972) <sup>4</sup>
Benthic worms	0.9 <sup>2</sup>	
Menhaden bait	0.9	Jones and Momot (1983) <sup>4</sup>
Insects	0.7	Moshiri and Goldman (1969) <sup>4</sup>

<sup>1</sup> Macrophytes plus detritus, periphyton and leaf litter (in wooded pond only).

<sup>2</sup> Digestibility coefficient determined by the digestibility study.

<sup>3</sup> Digestibility coefficient estimated by the author.

<sup>4</sup> No digestibility coefficient value for Procambarus clarkii and Procambarus acutus acutus, thus the value was obtained from similar species.

$$\begin{aligned}
 P_i &= \text{percentage of the } i^{\text{th}} \text{ food found in a crawfish's} \\
 &\quad \text{stomach as determined by the points method;} \\
 D_i &= \text{digestibility coefficient for the } i^{\text{th}} \text{ food;} \\
 \delta^{13}\text{C}_i &= \delta^{13}\text{C of the } i^{\text{th}} \text{ food by mass spectrometry;} \\
 \delta^{13}\text{C}_{\text{crawfish}} &= \text{calculated crawfish } \delta^{13}\text{C value.}
 \end{aligned}$$

The proportion of each food contributed to crawfish tissue elaboration ( $F_i$ ) is calculated as follows:

$$F_i = \frac{\left[ (P_i * D_i) / \sum_{i=1}^n (P_i * D_i) \right] * \delta^{13}\text{C}_i}{\delta^{13}\text{C}_{\text{crawfish}}} \quad (3)$$

where

$$F_i = \text{fractional contribution of the } i^{\text{th}} \text{ food to the} \\
 \text{elaboration of crawfish tissue.}$$

### Calculation Example

Assume that three food components (A, B and C) were found in a crawfish's stomach. The percentage of each food was estimated using the points method. Digestibility coefficients and the  $\delta^{13}\text{C}$  of each food were determined by digestibility study and mass spectrometer, respectively. Using equations 2 and 3, the  $\delta^{13}\text{C}$  value of crawfish and fractional contributions of each food to the elaboration of crawfish tissue were calculated. Crawfish  $\delta^{13}\text{C}$  value by calculation was -18.6 and foods A, B and C contributed 64.5%, 7% and 28.5%, respectively, to crawfish growth (Table 3).

Table 3. Example calculation of crawfish  $\delta^{13}\text{C}$  value and relative contribution of each food component to crawfish tissue.

Food	$P_i$	$D_i$	$P_i * D_i$	$\delta^{13}\text{C}_i$	$\left[ \frac{P_i * D_i}{\sum P_i * D_i} * \delta^{13}\text{C}_i \right]$	$F_i$ (%)
A	0.6	0.6	0.36	-20.0	-12.0	64.5
B	0.1	0.3	0.03	-25.0	-1.3	7.0
C	0.3	0.7	0.21	-15.0	-5.3	28.5
Sum	1.0		0.60		-18.6	100.0

$P_i$  is the percentage that the  $i^{\text{th}}$  food (in weight) makes up of the stomach contents by the points method (range: 0 to 1.0).

$D_i$  is the digestibility coefficient for the  $i^{\text{th}}$  food by crawfish (range: 0 to 1.0).

$\delta^{13}\text{C}_i$  is  $\delta^{13}\text{C}$  of the  $i^{\text{th}}$  food by mass spectrometry.

$F_i$  is the fractional contribution of the  $i^{\text{th}}$  food to the elaboration of crawfish tissue.

### Statistical Analysis

Data from stomach content analysis study were analyzed by an analysis of variance with a 2\*3\*3\*4 factorial arrangement to determine if statistical differences existed in the feeding habits of P. clarkii and P. acutus acutus. Main effects were crawfish species (2), crawfish size (3), pond type (3) and season (4). Response variables were the total scores (calculated from the points method) for macrophytes, seeds and aquatic fauna found in crawfish stomachs.

The  $\delta^{13}\text{C}$  values of crawfish obtained by spectrometry were analyzed by analysis of variance with pond type, season and species as main effects. A paired-comparison t-test was employed to determine difference between  $\delta^{13}\text{C}$  values of crawfish calculated from equation 2 and  $\delta^{13}\text{C}$  values determined from mass spectrometry. The linear relationship between the two  $\delta^{13}\text{C}$  values was calculated using the Pearson product-moment correlation test (Steel and Torrie 1980).

## Results

### Stomach Content Analysis

Crawfish stomach content analysis by frequency of occurrence demonstrated that the diet of P. clarkii was principally macrophytes<sup>5</sup> (91% occurrence), animal material (89% occurrence) and plant seeds (48% occurrence) (Table 4). Leaf litter occurred in 91% of the stomachs of P. clarkii collected from the wooded pond. The diet of P. acutus acutus was similar to that of P. clarkii. Macrophytes (91% occurrence), animal material (90% occurrence) and plant seeds (58% occurrence) were the most common items in stomachs of P. acutus acutus (Table 5). No P. acutus acutus were collected from the wooded pond. Macrophytes (living plant material dominating in November and January, and plant detritus dominating in March and May) and most animal material in crawfish stomachs could not be assigned to specific taxonomic categories because of the advanced stage of digestion.

Plant seeds present in the stomachs of crawfish were umbrella-sedge, wild millet, sprangletop (Leptochloa filiformis) and smartweed (Tables 4 and 5). A few species of filamentous algae were present in crawfish stomachs but only Spirogyra spp. could be identified. Identifiable animal material found in crawfish stomachs included oligochaetes, crustaceans (copepods, cladocerans, isopods and ostracods), chironomid larvae, coleopterans and dragonfly naiads. Unidentified aquatic insects and insect eggs were also encountered.

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<sup>5</sup> Macrophytes plus detritus, periphyton and leaf litter (in wooded pond only).

Table 4. Stomach content analysis, by frequency of occurrence, for Procambarus clarkii in rice, open and wooded ponds, Indigo Island Crawfish and Migratory Waterfowl Experimental Station, Iberville Parish, Louisiana, November 1985 through May 1986.

Food	% Occurrence		
	Rice (N=80)	Open (N=75)	Wooded (N=80)
Macrophytes <sup>1</sup>	94	88	91
Animal material (unidentified)	91	87	89
Filamentous algae	13	3	0
Seed	(64)	(43)	(38)
<u>Cyperus iria</u>	53	7	0
<u>Echinochloa colonum</u>	20	15	1
<u>Leptochloa filiformis</u>	3	17	6
<u>Polygonum punctatum</u>	0	0	14
Unidentified	3	21	30
Annelida			
Oligochaeta	0	0	4
Crustacea	(21)	(9)	(5)
Cladocera	14	5	3
Copepoda	10	3	0
Isopoda	1	0	0
Ostracoda	4	1	3
Insecta	(21)	(5)	(6)
Chironomidae	5	1	6
Unidentified	19	5	4
Bait			
<u>Brevoortia patronus</u>	9	0	0
Empty	6	12	9

<sup>1</sup> Macrophytes plus detritus, periphyton and leaf litter (in wooded pond only).

Table 5. Stomach content analysis, by frequency of occurrence, for Procambarus acutus acutus in rice and open ponds, Indigo Island Crawfish and Migratory Waterfowl Experimental Station, Iberville Parish, Louisiana, November 1985 through May 1986.

Food	% Occurrence	
	Rice (N=63)	Open (N=63)
Macrophytes <sup>1</sup>	95	86
Animal material (unidentified)	94	86
Filamentous algae	22	2
Seed	(62)	(54)
<u>Cyperus iria</u>	59	18
<u>Echinochloa colonum</u>	11	24
<u>Leptochloa filiformis</u>	6	21
Unidentified	3	18
Crustacea	(10)	(5)
Cladoceran	5	3
Copepoda	5	5
Isopoda	2	2
Insecta	(11)	(10)
Chironomidae	3	2
Unidentified	11	10
Vertebrata		
Amphibia		
<u>Rana catesbeiana</u>	0	2
Bait		
<u>Brevoortia patronus</u>	6	0
Empty	5	14

<sup>1</sup> Macrophytes plus detritus, periphyton and leaf litter (in wooded pond only).

Analysis of stomach contents by the points method (Tables 6 and 7) indicated that crawfish fed principally on plant material and to a lesser extent on aquatic fauna. P. clarkii and P. acutus acutus consumed similar quantities of macrophytes ( $\bar{X} = 47\%$ ) and plant seeds ( $\bar{X} = 8\%$ ) ( $P > 0.05$ , Appendix Tables 1 and 2) but P. acutus acutus consumed more animal material ( $\bar{X} = 10\%$ ) than did P. clarkii ( $\bar{X} = 5\%$ ) ( $P < 0.05$ , Appendix Table 3). Menhaden ( $\bar{X} = 20\%$ ), used as crawfish bait in the rice pond only, was consumed by P. clarkii and P. acutus acutus in May. The amount of food consumed by crawfish (degree of fullness) was lowest in mid-winter (56% in January) and highest in spring (72% in March and 71% in May) (Tables 6 and 7).

The amounts of macrophyte and seed consumed by P. clarkii and P. acutus acutus differed among ponds and changed during the October through May production season ( $P < 0.05$ , Appendix Tables 1 and 2). The amount of animal material consumed by crawfish increased from 2% in November to 10% in May ( $P < 0.05$ , Appendix Table 3). No differences were found in the amounts of macrophyte, animal material and aquatic plant seed consumed by small ( $< 55$  mm TL), medium (55-75 mm TL) and large crawfish ( $> 75$  mm TL) ( $P > 0.05$ , Appendix Tables 1, 2 and 3).

The amount of macrophyte consumed by crawfish in the rice pond was high (41%), while plant seeds (13%), algae (3%), animal matter (7%) and bait (5%) were consumed in lesser amounts (Tables 6 and 7). Consumption of macrophytes decreased from fall through spring with a concomitant increase in the consumption of animal matter. In this study, it was obvious that crawfish consumed only rice stems, leaves and roots. Rice grain was not found in crawfish stomachs at any time.



Table 6. Stomach content analysis, by the points method, for Procambarus clarkii in rice, open and wooded ponds, Indigo Island Crawfish and Migratory Waterfowl Experimental Station, Iberville Parish, Louisiana, November 1985 through May 1986.

Date	N	Size (mm)	Macro- phytes <sup>1</sup>	Seeds	Algae	Animal	Bait <sup>2</sup>	Total <sup>3</sup>
<u>Rice</u>								
Nov	20	35-75	63	8	0	2	0	73
Jan	20	40-55	49	<1	0	4	0	54
Mar	20	55-100	28	37	2	4	0	71
May	20	70-95	38	2	1	7	22	70
Weighted mean			45	12	1	4	6	67
<u>Open</u>								
Nov	20	35-85	64	3	0	1	0	68
Jan	20	40-100	44	3	0	4	0	51
Mar	20	55-100	55	2	1	5	0	62
May	20	60-90	38	<1	<1	18	0	57
Weighted mean			50	2	<1	7	0	59
<u>Wooded</u>								
Nov	20	35-80	21	15	0	2	0	38
Jan	20	50-80	47	18	0	3	0	68
Mar	20	45-90	64	2	0	10	0	76
May	20	65-100	67	0	0	6	0	73
Weighted mean			50	9	0	5	0	64
Overall weighted mean			48	8	<1	5		63

<sup>1</sup> Macrophytes plus detritus, periphyton and leaf litter (in wooded pond only).

<sup>2</sup> Menhaden used in the rice pond, mullet and gizzard shad used in the open and wooded pond.

<sup>3</sup> Mean total amount of food in crawfish's stomach or degree of fullness.

Table 7. Stomach content analysis by the points method for Procambarus acutus acutus in rice, open and wooded ponds, Indigo Island Crawfish and Migratory Waterfowl Experimental Station, Iberville Parish, Louisiana, November 1985 through May 1986.

Date	N	Size (mm)	Macro-phytes <sup>1</sup>	Seeds	Algae	Animal	Bait <sup>2</sup>	Total <sup>3</sup>
<u>Rice</u>								
Nov	20	35-65	57	5	0	4	0	66
Jan	20	45-80	50	3	0	6	0	59
Mar	13	70-95	16	42	9	3	0	70
May	10	75-105	25	4	12	24	18	83
Weighted mean			37	13	5	9	5	70
<u>Open</u>								
Nov	20	35-60	72	1	0	2	0	75
Jan	7	75-100	40	5	0	2	0	46
Mar	16	75-100	49	5	0	28	0	82
May	20	75-100	60	<1	<1	11	0	72
Weighted mean			55	3	<1	11	0	69
Overall weighted mean			46	8	3	10	2	69

<sup>1</sup> Macrophytes plus detritus, periphyton and leaf litter (in wooded pond only).

<sup>2</sup> Menhaden used in the rice pond, mullet and gizzard shad used in the open pond.

<sup>3</sup> Mean total amount of food in crawfish's stomach or degree of fullness.

Plant seeds comprised 40% of the stomach contents in March but only 2-6% in November, January and May. Menhaden was found in P. clarkii and P. acutus acutus stomachs only in May (20%) (Tables 6 and 7). About 6% of the crawfish had empty stomachs during the production season, with a peak in May (13% empty) (Table 8).

In the open pond, crawfish consumed 53% macrophytes, 2% plant seeds, <1% algae and 9% animal matter (Tables 6 and 7) averaged over the production season. The amount of vegetative material consumed by crawfish remained relatively constant throughout the season but the consumption of animal matter increased from 2% in November to 15% in May ( $P < 0.01$ , Tables 6 and 7). Crawfish with empty stomachs averaged 13% in the open pond but 43% of the crawfish sampled in May in the open pond had empty stomachs (Table 8).

Vegetative material consumed by P. clarkii in the wooded pond increased from 21% in November to 67% in May with an overall seasonal mean of 50%. Plant seeds consumed by P. clarkii were 15% in November and 18% in January and declined to 2% and 0% in March and May, respectively (Table 6). No algae were found in the stomachs of P. clarkii collected from the wooded pond. On average, 9% of the crawfish collected in the wooded pond had empty stomachs and most of these were observed in November (20%).

Table 8. Percent of Procambarus clarkii and Procambarus acutus acutus with empty stomachs in rice, open, and wooded ponds, Indigo Island Crawfish and Migratory Waterfowl Experimental Station, Iberville Parish, Louisiana, November 1985 through May 1986.

Date	Rice		Open		Wooded		Water Temperature (°C)
	N	(%)	N	(%)	N	(%)	
20 Nov 85	40	8	40	0	20	20	16
22 Jan 86	40	3	27	3	20	5	12
25 Mar 86	33	0	36	5	20	0	17
13 May 86	30	13	40	43	20	10	24
Mean		6		13		9	

Stable Carbon Isotope Analysis of Crawfish  
and Potential Foods

The  $\delta^{13}\text{C}$  values of crawfish and potential crawfish foods are summarized in Tables 9, 10 and 11. The  $\delta^{13}\text{C}$  values of P. clarkii ( $\bar{X} \pm \text{SE} = -22.6 \pm 0.8$ ) were not different from P. acutus acutus ( $\bar{X} = -21.0 \pm 0.7$ ) ( $P > 0.05$ , Appendix Table 4), which indicated that the two species subsisted on similar foods in these ponds. Crawfish  $\delta^{13}\text{C}$  values changed from November through May ( $P < 0.05$ , Appendix Table 4) indicating a shift in food habits during the production season. The  $\delta^{13}\text{C}$  values of crawfish collected from the three ponds differed ( $P < 0.01$ , Appendix Table 4), indicating differences in major food sources for crawfish in each pond. Crawfish in the rice pond had  $\delta^{13}\text{C}$  values from -20.7 to -26.9, with a mean of  $-23.5 \pm 2.3$  (Table 9), while the  $\delta^{13}\text{C}$  value of crawfish from the open pond ranged from -16.7 to -21.0 with a mean of  $-18.1 \pm 1.5$  (Table 10). The  $\delta^{13}\text{C}$  values of crawfish from the wooded pond were lower than those from the rice and open ponds, ranging from -20.9 to -29.4 with a mean of  $-26.7 \pm 3.9$  ( $P < 0.05$ , Table 11).

The  $\delta^{13}\text{C}$  values of P. clarkii and P. acutus acutus were similar to many of their potential food sources (Tables 9, 10 and 11). The  $\delta^{13}\text{C}$  values of plants ranged from -12.4 to -38.5, which included both  $\text{C}_3$  and  $\text{C}_4$  plants. The difference between  $\text{C}_3$  and  $\text{C}_4$  plants is based on the presence or absence of the  $\text{C}_4$ -dicarboxylic acid cycle of carbon fixation (Caswell et al. 1973).  $\text{C}_3$  plants, which utilize only the Calvin cycle, have  $\delta^{13}\text{C}$  values that typically range from -24 to -34, while  $\text{C}_4$  plants, which employ both Calvin and dicarboxylic acid cycles,

Table 9. The  $\delta^{13}\text{C}$  values of crawfish and potential foods from the rice pond, Indigo Island Crawfish and Migratory Waterfowl Experimental Station, Iberville Parish, Louisiana, November 1985 through May 1986.

Source	Nov	Jan	Mar	May
Crawfish <sup>1</sup>				
<u>P. clarkii</u>	-20.7	-26.3	-24.4	-22.1
<u>P. acutus acutus</u>	-21.6	-26.9	-24.0	-21.6
Zooplankton				
micro-zooplankton	-21.0	-24.3	-26.8	-23.1
macro-zooplankton	-20.2	-26.9	-26.5	-22.8
Bottom sediment	-21.3	-22.2	-22.2	-22.9
Periphyton	-24.9	-27.3	-25.5	-21.9
Detritus	-15.1	-19.8	-24.4	-25.0
Filamentous algae				
<u>Pithophora</u> spp.	-20.9			
<u>Oscillatoria</u> spp.		-15.8		
<u>Spirogyra</u> spp.		-20.9	-20.4	-21.9
<u>Vouheria</u> spp. + Diatom		-38.5	-36.3	
Macrophyte				
<u>Oryza sativa</u>	-25.4	-27.4		
<u>Alternanthera</u>				
<u>philoxeroides</u>	-27.8	-26.4	-26.8	-27.1
<u>Panicum dichotomiflorum</u>	-14.6	-13.6		
<u>Ludwigia peploides</u>	-30.8	-30.5	-31.7	
<u>Echinochloa colonum</u>	-13.8			
Annelida				
Oligochaeta			-19.2	
Insecta				
Chironomidae larvae	-23.6	-23.6	-23.4	-23.4
Odonata naiads	-27.2			
Corixidae	-26.6	-27.1	-27.4	-27.9
Mollusca				
<u>Physa</u> spp.	-26.6			

Table 9. (Continued)

Source	Nov	Jan	Mar	May
Bait <u>Brevoortia patronus</u>				-22.1

<sup>1</sup> For a particular month and species, the mean  $\delta^{13}\text{C}$  value of crawfish was calculated from three samples with standard error within  $\pm 0.6$ .

Table 10. The  $\delta^{13}\text{C}$  values of crawfish and potential foods from the open pond, Indigo Island Crawfish and Migratory Waterfowl Experimental Station, Iberville Parish, Louisiana, November 1985 through May 1986.

Source	Nov	Jan	Mar	May
Crawfish <sup>1</sup>				
<u>P. clarkii</u>	-19.3	-17.6	-16.7	-16.9
<u>P. acutus acutus</u>	-21.0	-18.6	-17.0	-17.8
Zooplankton				
micro-zooplankton	-22.2	-24.3	-23.7	-21.1
macro-zooplankton	-21.6	-22.3	-22.2	-18.5
Bottom sediment	-21.4	-21.1	-21.2	-19.5
Periphyton	-22.0	-19.1	-17.6	-16.6
Detritus	-14.0	-14.0	-12.4	-13.3
Filamentous algae				
<u>Pithophora</u> spp.	-20.9			
<u>Oscillatoria</u> spp.				-15.5
<u>Spirogyra</u> spp.		-21.3	-19.8	
Macrophyte				
<u>Panicum dichotomiflorum</u>	-13.1			
<u>Cyperus iria</u>	-12.4			
Annelida				
Oligochaeta			-21.2	
Crustacean				
Asellidae		-23.4		
Insecta				
Chironomidae larvae		-20.2	-20.2	-20.2
Odonata naiads	-29.8	-24.0	-28.5	
Corixidae	-29.2	-24.9	-27.4	-27.9
Lestidae		-23.1		
Baetidae		-27.1		
Mollusca				
<u>Physa</u> spp.	-22.0	-20.3		
Vertebrata				
Pisces				
<u>Gambusia affinis</u>		-21.4		-23.8
<u>Lepomis cyanellus</u>				-24.4
<u>Ictalurus melas</u>				-24.2
Amphibian				
<u>Rana catesbeiana</u>	-24.1			



Table 10. (Continued)

Source	Nov	Jan	Mar	May
Bait				
<u>Mugil cephalus</u>				-23.5
<u>Dorosoma cepedianum</u>				-19.8

<sup>1</sup> For a particular month and species, the mean  $\delta^{13}\text{C}$  value of crawfish was calculated from three samples with standard error within  $\pm 0.6$ .

Table 11. The  $\delta^{13}\text{C}$  values of crawfish and potential foods from the wooded pond, Indigo Island Crawfish and Migratory Waterfowl Experimental Station, Iberville Parish, Louisiana, November 1985 through May 1986.

Source	Nov	Jan	Mar	May
Crawfish <sup>1</sup>				
<u>P. clarkii</u>	-20.9	-27.9	-29.4	-28.5
Zooplankton				
micro-zooplankton	-26.2	-22.2	-26.1	-27.3
macro-zooplankton	-25.9	-25.9	-25.2	-26.6
Bottom sediment	-26.3	-25.6	-25.9	-27.8
Periphyton	-27.2	-27.5	-28.7	-30.1
Detritus	-25.8	-28.5	-27.0	-27.2
Leaf litter	-31.6	-30.3	-29.6	-28.7
Filamentous algae				
<u>Spirogyra</u> spp.		-20.3	-20.6	
Macrophyte				
<u>Panicum dichotomiflorum</u>	-13.1			
<u>Polygonum punctatum</u>	-27.2			
<u>Alternanthera</u>				
<u>philoxeroides</u>		-28.6		-28.9
<u>Eichhornia crassipes</u>				-28.9
<u>Lemna minor</u>				-30.4
Annelida				
Oligochaeta				-30.7
Crustacean				
Palaemonidae		-32.1		-32.9
Insecta				
Chironomidae larvae			-25.3	-33.7
Odonata naiads			-29.9	
Dysticidae	-31.4	-32.7	-32.0	-32.3
Mollusca				
<u>Physa</u> spp.	-27.1			

Table 11. (Continued)

Source	Nov	Jan	Mar	May
Vertebrata				
Pisces				
<u>Gambusia affinis</u>		-33.8	-32.8	-32.7
<u>Lepomis cyanellus</u>				-35.0
<u>Notropis spp.</u>				-36.1

<sup>1</sup> For a particular month and species, the mean  $\delta^{13}\text{C}$  value of crawfish was calculated from three samples with standard error within  $\pm 0.6$ .

have  $\delta^{13}\text{C}$  values ranging from -6 to -19 (Smith and Epstein 1971). The  $\delta^{13}\text{C}$  of aquatic fauna collected from the ponds ranged from -18.5 to -36.1. Zooplankton, which consisted mostly of cladocerans, copepods, ostracods and isopods, had a mean  $\delta^{13}\text{C}$  value of  $-23.9 \pm 2.5$ .

The  $\delta^{13}\text{C}$  values of bottom sediments remained relatively constant from November through May ( $\pm 2$  parts per mil). Periphyton  $\delta^{13}\text{C}$  values were most similar to the  $\delta^{13}\text{C}$  values of crawfish in the three ponds except in November (Tables 9, 10 and 11). The  $\delta^{13}\text{C}$  of detritus in the rice pond changed from November through May, but detritus in open and wooded ponds had similar  $\delta^{13}\text{C}$  values from November through May.

There were no differences ( $P > 0.05$ , Appendix Table 5) between  $\delta^{13}\text{C}$  values of crawfish determined by mass spectrometry and values calculated from equation 2 (Table 12). This indicates that equation 2 (developed in this study) could be used to predict  $\delta^{13}\text{C}$  of crawfish. The linear relationship (Pearson correlation coefficient) between the measured and calculated  $\delta^{13}\text{C}$  values was 0.93 ( $P < 0.01$ ). Equation 3 was developed to predict the contribution of each food source to growth of *P. clarkii* and *P. acutus acutus* (Appendix Table 6). Using equation 3, macrophytes contributed 90% of crawfish growth in November, 85% in January, 61% in March and 56% in May, while aquatic fauna (insects and zooplankton) contributed 6% of crawfish growth in November, 12% in January, 23% in March and 28% in May (Tables 13 and 14).

Table 12. Comparison of  $\delta^{13}\text{C}$  values determined by mass spectrometry (MS) and values calculated from equation 2 (EQ) for Procambarus clarkii and Procambarus acutus acutus from rice, open, and wooded ponds, Indigo Island Crawfish and Migratory Waterfowl Experimental Station, Iberville Parish, Louisiana, November 1985 through May 1986.

Pond	Date	<u>P. clarkii</u>			<u>P. acutus acutus</u>		
		MS	EQ	Diff <sup>1</sup>	MS	EQ	Diff <sup>1</sup>
Rice	Nov	-20.7	-21.3	0.6	-21.6	-21.5	0.1
	Jan	-26.3	-25.0	1.3	-26.9	-24.9	2.0
	Mar	-24.4	-21.1	3.3	-24.0	-21.2	2.8
	May	-22.1	-23.3	1.2	-21.6	-23.3	1.7
Open	Nov	-19.3	-18.1	1.2	-21.0	-18.3	2.7
	Jan	-17.6	-17.5	0.1	-18.6	-16.8	1.8
	Mar	-16.7	-16.4	0.3	-17.0	-18.7	1.7
	May	-16.9	-17.8	0.9	-17.8	-17.0	0.8
Wooded	Nov	-20.9	-22.3	1.4			
	Jan	-27.9	-27.5	0.4			
	Mar	-29.4	-28.5	0.9			
	May	-28.5	-29.1	0.6			

<sup>1</sup> Difference between the  $\delta^{13}\text{C}$  values of crawfish determined by mass spectrometry and from equation 2. Difference between these two values was within  $\pm 1.0$  part per mil for 45% of the observations,  $\pm 2.0$  parts per mil for 40% of the observations and  $\pm 3.0$  parts per mil for 15% of the observations.

Table 13. Percent contribution of macrophytes, plant seeds, algae, bait and animal material to the growth of Procambarus clarkii in rice, open and wooded ponds, Indigo Island Crawfish and Migratory Waterfowl Experimental Station, Iberville Parish, Louisiana, November 1985 through May 1986.

Pond	Date	Macrophytes <sup>1</sup>	Seeds	Algae	Animal	Bait <sup>2</sup>
Rice	Nov	93	3	0	4	0
	Jan	91	0	0	9	0
	Mar	61	18	5	16	0
	May	46	1	2	13	38
Open	Nov	95	2	0	3	0
	Jan	78	2	0	20	0
	Mar	79	1	1	19	0
	May	52	0	0	48	0
Wooded	Nov	78	13	0	9	0
	Jan	82	10	0	8	0
	Mar	81	1	0	18	0
	May	87	0	0	13	0

<sup>1</sup> Macrophytes plus detritus, periphyton and leaf litter (in wooded pond only).

<sup>2</sup> Menhaden used in the rice pond, mullet and gizzard shad used in the open and wooded pond.

Table 14. Percent contribution of macrophytes, plant seeds, algae, bait and animal material to the growth of Procambarus acutus acutus in rice, open and wooded ponds, Indigo Island Crawfish and Migratory Waterfowl Experimental Station, Iberville Parish, Louisiana, November 1985 through May 1986.

Pond	Date	Macrophytes <sup>1</sup>	Seeds	Algae	Animal	Bait <sup>2</sup>
Rice	Nov	90	2	0	8	0
	Jan	83	1	0	17	0
	Mar	38	21	31	10	0
	May	26	1	16	34	23
Open	Nov	94	1	0	5	0
	Jan	90	3	0	7	0
	Mar	45	2	0	53	0
	May	69	0	0	31	0

<sup>1</sup> Macrophytes plus detritus, periphyton and leaf litter (in wooded pond only).

<sup>2</sup> Menhaden used in the rice pond, mullet and gizzard shad used in the open pond.

As indicated by stomach content analysis, the contribution of animal matter to growth as determined by  $\delta^{13}\text{C}$  value was large in spring. Plant seeds and algae contributed 4% and 3%, respectively, to crawfish growth (Tables 13 and 14). Menhaden, used only in the rice pond, contributed 31% to growth of crawfish in May (Tables 13 and 14).

Comparisons between percentage of foods in crawfish stomachs (S, calculated from the points method) and percent contribution of foods to growth (G, calculated from equations 2 and 3) of *P. clarkii* and *P. acutus acutus* are shown in Tables 15 and 16. The G/S ratio indicates the nutritional value of each food to crawfish. G/S ratio of macrophytes (1.08) is lower than G/S ratio of animal material (1.82). Algae and plant seeds have G/S ratios of 1.34 and 0.35, respectively.



Table 15. Comparisons between percentage of foods in crawfish stomachs (S) and percent contribution of foods to growth (G) of Procambarus clarkii in rice, open and wooded ponds, Indigo Island Crawfish and Migratory Waterfowl Experimental Station, Iberville Parish, Louisiana, November 1985 through May 1986.

Date	Macrophytes <sup>1</sup>		Seeds		Algae		Animal		Bait <sup>2</sup>	
	S	G	S	G	S	G	S	G	S	G
	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
<u>Rice</u>										
Nov	86	93	11	3	0	0	3	4	0	0
Jan	91	91	1	0	0	0	8	9	0	0
Mar	39	61	52	18	3	5	6	16	0	0
May	54	46	3	1	2	2	10	13	31	38
<u>Open</u>										
Nov	94	95	5	2	0	0	1	3	0	0
Jan	86	78	6	2	0	0	8	20	0	0
Mar	87	79	3	1	2	1	8	19	0	0
May	67	52	1	0	1	0	31	48	0	0
<u>Wooded</u>										
Nov	55	78	40	13	0	0	5	9	0	0
Jan	69	82	27	10	0	0	4	8	0	0
Mar	84	81	3	1	0	0	13	18	0	0
May	92	87	0	0	0	0	8	13	0	0

<sup>1</sup> Macrophytes plus detritus, periphyton and leaf litter (in wooded pond only).

<sup>2</sup> Menhaden used in the rice pond, mullet and gizzard shad used in the open and wooded pond.

Table 16. Comparisons between percentage of foods in crawfish stomachs (S) and percent contribution of foods to growth (G) of Procambarus acutus acutus in rice, open and wooded ponds, Indigo Island Crawfish and Migratory Waterfowl Experimental Station, Iberville Parish, Louisiana, November 1985 through May 1986.

Date	Macrophytes <sup>1</sup>		Seeds		Algae		Animal		Bait <sup>2</sup>	
	S	G	S	G	S	G	S	G	S	G
	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
<u>Rice</u>										
Nov	86	90	8	2	0	0	6	8	0	0
Jan	85	83	5	1	0	0	10	17	0	0
Mar	23	38	60	21	13	31	4	10	0	0
May	30	26	5	1	14	16	29	34	22	23
<u>Open</u>										
Nov	96	94	1	1	0	0	3	5	0	0
Jan	85	90	11	3	0	0	4	7	0	0
Mar	60	45	6	2	0	0	34	53	0	0
May	83	69	1	0	1	0	15	31	0	0

<sup>1</sup> Macrophytes plus detritus, periphyton and leaf litter (in wooded pond only).

<sup>2</sup> Menhaden used in the rice pond, mullet and gizzard shad used in the open and wooded pond.

## Discussion

### Stomach Content Analysis of Crawfish

Diets of P. clarkii and P. acutus acutus, as determined by stomach content analysis, were similar to diets reported for P. clarkii and P. acutus acutus by Huner and Naqvi (1986). In this study, P. clarkii and P. acutus acutus consumed mostly vegetative material when vegetation was available. Animal material must be important as a nutritional source for crawfish since animal material was found in most stomachs. However, the importance of animal material may be underestimated because of the low percentage found in most stomachs, possibly because of rapid digestion and absorption of animal tissue (Moshiri and Goldman 1969). Huner and Naqvi (1986) reported that P. clarkii and P. acutus acutus (25-80 mm TL) consumed 90% plant detritus and/or living plant material, and 10% aquatic fauna (cladocerans, ostracods, copepods, amphipods, mysid shrimps, aquatic insects and other arthropods) in rice ponds.

Diets of P. clarkii and P. acutus acutus were also similar to diets of coolwater and coldwater crawfishes, Orconectes immunis (Tack 1941), Orconectes rusticus and Cambarus tenebrosus (Prins 1968), Astacus (Hessen and Skurdal 1984; Westman et al. 1986) and Pacifastacus leniusculus (Mason 1975). Tack (1941) reported that stomach contents of the coolwater crawfish, O. immunis, contained 84% plant fragments, 11% detritus, and 5% animal matter (Daphnia spp., Asselus spp., Chironomus spp. and other unidentified insect species). Prins (1968) reported that detritus, vascular aquatic plants (Myriophyllum

heterophyllum, Nasturtium officinale, Fissidens fontanus, Myosotis scorpioides) and algae (Cladophora fracta, Vaucheria spp.) were the most important foods of O. rusticus (11-28 mm carapace length, CL) and C. tenebrosus (15-56 mm CL). Hessen and Skurdal (1984) and Westman et al. (1986) concluded that Noble crawfish, A. astacus, a coldwater species (13-124 mm TL), was basically herbivorous, with aquatic fauna (mollusks, insect larvae, crawfish, bryozoans and oligochaetes) contributing supplementally to its nutrition. Mason (1975) observed that adult P. leniusculus consumed mainly plant material (>90%). Most crawfish species have similar feeding habits despite differences in habitat. However, diet of some crawfish species, such as Orconectes propinquus (Vannote 1963; Capelli 1980), differed from diets of P. clarkii and P. acutus. O. propinquus ( $\geq 17$  mm CL) consumed principally animal matter (dipteran and chironomid larvae, heptageniid and ephemeropteran nymphs, and other crawfish) with diatoms, algae (Cladophora), plant detritus and zooplankton (rotifers, copepods, cladocerans and amphipods) occasionally found in stomach contents.

Food habits of P. clarkii were similar to food habits of P. acutus acutus, but P. acutus acutus consumed more animal material than did P. clarkii. P. acutus acutus is usually found in lotic environments (Huner and Barr 1984) and might require more energy for daily activity than does P. clarkii, a common inhabitant of lentic swamp and marsh ponds (Huner and Barr 1984). Laboratory digestibility studies of animal and plant food materials fed to P. clarkii and P. acutus acutus showed that animal matter provided more energy (averaged 4,100 cal/g dry weight) than plant material (averaged 3,500 cal/g dry weight). The

digestibility coefficient for animal matter (80-90%) was higher than that for plant matter (60-70%). Moshiri and Goldman (1969) reported that digestibility of animal material (chicken) fed to P. leniusculus was higher than that of plant material (lettuce and leaves). The lower digestibility of plant matter might be due to the presence of cellulose and other indigestible materials in plant tissues (Halver 1972).

It has been reported that the type and amount of foods found in crawfish stomachs are reflected to a degree by the availability of foods in lakes or ponds (Abrahamsson 1966; Mason 1975; Capelli 1980; Huner and Naqvi 1986). My results agree with these previous findings. In March and May, crawfish stomachs, collected from the rice and open ponds, contained less vegetative material than those in November and January. Decline of vegetative material in crawfish stomachs corresponded to the decline of vegetation in ponds. None of the original emergent macrophytes present in the rice and open ponds at flooding was visible by late-February. In contrast, the amount of vegetative material in crawfish stomachs collected from the wooded pond increased from November through May, which coincided with the increase in leaf litter in the pond. Leaves from trees in the wooded pond began to drop in the fall and became abundant in late December when remaining attached leaves were killed by frost.

Animal material found in crawfish stomachs increased from November through May and consisted mostly of insects (larvae and adults of chironomids, odonates and hemipterans). Zooplankton (cladocerans, copepods, isopods and ostracods) was present in small numbers in crawfish stomachs throughout the November-May production season. The

increase in animal material in crawfish stomachs coincided with the increase in animal organisms in ponds (Witzig 1980; Huner and Naqvi 1986). Witzig (1980) found the number of aquatic insects in crawfish ponds increased from November through May and was associated with the degree of decomposition of pond vegetation. Huner and Naqvi (1986) reported that the abundance of macro-invertebrates (insects and gastropods) in a 20-ha commercial crawfish pond increased from November through late April.

In this study, there were no differences in feeding habits among different sizes of crawfish and it was confirmed by the relatively constant amount of animal material found in crawfish stomachs collected from the wooded pond throughout the November-May season. Prins (1968) and Hessen and Skurdal (1984) reported similar feeding habits between juvenile and adult O. rusticus and A. astacus. However, other investigators (Mason 1975; Momot et al. 1983; Huner and Naqvi 1986) reported differences in feeding habits for juvenile and adult crawfish. Huner and Naqvi (1986) reported that P. clarkii and P. acutus acutus over 45-mm TL consumed more insects than did smaller crawfish. Mason (1975) observed that 65% of the diet of juvenile P. leniusculus was animal matter (ephemeropteran nymphs and chironomid larvae), but adult P. leniusculus consumed mainly plant material (>90%). Momot et al. (1978) reported that juvenile O. virilis fed more extensively on aquatic fauna (chironomid larvae, ostracods, cladocerans, small dragonfly naiads, crayfish and other arthropods) than did adults.

Various food groups were always found together in the same stomach, indicating that P. clarkii and P. acutus acutus were

opportunistic feeders, but that they also displayed preferences for certain types of food. Menhaden bait in the rice pond was found in crawfish stomachs in large amount in May, but gizzard shad and mullet, used as crawfish baits in the open and wooded pond, were not found in any crawfish stomachs. The results of this study, that P. clarkii and P. acutus acutus exhibit opportunistic feeding habits with preferences for some types of food, agree with the previous findings of other crawfish species (Abrahamsson 1966; Mason 1975; Seroll and Coler 1975; Reynolds 1979; Capelli 1980).

Stable Carbon Isotope Analysis of Crawfish  
and Potential Foods

Seasonal  $\delta^{13}\text{C}$  values of P. clarkii and P. acutus acutus collected from the same pond were similar ( $\pm 2$  parts per mil), indicating that both P. clarkii and P. acutus acutus occupied similar ecological niches and competed for the same food. DeNiro and Epstein (1978) and Fry et al. (1978) reported that animals (insects, brine shrimp, snails, mice and grasshoppers) with similar  $\delta^{13}\text{C}$  values (within 1.8-2.0 parts per mil) had similar diets.

The  $\delta^{13}\text{C}$  values of P. clarkii and P. acutus acutus were similar to the  $\delta^{13}\text{C}$  values of periphyton in the three ponds throughout the season. Periphyton could not be directly identified from crawfish stomachs; however, I frequently observed crawfish grazing on periphyton attached to the submerged portion of plant stems. Momot et al. (1978) reported that O. virilis grazed on periphyton attached to pine (Pinus spp.) needles, oak (Quercus spp.) and aspen (Populus spp.) leaves. Chien (1980) concluded that crawfish ponds with good rice biomass provided substrate for good periphyton growth and that periphyton provided excellent nutrition, resulting in good crawfish growth and high yields. The close similarity in  $\delta^{13}\text{C}$  values of crawfish and periphyton, supported by my field observations and studies by Momot et al. (1978) and Chien (1980), indicated that crawfish probably consumed and assimilated a significant amount of periphyton and that periphyton is a significant contributor to crawfish growth.

The  $\delta^{13}\text{C}$  values of crawfish in rice, open and wooded ponds differed, indicating differences in food habits among crawfish in



rice, open and wooded ponds. The type of dominant macrophytes present in each pond appears to have been a major factor affecting differences in crawfish  $\delta^{13}\text{C}$  values, because the total amount of food consumed by crawfish from the three ponds was similar. Foods were primarily macrophytes, with lesser amounts of aquatic insect, zooplankton, algae and plant seed.

In the rice and open pond, crawfish growth in November resulted from consumption of dominant macrophytes such as rice or natural vegetation (90-95%) and zooplankton and aquatic insects (3-8%). In the wooded pond,  $\delta^{13}\text{C}$  values of crawfish in November were much higher than in January, March and May. Stomach contents of crawfish and  $\delta^{13}\text{C}$  values of dominant macrophytes in the wooded pond in November indicated that crawfish grew on a diet of leaf litter and fall panicum.

The  $\delta^{13}\text{C}$  values of crawfish in the rice and open ponds were higher from January through May. This temporal change paralleled the change in  $\delta^{13}\text{C}$  of periphyton and a shift to increased consumption of animal material by crawfish. Conversely,  $\delta^{13}\text{C}$  values of crawfish in the wooded pond remained relatively constant from January through May because leaf litter was the major food for crawfish in this environment throughout the year.

Stomach content analysis and stable carbon isotope ratios indicated that crawfish growth resulted from consumption of a mixed diet, principally periphyton, macrophytes and animal material. Chien (1980) reported that periphyton growth depended primarily on the type and total biomass of macrophytes. Thus, commercial crawfish ponds should be managed to provide good forage biomass for production of

periphyton for crawfish. Practices such as selection of good cultivated forage (ease of culture, cost, biomass production, rate of biomass degradation) and fertilization in summer maximize vegetative biomass. Failure to establish a good vegetative crop could lead to reduced crawfish growth and yield. Cultivation of forages such as rice and sorghum is recommended for crawfish ponds (Brunson and Taylor 1987; Brunson and Griffin 1988), rather than natural vegetation (Brunson 1987) or leaf litter (Huner and Barr 1984). Because crawfish consume a mixed diet of plant and animal material, reasonably priced formulated feed could provide supplemental food for crawfish.

APPARENT DIGESTIBILITY COEFFICIENTS AND NUTRITIONAL  
QUALITY OF NATURAL FOODS AND FORMULATED DIETS FOR  
RED SWAMP CRAWFISH AND WHITE RIVER CRAWFISH

**Objectives**

Objectives of this study were to determine daily consumption rate and digestibility coefficients for five natural diets (rice stems and leaves, alligatorweed, filamentous algae, rice detritus and earthworms) and two formulated diets (Purina Jumbo crawfish bait and Zeigler shrimp ration) for juvenile P. clarkii and P. acutus acutus; estimate gross growth efficiency ( $K_1$ ) and net growth efficiency ( $K_2$ ) of juvenile P. clarkii and P. acutus acutus fed the seven diets; and develop energy budgets for juvenile P. clarkii and P. acutus acutus fed the seven diets.

**Materials and Methods**

Diet Preparation

Five natural foods typically found in commercial crawfish ponds and known to be consumed by P. clarkii and P. acutus acutus were evaluated: filamentous algae (Spirogyra sp.), vascular aquatic plant (stems and leaves of mature alligatorweed), vascular semi-aquatic plant (stems and leaves of mature rice), earthworms (Lumbricus terrestris) and rice detritus (120-day-old decomposed rice stems and leaves). Two formulated diets were also tested: crawfish bait (Purina Jumbo, Ralston Purina, Checkerboard Square, St. Louis, Missouri) and shrimp ration (45% protein, Zeigler Brothers, Gardners, Pennsylvania).

Filamentous algae, alligatorweed, rice, and rice detritus were collected from crawfish ponds located at the Ben Hur Research Farm, Louisiana Agricultural Experiment Station, Louisiana State University Agricultural Center, Baton Rouge. Live earthworms were obtained from a local bait store in Baton Rouge. Filamentous algae and earthworms were washed with tap water to remove adhering debris. All foodstuffs (except shrimp ration) were dried at 90°C for 48 hours and ground to a particle size of 1 mm diameter and blended for 20 min with non-nutritive powdered cellulose (filler) and carboxymethylcellulose (binder) (Halver 1972). Amounts of binder and filler added to each diet differed depending upon the nature of the foodstuff (Table 17). Diets with higher fiber content required larger amounts of filler and binder to produce satisfactory pellets. A sufficient quantity of water (50-100% of diet dry weight) was added to the dry mixture, blended for 15 min and pelleted through a  $\frac{1}{2}$  HP commercial meat grinder fitted with a 3-mm diameter die. Pellets were air-dried for 48 hours to approximately 10% moisture, broken into small pieces (2-3 cm L) and stored in plastic bags at 4°C until used. Shrimp ration (3 mm D x 5 mm L) was used as manufactured. Proximate analysis of the seven diets (Table 18) was conducted by the Feeds and Fertilizer Laboratory, Louisiana Agricultural Experiment Station, Louisiana State University Agricultural Center, Baton Rouge.

Table 17. Composition of test diets (% dry matter) fed to Procambarus clarkii and Procambarus acutus acutus in the digestibility and growth studies.

Diet	Feed (%)	Filler <sup>a</sup> (%)	Binder <sup>b</sup> (%)
Shrimp ration <sup>c</sup>	100	0	0
Crawfish bait <sup>d</sup>	97	0	3
Worm	87	10	3
Rice	65	25	10
Algae	65	25	10
Rice detritus	65	25	10
Alligatorweed	90	0	10

<sup>a</sup> Powdered cellulose (Celufil; United States Biochemical Corporation, Cleveland, Ohio).

<sup>b</sup> Carboxymethylcellulose.

<sup>c</sup> 45% protein, Zeigler Brothers, Gardners, Pennsylvania, used as purchased.

<sup>d</sup> Purina Jumbo, Ralston Purina, Checkerboard Square, St. Louis, Missouri.

Table 18. Proximate analysis (% dry matter), energy (kcal/g dry weight) and protein:energy ratio of test diets fed to Procambarus clarkii and Procambarus acutus acutus in the digestibility and growth studies, before adjustment of leaching loss.

Diet					Nitrogen-		P:E Ratio <sup>b</sup>
	Crude	Fat	Fiber	Ash	free	Gross	
	Protein				extract <sup>a</sup>	Energy	
	(%)	(%)	(%)	(%)	(%)	(kcal/g)	(mg/kcal)
Shrimp ration <sup>c</sup>	45.5	9.6	3.8	12.5	28.6	4.4	103
Crawfish bait <sup>d</sup>	21.8	1.0	4.9	5.7	66.6	3.9	59
Earthworms	47.8	3.8	10.5	15.2	22.7	4.1	117
Rice	8.5	2.1	30.6	10.6	48.2	3.6	24
Algae	6.4	1.8	24.5	9.2	58.1	3.3	19
Rice detritus	3.4	0.3	43.9	12.9	39.5	3.4	10
Alligatorweed	19.2	0.3	12.6	13.3	54.6	3.7	52

<sup>a</sup> Nitrogen-free extract represents the more digestible carbohydrate fraction which includes sugars and starch.

<sup>b</sup> Protein:energy ratio.

<sup>c</sup> 45% protein, Zeigler Brothers, Gardners, Pennsylvania.

<sup>d</sup> Purina Jumbo, Ralston Purina, Checkerboard Square, St. Louis, Missouri.

### Determination of Leaching Loss

Five samples of each diet were dried at 90°C for 24 hours, weighed ( $\pm 1$  mg) and placed in 200 ml of distilled water. After immersion for 90 min, each sample was filtered through a 79- $\mu$  mesh Nitex sieve, dried at 90°C for 24 hours and weighed ( $\pm 1$  mg) (Hasting 1964; Balazs 1973; Cuzon et al. 1981). Percent dry weight loss of a diet (Table 19) was determined as follows:

$$\text{Percent dry wt loss} = \frac{\text{final dry wt} - \text{initial dry wt}}{\text{initial dry wt}} * 100 \quad (4)$$

Juvenile P. clarkii and P. acutus acutus (45-55 mm TL) for digestibility and growth studies were collected from experimental crawfish ponds at Ben Hur Research Farm and maintained in polyethylene tanks at 22°C water temperature. Crawfish were conditioned to the test diets for 6 days prior to each experiment. Feeding was terminated 2 days before a test to void the gut contents.

### Digestibility Study

Fifty-six rectangular plastic trays (14 cm L x 7 cm W x 5 cm H), each containing 200 ml of dechlorinated water, were used as feeding containers, and 56 2-liters wide-mouth Erlenmeyer flasks were used for fecal collection. Twenty-eight treatment combinations (7 diets x 2 species x 2 sexes) with two replications per treatment combination were randomly assigned to the 56 containers (1 crawfish per container).

Table 19. Percent dry weight loss of test diets fed to Procambarus clarkii and Procambarus acutus acutus in the digestibility and growth studies after immersion in distilled water for 90 min.

Diet	Percent dry weight loss (%)
Shrimp ration <sup>a</sup>	13
Crawfish bait <sup>b</sup>	8
Worm	40
Rice	11
Algae	13
Rice detritus	5
Alligatorweed	16

<sup>a</sup> 45% protein, Zeigler Brothers, Gardners, Pennsylvania.

<sup>b</sup> Purina Jumbo, Ralston Purina, Checkerboard Square, St. Louis, Missouri.



Diets were dried at 90°C for 24 hours, weighed ( $\pm 1$  mg), and allotted to each crawfish in excess of anticipated consumption, generally greater than 5% of total body weight. Crawfish were conditioned to the containers and dietary regime for three days prior to initiation of digestibility determinations (Cho et al. 1982). Crawfish were allowed to feed for 90 min (Jones and Momot 1983), removed, rinsed and placed in a fecal collection flask containing two liters of water. Uneaten food in the feeding container was collected by filtering the water through a 79- $\mu$  mesh Nitex sieve. The material was transferred to a clean, pre-weighed 250 ml beaker by rinsing with distilled water, dried at 90°C for 24 hours, and weighed to the nearest 1 mg (de la Noüe and Choubert 1985). Dry weight of ingested food was the difference between initial weight of food provided to crawfish (after adjustment for leaching loss) and weight of uneaten food. Crawfish were removed from the fecal collection flasks after 22 hours. Water and feces were then filtered through pre-weighed 11- $\mu$  filters. Filters and feces were dried at 90°C for 24 hours and weighed to the nearest 1 mg (de la Noüe and Choubert 1985).

Feeding and fecal collection continued for five consecutive days with the same crawfish. The experiment was then repeated using a different group of crawfish. Food consumption rate (g dry weight food/g wet weight crawfish/day) and fecal elimination rate (g dry weight feces/g wet weight crawfish/day) were determined daily. Gross energy (cal/g dry weight) of food (IE) and feces (FE) were determined using an adiabatic bomb calorimetry (Model 1252; Parr Instrument, Co., Moline, Illinois) and caloric content (cal/g dry weight crawfish/day)

of ingested food and eliminated feces was calculated.

Data were analyzed by an analysis of variance using a randomized block design with a split plot arrangement of treatments. Main effects were diet (7), crawfish species (2), sex (2) and day of experiment (5). Response variables were food consumption (I; g dry weight food/g wet weight crawfish/day), fecal elimination (F; g dry weight feces/g wet weight crawfish/day), apparent dry matter digestibility (ADMD) and apparent energy digestibility (AED).

#### Growth Study

Forty-two 40-l polyethylene tanks (45 cm L x 30 cm W x 30 cm D) containing 30 l of dechlorinated tap water, were used as test units in the growth study. Each tank was partitioned by plexiglass dividers into five compartments (four compartments with 15 cm L x 15 cm W per compartment and one compartment with 30 cm L x 15 cm W). Water was exchanged among compartments through nine holes (1 cm diameter) in the plexiglass. Water temperature during the 63-day study was maintained at  $22 \pm 2^{\circ}\text{C}$ . Water hardness was adjusted to 100 mg/l as  $\text{CaCO}_3$  by addition of calcium chloride (de la Bretonne et al. 1969). Dissolved oxygen was maintained at  $\geq 50\%$  oxygen saturation with compressed air bubbled into each tank. Water was exchanged in each tank every three or four days to maintain good water quality.

Five P. clarkii (45-55 mm TL) were randomly assigned to each of 21 tanks and five P. acutus acutus (45-55 mm TL) were assigned to each of the 21 remaining tanks. One crawfish per compartment was used to prevent cannibalism. The seven diets were randomly assigned to the 42

tanks (three replications per diet per species). Crawfish were acclimated to the tanks and fed for seven days before initiation of the experiment (Cho et al. 1982). Crawfish were fed ad libitum for 63 days. The total length ( $\pm 1$  mm) and weight ( $\pm 0.01$  g) of each crawfish was measured at day 0, day 32, and day 63. Crawfish were observed for molts daily. Caloric content of whole crawfish (cal/g dry weight crawfish), before and after the experiment, and caloric content of crawfish exuvia (EE, cal/g dry weight crawfish), were determined using bomb calorimetry. Energy retention (GE, cal/g dry weight crawfish/63 days) was calculated as the difference between the mean caloric content of crawfish before and after the feeding experiment.

Data were analyzed using analysis of variance with a completely randomized design and a split plot arrangement of treatments. Main effects were diet (7), crawfish species (2) and sex (2). Response variables were energy retained (GE; cal/g dry weight crawfish/day) and energy expended for ecdysis (EE; cal/g dry weight crawfish/day).

#### Energy Budget

An energy budget for P. clarkii and P. acutus acutus fed the seven diets was calculated as follows (Warren and Davis 1967):

$$DE = IE - FE = GE + EE + HE \quad (5)$$

where

DE = energy of food digested;

IE = energy of food consumed;

FE = energy of fecal matter;

GE = energy retention;

EE = energy utilized in ecdysis (energy in shedded exoskeleton); and

HE = energy associated with basal metabolism, specific dynamic action (digestive process, biosynthesis and metabolic waste production) and activity.

Energy (cal/g dry weight crawfish/day) of ingested food (IE) and feces (FE) was determined after adjustment for energy contributed by cellulose added to the diets. Energy retention (GE) and energy associated with ecdysis (EE) were determined from the growth study. Energy loss associated with metabolism (HE) was calculated from the difference between digested energy (DE), energy retained (GE) and energy for ecdysis (EE) (Warren and Davis 1967). Crawfish used in this study were immature, thus caloric utilization in gamete production was not included in the energy budget. Energy for waste excretion in crustaceans is so small compared to other energy components (Nelson et al. 1977; Villarreal 1987) that it was not incorporated in the energy budget.

Apparent digestibility coefficients for dry matter (ADMD) and energy (AED) were calculated using the total fecal collection method as follows:

$$\text{ADMD}(\%) = \frac{\text{DM food consumed} - \text{DM feces collected}}{\text{DM food consumed}} * 100 \quad (6)$$

$$\text{AED}(\%) = \frac{\text{food energy ingested} - \text{fecal energy collected}}{\text{food energy ingested}} * 100 \quad (7)$$

Gross growth efficiency ( $K_1$ ) and net growth efficiency ( $K_2$ ) for the seven diets were calculated as follows (Jones and Momot 1983):

$$K_1 = \frac{\text{growth energy retained}}{\text{food energy ingested}} * 100 \quad (8)$$

$$K_2 = \frac{\text{growth energy retained}}{\text{food energy ingested} - \text{fecal energy collected}} * 100 \quad (9)$$

## Results

### Food Consumption and Fecal Elimination

Food consumption and fecal elimination rates were not different between species or sexes ( $P > 0.05$ , Appendix Tables 7 and 8), but consumption and elimination rates differed among diets ( $P < 0.01$ , Appendix Tables 7 and 8). Mean daily food consumption rate (g dry weight food/g wet weight crawfish) ranged from 1.6% (rice detritus) to 5.8% (earthworms) and fecal elimination rate (g dry weight feces/g wet weight crawfish) ranged from 0.5% (rice detritus and algae) to 1.3% (earthworms and rice) (Table 20). Crawfish consumed the animal diet (earthworms;  $\bar{X} = 6\%$ ) and formulated diets (shrimp ration and crawfish bait;  $\bar{X} = 5\%$ ) much quickly and in greater quantities than they consumed plant diets (rice, algae, alligatorweed, and detritus;  $\bar{X} = 2\%$ ) ( $P < 0.05$ , Table 20). Fecal elimination rate followed a trend similar to that of food consumption. Elimination rate depended upon the amount of food consumed and the amount of non-digestible matter in the diet.

### Digestibility Coefficients

Apparent dry matter and energy digestibility coefficients for the seven diets did not differ between species or sexes ( $P > 0.05$ , Appendix Tables 9 and 10); however, there were differences among digestibility coefficients for the seven diets ( $P < 0.01$ , Appendix Tables 9 and 10). Apparent dry matter digestibility coefficients (ADMD) ranged 60-85% and were similar to apparent energy digestibility coefficients (AED, range: 63-88%) (Table 21). Crawfish bait, earthworms, shrimp ration

Table 20. Mean daily food consumption and fecal elimination rates ( $\pm$  SE) (g dry weight of food or feces/g wet weight of crawfish) for Procambarus clarkii and Procambarus acutus acutus combined, after adjustment of leaching loss.

Diet	N	Consumption	Elimination
		rate (%)	rate (%)
Shrimp ration	80	$5.1 \pm 0.4^b$	$1.1 \pm 0.1^b$
Crawfish bait	80	$4.2 \pm 0.4^c$	$0.6 \pm 0.1^d$
Earthworms	80	$5.8 \pm 0.5^a$	$1.3 \pm 0.2^a$
Rice	78	$3.4 \pm 0.2^d$	$1.3 \pm 0.1^a$
Algae	80	$2.2 \pm 0.1^e$	$0.5 \pm 0.0^d$
Rice detritus	80	$1.6 \pm 0.2^f$	$0.5 \pm 0.1^d$
Alligatorweed	80	$2.3 \pm 0.2^e$	$0.9 \pm 0.1^c$

Means in the same column followed by the same letter are not different ( $\alpha = 0.05$ ); Tukey's studentized range test.

Table 21. Apparent dry matter digestibility coefficients and apparent energy digestibility coefficients for seven diets fed to Procambarus clarkii and Procambarus acutus acutus combined, after adjustment of leaching loss.

Diet	N	Apparent digestibility coefficient	
		Dry matter (%)	Energy (%)
Shrimp ration	80	77 <sup>b</sup>	87 <sup>a</sup>
Crawfish bait	80	85 <sup>a</sup>	88 <sup>a</sup>
Earthworms	80	77 <sup>b</sup>	88 <sup>a</sup>
Rice	78	60 <sup>d</sup>	64 <sup>d</sup>
Algae	80	76 <sup>b</sup>	84 <sup>b</sup>
Rice detritus	80	69 <sup>c</sup>	70 <sup>c</sup>
Alligatorweed	80	62 <sup>d</sup>	63 <sup>d</sup>

Means in the same column followed by the same letter are not different ( $\alpha = 0.05$ ); Tukey's studentized range test.



and algae (ADMD, range: 77-85%; AED, range: 87-88%) were more efficiently digested by P. clarkii and P. acutus acutus than rice, rice detritus and alligatorweed (ADMD, range: 60-69%; AED, range: 63-70%) ( $P < 0.05$ , Table 21).

### Growth and Ecdysis

Survival of P. clarkii and P. acutus acutus (Tables 22 and 23) fed the seven diets ranged from 80 to 100%. Mean growth of P. clarkii ranged from 0.1 to 6.4 g wet weight and from 0 to 19 mm in 63 days (Table 22), while growth of P. acutus acutus ranged from -0.1 to 5 g wet weight and from 0 to 12 mm (Table 23). Crawfish fed shrimp ration and crawfish bait molted an average of 2.6 times in 63 days, 1.3 times when fed earthworms or rice, and less than one molt when fed algae, rice detritus or alligatorweed.

Energy retained in crawfish and energy expended in ecdysis (energy in shedded exoskeleton, cal/g dry weight crawfish/day) differed between P. clarkii and P. acutus acutus and among diets ( $P < 0.01$ , Appendix Tables 11 and 12), but not between sexes ( $P > 0.05$ , Appendix Tables 11 and 12). The mean increase in growth was 33.8 cal/g dry weight crawfish/day for P. clarkii (Table 24) and was 2.4 times greater than for P. acutus acutus, which was 14.2 cal/g dry weight crawfish/day (Table 25). Energy expended in ecdysis averaged 6.3 cal/g dry weight crawfish/day for P. clarkii (Table 24) and 4.9 cal/g dry weight crawfish/day for P. acutus acutus (Table 25).

Table 22. Mean survival (S), initial weight (IWT; g wet weight) and length (ILT; mm), final weight (FWT; g wet weight) and length (FLT; mm), and mean number of molts of juvenile Procambarus clarkii ( $\pm$  SE) fed seven diets at 22°C for 63 days.

Diet	N	S	IWT	FWT	ILT	FLT	Number
							of
		(%)	(g)	(g)	(mm)	(mm)	molts
Shrimp ration	15	100	3.1 $\pm$ 0.1	9.5 $\pm$ 0.2	50 $\pm$ 0.8	69 $\pm$ 1.3	3.1 $\pm$ 0.1
Crawfish bait	15	100	2.7 $\pm$ 0.2	6.5 $\pm$ 0.4	48 $\pm$ 0.7	61 $\pm$ 0.9	2.7 $\pm$ 0.2
Earthworms	15	100	2.9 $\pm$ 0.1	3.7 $\pm$ 0.2	50 $\pm$ 0.8	53 $\pm$ 0.8	1.5 $\pm$ 0.1
Rice	15	93	2.9 $\pm$ 0.2	3.4 $\pm$ 0.2	49 $\pm$ 0.7	53 $\pm$ 0.9	1.4 $\pm$ 0.1
Algae	15	93	2.7 $\pm$ 0.1	2.9 $\pm$ 0.1	48 $\pm$ 0.8	49 $\pm$ 0.8	0.6 $\pm$ 0.1
Rice detritus	15	80	2.8 $\pm$ 0.1	2.9 $\pm$ 0.1	49 $\pm$ 0.4	50 $\pm$ 0.6	0.4 $\pm$ 0.1
Alligatorweed	15	100	3.0 $\pm$ 0.1	3.1 $\pm$ 0.2	50 $\pm$ 0.7	50 $\pm$ 0.8	0.3 $\pm$ 0.1

Table 23. Mean survival (S), initial weight (IWT; g wet weight) and length (ILT; mm), final weight (FWT; g wet weight) and length (FLT; mm), and mean number of molts of juvenile Procambarus acutus acutus ( $\pm$  SE) fed seven diets at 22°C for 63 days.

Diet	N	S	IWT	FWT	ILT	FLT	Number
							of
		(%)	(g)	(g)	(mm)	(mm)	molts
Shrimp ration	15	100	3.9 $\pm$ 0.3	8.9 $\pm$ 0.7	52 $\pm$ 1.0	64 $\pm$ 1.3	2.3 $\pm$ 0.2
Crawfish bait	15	100	3.6 $\pm$ 0.3	6.7 $\pm$ 0.4	51 $\pm$ 1.0	60 $\pm$ 1.0	2.3 $\pm$ 0.2
Earthworms	15	100	3.3 $\pm$ 0.2	4.0 $\pm$ 0.3	50 $\pm$ 1.0	53 $\pm$ 1.1	1.3 $\pm$ 0.2
Rice	14	93	3.7 $\pm$ 0.2	4.1 $\pm$ 0.2	52 $\pm$ 0.8	54 $\pm$ 1.1	0.9 $\pm$ 0.2
Algae	14	93	3.7 $\pm$ 0.3	3.9 $\pm$ 0.3	52 $\pm$ 1.2	53 $\pm$ 1.1	0.4 $\pm$ 0.1
Rice detritus	13	87	3.6 $\pm$ 0.2	3.5 $\pm$ 0.2	51 $\pm$ 1.2	51 $\pm$ 1.1	0.2 $\pm$ 0.1
Alligatorweed	14	93	4.0 $\pm$ 0.2	4.2 $\pm$ 0.3	53 $\pm$ 1.0	54 $\pm$ 1.2	0.5 $\pm$ 0.1

Table 24. Energy budget of juvenile Procambarus clarkii fed seven diets in laboratory study (cal/g dry weight crawfish/day), after adjustment of leaching loss.

Diet	Food consumed	Feces eliminated	Food digested	Growth	Ecdysis	Metabolism <sup>1</sup>
Shrimp ration	327	41	286	132	14	140
Crawfish bait	306	30	276	79	12	185
Earthworms	390	45	345	17	6	322
Rice	142	52	90	9	6	75
Algae	91	13	78	0	3	75
Rice detritus	65	22	43	-4	2	45
Alligatorweed	133	48	85	-8	1	92

<sup>1</sup> Metabolism was calculated as energy of food digested, minus growth and ecdysis. It included energy for basal metabolism, specific dynamic action (digestive process, biosynthesis and metabolic waste production) and activity.

Table 25. Energy budget of juvenile Procambarus acutus acutus fed seven diets in laboratory study (cal/g dry weight crawfish/day), after adjustment of leaching loss.

Diet	Food consumed	Feces eliminated	Food digested	Growth	Ecdysis	Metabolism <sup>1</sup>
Shrimp ration	344	46	298	86	10	202
Crawfish bait	213	25	188	46	10	132
Earthworms	288	29	259	4	6	249
Rice	140	45	95	-9	3	101
Algae	80	11	69	-12	2	79
Rice detritus	54	11	43	-12	1	54
Alligatorweed	119	45	74	-11	2	83

<sup>1</sup> Metabolism was calculated as energy of food digested, minus growth and ecdysis. It included energy for basal metabolism, specific dynamic action (digestive process, biosynthesis and metabolic waste production) and activity.

P. clarkii ( $\bar{X}$  = 132 cal/g dry weight crawfish/day) and P. acutus acutus ( $\bar{X}$  = 86 cal/g dry weight crawfish/day) fed shrimp ration grew faster than crawfish fed the other diets (Tables 24 and 25). Crawfish bait (46-79 cal/g dry weight crawfish/day) and earthworms (4-17 cal/g dry weight crawfish/day) were the second and third best diets, respectively (Tables 24 and 25). P. clarkii (9 cal/g dry weight crawfish/day) fed rice grew slightly but P. acutus acutus (-9 cal/g dry weight/crawfish/ day) fed rice exhibited negative "growth", apparently because tissues were catabolized to supply energy for metabolism. Crawfish fed algae, alligatorweed and rice detritus did not grow and also catabolized tissue to satisfy energy needs.

Energy expended in ecdysis for P. clarkii ( $\bar{X}$  = 2,906 cal/g dry weight crawfish) was similar to that for P. acutus acutus ( $\bar{X}$  = 2,918 cal/g dry weight crawfish). Because crawfish fed shrimp ration and crawfish bait molted more frequently than crawfish fed algae, rice alligatorweed, earthworms and rice detritus, the total energy cost of ecdysis for crawfish fed "high quality" diets was higher (10 to 14 cal/g dry weight crawfish/day) than that for crawfish fed "low quality" diets (1 to 6 cal/g dry weight crawfish/day) (Tables 24 and 25).

P. clarkii and P. acutus acutus fed formulated rations (crawfish bait and shrimp ration) converted 22-40% of ingested energy ( $K_1$ ) and 25-46% of digested energy ( $K_2$ ) to growth (Table 26). Conversely, P. clarkii and P. acutus acutus fed earthworms converted only 1-4% of ingested energy and 2-5% of digested energy into growth. P. clarkii fed rice had  $K_1$  and  $K_2$  values of 6% and 10%, respectively, but P. acutus acutus fed rice had  $K_1$  and  $K_2$  values of -6% and -9%. Crawfish

fed algae, alligatorweed and rice detritus catabolized tissue to meet energy needs.

Table 26. Mean gross growth efficiency ( $K_1$ ) and net growth efficiency ( $K_2$ ) for Procambarus clarkii and Procambarus acutus acutus fed seven diets in 63-day growth study, after adjustment of leaching loss.

Diet	<u>P. clarkii</u>			<u>P. acutus acutus</u>		
	N	$K_1$	$K_2$	N	$K_1$	$K_2$
		(%)	(%)		(%)	(%)
Shrimp ration	15	40	46	15	25	29
Crawfish bait	15	26	29	15	22	25
Earthworms	15	4	5	15	1	2
Rice	14	6	10	14	-6	-9
Algae	14	0	0	14	-15	-17
Rice detritus	12	-6	-9	13	-22	-28
Alligatorweed	15	-6	-9	14	-9	-15



## Discussion

Daily food consumption by juvenile P. clarkii and P. acutus acutus fed natural and formulated diets in the present study was similar to that reported for P. clarkii (Brown et al. 1986) and for other crawfish species fed a variety of natural and formulated diets (Moshiri and Goldman 1969; Kossakowski 1975; Mason 1975; Tcherkashina 1977; Jones and Momot 1983; Villarreal 1987) (Table 27). Brown et al. (1986) reported mean food consumption for adult P. clarkii fed various feedstuffs ranged from 0.4% to 2.7% of body weight. Kossakowski (1975) found that the daily consumption of Orconectes limosus fed macrophytes and mollusks was 5% to 7% of body weight.

P. clarkii and P. acutus acutus consumed more animal diet (earthworms) and formulated diets containing fish meal (shrimp ration and crawfish bait) than plant diets (rice, algae, alligatorweed and rice detritus). High consumption of earthworms, shrimp ration and crawfish bait was probably due to substances in these diets that stimulated chemoreception and immediately attracted crawfish to the diets. Palatability was another factor affecting animal consumption of crawfish. Feeding studies have shown that formulated diets containing fish and shrimp meal were readily accepted by P. clarkii (Clark et al. 1975; Huner and Meyers 1979; Hubbard et al. 1986). It is also well established that attractiveness and palatability of diets are important factors in crustacean feeding (New 1976).

Apparent digestibility of a foodstuff is a useful indicator of food utilization. Apparent dry matter digestibility (ADMD) coefficients for diets fed to P. clarkii and P. acutus acutus were

Table 27. Comparisons of daily food consumption (I) as % body wet weight, apparent dry matter digestibility coefficient (ADMD), apparent energy digestibility coefficient (AED), gross growth efficiency ( $K_1$ ) and net growth efficiency ( $K_2$ ), expressed as %, for various species of crawfish.

Species	I	ADMD	AED	$K_1$	$K_2$	Source
<u>Astacus</u> <u>leptodactylus</u>	4.4-16			17-60	21-75	Tcherkashina (1977)
<u>Cherax</u> <u>destructor</u>					54	Woodland (1969)
<u>Cherax</u> <u>tenuimanus</u>	2.1-2.6		61-80	23-60	38-75	Villarreal (1987)
<u>Orconectes</u> <u>limosus</u>	5.0-7.0	46		7	26	Kossakowski (1975)
<u>Orconectes</u> <u>virilis</u>	2.4-2.8	70-90			24	Jones and Momot (1981)
<u>Pacifastacus</u> <u>leniusculus</u>	0.4-4.4	40-64				Moshiri and Goldman (1969)
	2.4-2.8	50		15	29	Mason (1975)
<u>Procambarus</u> <u>acutus acutus</u>	1.5-4.3	60-84	63-89	-22-25	-28-29	This study
<u>Procambarus</u> <u>clarkii</u>	1.6-6.8	59-87	62-90	-6-40	-9-46	This study
	0.4-2.7	25-89				Brown et al. (1986)
		20-45				Wiernicki (1984)

1-11% lower than their respective apparent energy digestibility (AED) coefficients. The ADMD coefficients for the diets used in this study are reasonable predictors of the AED coefficients.

Digestibility coefficients for the diets (60-85% for ADMD and 63-88% for AED) fed to P. clarkii and P. acutus acutus in this study were similar to the digestibility coefficients for diets fed to P. clarkii (Brown et al. 1986) and O. virilis (Jones and Momot 1983). Brown et al. (1986) reported that the ADMD coefficients for wheat gluten, fish meal, casein, chitin, soybean meal, wheat bran and rice bran fed to P. clarkii ranged 61-89%. ADMD coefficients for a formulated diet and fish fed to O. virilis were 70% and 90%, respectively (Jones and Momot 1983). However, digestibility of diets in this study was high compared to digestibility of similar diets fed to P. clarkii (Wiernicki 1984) and other crawfish species (Moshiri and Goldman 1969, Kossakowski 1975, Mason 1975). Wiernicki (1984) reported the ADMD coefficient for elodea (Egera densa) fed to P. clarkii ranged 20-45% depending upon size of crawfish and stage of diet decomposition. ADMD coefficients for macrophytes, chicken and insects fed to Pacifastacus leniusculus were 44%, 50% and 64%, respectively (Moshiri and Goldman 1969; Mason 1975). Kossakowski (1975) reported 46% as the ADMD coefficient for a mixed diet (mollusks and macrophytes) fed to Orconectes limosus. Digestibility was related to the composition of the diet. ADMD and AED coefficients obtained in this study indicated that shrimp ration, crawfish bait and earthworms, which were low in fiber content, were more digestible for crawfish than high fiber plant diets (rice, rice detritus and algae).

Although there was not a wide discrepancy of ADMD (62-85%) and AED (63-89%) coefficients among the seven diets tested, poor growth of P. clarkii and P. acutus acutus fed some of the diets in this study indicated apparent nutritional deficiencies. Thus, digestibility coefficients can be used as gross indicators of the usefulness of a diet as a potential food source but cannot provide information on the nutritional quality of the diet. Animal growth rate and survival are generally used as decisive indicators of the nutritional value of a diet.

Growth and survival of P. clarkii and P. acutus acutus fed formulated diets (shrimp ration and crawfish bait) was superior to growth and survival of crawfish fed the natural diets. Shrimp ration was the best diet among those evaluated. It is a balanced diet formulated to meet the nutritional requirements of shrimp in intensive aquaculture. Crawfish bait was not developed as a crawfish feed but contains ingredients that attract crawfish and that may benefit crawfish growth indirectly.

Poor growth of crawfish fed rice, rice detritus and algae was possibly due to protein deficiency, because crude protein (3-9%) and protein:energy (P:E) ratios (10-24 mg/Kcal) of these diets were six times lower than the optimum P:E ratio. Hubbard et al. (1986) fed formulated diets containing various P:E ratios to juvenile P. clarkii and evaluated effects on growth and body composition. Growth data indicated that the optimum P:E ratio was between 80 and 120 mg/Kcal. The protein requirement of P. clarkii and P. acutus acutus maintained in closed systems is estimated to be 25-35% (Tarshis 1978; Huner et

al. 1975; Huner and Meyer 1979).

Crawfish fed rice detritus (120-day-old, decomposed in pond) exhibited negative growth, which indicated that the rice detritus diet was not a "nutritive" food for crawfish. Jones and Momot (1983) fed pine litter to *O. virilis* and reported negative crawfish growth. However, several investigators have reported that decaying rice straw and associated microorganisms are the main food source for crawfish in rice ponds (Chien 1978; Chien 1980; Day 1983; Huner and Barr 1984). Russel-Hunter (1971) stated that detritus must attain a C:N ratio of 17 or lower before it becomes suitable for animal nutrition. Chien (1980) studied the decay dynamics of rice straw in ponds and concluded that the average C:N ratio of standing rice straw dropped below 17 after four or five months of decomposition. Day (1983) reported the C:N ratio of rice straw never dropped to 17. The C:N ratio of the rice detritus used in my study was probably above 17 because it had decomposed in the pond for only four months. In addition, after adding 25% cellulose to rice detritus, the nutritive value of the diet was greatly reduced. Thus, the quality of the rice detritus diet in this study could not be used to represent the quality of rice detritus in the pond. However, the poor growth of crawfish fed rice detritus indicated that the fiber content of rice detritus was definitely not a sufficient source of nutrition for crawfish.

Alligatorweed, relatively high in protein (19%) and low in fat (0.3%), did not provide adequate nutrition for crawfish growth. Dietary lipids are important sources of energy, essential fatty acids and sterols (National Research Council 1983). There is limited information

concerning the use of lipid by crawfish. Davis and Robinson (1986) fed purified diets containing different levels of lipid to juvenile P. acutus acutus and found that crawfish grew equally well on diets containing 0-6% dietary lipid. However, they believed that crawfish fed 0% dietary lipid would have grown poorly if the experiment had continued for a longer period of time.

Results from my growth study supported results from published field studies that ponds with alligatorweed do not produce crawfish in excess of 800 kg/ha, such ponds become food deficiency and crawfish become stunted (Chien and Avault 1980; Garces and Avault 1985). A dissimilarity between the  $\delta^{13}\text{C}$  value of alligatorweed and the  $\delta^{13}\text{C}$  value of crawfish in ponds containing alligatorweed confirm that crawfish do not consume alligatorweed in large quantities.

The earthworm diet, despite a high protein level (48%) and high P:E ratio (117), did not provide good growth for crawfish. The poor growth response was possibly due to a deficiency in necessary nutrients, anti-nutritional compounds or other unexplained effects. Further studies are required in order to understand the quality of earthworms as a food source for crawfish.

P. clarkii had higher growth efficiency than P. acutus acutus when both were fed the same diet. P. clarkii fed shrimp ration had a net growth efficiency ( $K_2$ ) value of 46% while P. acutus acutus had a  $K_2$  value of 29%. It is possible that the higher  $K_2$  value of P. clarkii indicates that P. clarkii converts digested energy into tissue more efficiently than does P. acutus acutus. Daily food consumption and diet digestibility coefficients were similar for both species, thus

growth differences between P. clarkii and P. acutus acutus were possibly due to differences in the amount of energy required for basal metabolism and specific dynamic action. Metabolic studies must be conducted to determine whether or not P. acutus acutus requires a greater amount of energy than P. clarkii for basal metabolism and specific dynamic action. Another possibility is that the two species have different requirements for dietary components other than energy. Lack of any essential nutrient will eventually limit growth.

The  $K_2$  values for P. acutus acutus fed shrimp ration (29%) were similar to those reported for other coolwater crawfishes (24% and 29%) (Kossakowski 1975; Mason 1975; Jones and Monot 1983). P. clarkii has a  $K_2$  (46%) value similar to those of the warmwater Australian crawfishes, Cherax destructor (54%, Woodland 1969) and Cherax tenuimanus (38-75%, Villarreal 1987).

The gross growth efficiency ( $K_1$ ) value of P. clarkii (26%) and P. acutus acutus (22%) fed formulated crawfish bait may be significant for crawfish growth in ponds. Small non-harvestable crawfish that enter baited traps and feed on the bait can benefit nutritionally. In commercial ponds, 75 crawfish traps are typically fished per ha (Pfister and Romaine 1983) and 0.15 kg of crawfish bait is usually used in each trap set daily (Osorio 1987). Total amount of crawfish bait used each day is 11.25 kg/ha which can produce about 1.35 kg of crawfish dry weight, assuming that 50% of the bait is consumed by small crawfish and  $K_1$  for pond crawfish is the same as  $K_1$  (24%) for crawfish in this study. About 5.4 kg wet weight of crawfish are produced per ha per day based on 75% water content in crawfish tissue. Thus, the

presence of crawfish bait alone could directly produce 648 kg/ha of crawfish biomass if ponds are fished 120 days in a season. Formulated bait may serve not only as bait, but also as a supplemental feed for crawfish.

Negative or low growth efficiency of P. clarkii and P. acutus fed either plant or animal material in my laboratory study indicated that crawfish likely utilize plant and animal materials as food in the "vegetation-dominated" grow-out ponds. Direct consumption of either macrophytes or animals probably cannot solely supply the nutritional needs of crawfish. Covich (1977) reported that P. acutus fed a mixed diet of plant (Elodea canadensis) and animal (Physa gyrina) material grew better than those limited exclusively to either plant or animal food sources.

My results are helpful in terms of projecting the minimum potential crawfish yield in commercial crawfish-vegetation ponds. The total dry weight biomass of rice (stems, leaves and roots only) in rice-crawfish double cropping systems averages 7,700 kg/ha (Brunson et al. 1988). If we assume that 70% of rice biomass is consumed by crawfish, and the  $K_1$  value of P. clarkii fed rice is about 6%, then the total crawfish yield could be about 323 kg dry weight/ha or 1,292 kg wet weight/ha. Study of energy utilization not only provides information on crawfish growth under particular environmental conditions but also provides a conceptual framework for examining the relationship between growth and environment (Warren and Davis 1967).

Further studies of energy requirements for basal metabolism, specific dynamic action and activity have to be developed in order to



complete energy budgets for *P. clarkii* and *P. acutus acutus*. The relationship between energy partitioning (growth and non-growth components) and environmental parameters such as temperature and dissolved oxygen must be studied in order to identify environmental conditions that maximize energy utilization by crawfish. Research on crawfish growth and digestibility of natural foods such as aquatic insects and mixed plant and animal materials should be conducted. Knowledge on the percentage of microorganisms and periphyton assimilated into crawfish tissues is needed to further understand the role of these microbes in crawfish nutrition under natural conditions. Information obtained from these studies will be useful in maximizing crawfish production in commercial ponds under existing cultural practices.

## CONCLUSIONS

1. P. clarkii and P. acutus acutus consumed mostly vegetation but animal material was important as a nutritional source.
2. Food habits of P. clarkii were similar to food habits of P. acutus acutus, but P. acutus acutus consumed more animal material than did P. clarkii.
3. Animal material found in crawfish stomachs increased from November through May. The increase in animal material in crawfish diets coincided with the increase in animal organisms in ponds and a decrease in vegetation.
4. Feeding habits among different sizes of crawfish (< 55 mm, 55-75 mm and > 75 mm TL) did not differ.
5. Seasonal  $\delta^{13}\text{C}$  values of P. clarkii and P. acutus acutus collected from the same pond were similar, indicating that both species occupied similar ecological niches and competed for the same food.
6. The  $\delta^{13}\text{C}$  values of P. clarkii and P. acutus acutus were most similar to the  $\delta^{13}\text{C}$  values of periphyton in the three ponds throughout the season.
7. The  $\delta^{13}\text{C}$  values of crawfish in rice, open and wooded ponds differed, indicating different types of foods contributed to crawfish growth in these ponds. The type of dominant macrophytes present in a pond appears to have been a major factor affecting differences in crawfish  $\delta^{13}\text{C}$  values.

8. The  $\delta^{13}\text{C}$  values of crawfish in the rice and open pond increased from January through May. The temporal change paralleled the change in  $\delta^{13}\text{C}$  of periphyton and a shift to increased consumption of animal material by crawfish.
9. The  $\delta^{13}\text{C}$  values of crawfish in the wooded pond remained relatively constant from January through May because leaf litter was the major food for crawfish throughout the year.
10. Predicted  $\delta^{13}\text{C}$  value for each crawfish could be obtained from a simple mixed model, based on the assumption that sources of crawfish carbon are in direct proportion to the amount of each food assimilated:

$$\delta^{13}\text{C}_{\text{crawfish}} = \sum_{i=1}^n \left[ \frac{(P_i * D_i)}{\sum_{i=1}^n (P_i * D_i)} * \delta^{13}\text{C}_i \right]$$

11. Crawfish growth resulted from consumption of a mixed diet, principally periphyton, macrophytes and insects.
12. Selection of good cultivated forage (ease of culture, cost, biomass production, rate of biomass degradation) and a summer fertilization program will maximize vegetative biomass and enhance crawfish growth and yield. Cultivation of rice as a forage for crawfish is recommended rather than volunteer vegetation or leaf litter.

13. In the laboratory, P. clarkii and P. acutus acutus consumed more animal diet (earthworms) and formulated diets containing fish meal (shrimp ration and crawfish bait) than plant diets (rice, algae, alligatorweed and rice detritus).
14. Apparent dry matter and energy digestibility coefficients indicated that shrimp ration, crawfish bait and earthworms, low in fiber content, were more digestible for crawfish than high fiber plant diets (rice, rice detritus and algae).
15. P. clarkii had higher gross and net growth efficiencies than P. acutus acutus when fed the same diet.
16. Growth and survival of crawfish fed shrimp ration and crawfish bait was superior to growth and survival of crawfish fed the natural diets.
17. Crawfish fed rice detritus exhibited negative growth, indicating that rice detritus was not a "nutritive" food for crawfish. Alligatorweed, although relatively high in protein (19%), did not provide adequate nutrition for crawfish growth. The earthworm diet, despite a high protein level (48%) and high P:E ratio (117), did not provide good crawfish growth.
18. Negative or low growth efficiency of P. clarkii and P. acutus acutus fed either plant or animal material suggested that crawfish required both plant and animal material to satisfy nutrient requirements in the "vegetation-dominated" environment of commercial grow-out ponds.

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## **APPENDIX**

Appendix Table 1. Analysis of variance of macrophytes found in stomachs of Procambarus clarkii and Procambarus acutus acutus, Indigo Island Crawfish and Migratory Waterfowl Experimental Station, Iberville Parish, Louisiana, November 1985 through May 1986.

Source	Degree of Freedom	Sum of Square	F Value	Pr > F
Species	1	191.0	0.38	0.54
Size	2	739.1	0.74	0.48
Pond	2	3264.8	3.25	0.04
Season	3	5043.5	3.35	0.02
Species*Size	2	59.0	0.06	0.94
Species*Pond	1	4532.6	9.03	0.01
Species*Season	3	1570.3	1.04	0.37
Size*Pond	4	2392.5	1.19	0.31
Size*Season	5	3989.9	1.59	0.16
Pond*Season	6	26258.4	8.72	0.01
Species*Size*Pond	2	488.8	0.49	0.61
Species*Size*Season	3	690.5	0.69	0.50
Species*Pond*Season	3	1085.6	1.08	0.34
Size*Pond*Season	5	2896.5	1.15	0.33
Error	267	133960.2		
Total	309	222482.9		



Appendix Table 2. Analysis of variance of aquatic plant seeds found in stomachs of Procambarus clarkii and Procambarus acutus acutus, Indigo Island Crawfish and Migratory Waterfowl Experimental Station, Iberville Parish, Louisiana, November 1985 through May 1986.

Source	Degree of Freedom	Sum of Square	F Value	Pr > F
Species	1	189.7	1.24	0.27
Size	2	207.4	0.68	0.51
Pond	2	4998.0	16.36	0.01
Season	3	2535.7	5.53	0.01
Species*Size	2	816.7	2.67	0.07
Species*Pond	1	552.6	3.62	0.06
Species*Season	3	628.9	1.37	0.25
Size*Pond	4	2598.8	4.25	0.01
Size*Season	5	3420.6	4.48	0.01
Pond*Season	6	9255.5	10.10	0.01
Species*Size*Pond	2	2054.0	6.73	0.01
Species*Size*Season	3	203.5	0.67	0.51
Species*Pond*Season	3	505.3	1.65	0.19
Size*Pond*Season	5	778.0	1.02	0.41
Error	267	40774.1		
Total	309	85674.5		

Appendix Table 3. Analysis of variance of animal material found in stomachs of Procambarus clarkii and Procambarus acutus acutus, Indigo Island Crawfish and Migratory Waterfowl Experimental Station, Iberville Parish, Louisiana, November 1985 through May 1986.

Source	Degree of Freedom	Sum of Square	F Value	Pr > F
Species	1	561.9	4.56	0.03
Size	2	123.3	0.50	0.61
Pond	2	335.2	1.36	0.26
Season	3	2010.5	5.43	0.01
Species*Size	2	41.3	0.17	0.85
Species*Pond	1	87.5	0.71	0.40
Species*Season	3	415.8	1.12	0.34
Size*Pond	4	995.4	2.02	0.09
Size*Season	5	489.5	0.79	0.55
Pond*Season	6	3652.4	4.94	0.01
Species*Size*Pond	2	10.1	0.04	0.96
Species*Size*Season	3	71.6	0.29	0.75
Species*Pond*Season	3	3628.2	14.71	0.01
Size*Pond*Season	5	1623.6	2.63	0.02
Error	267	32928.1		
Total	309	51760.1		

Appendix Table 4. Analysis of variance for differences in  $\delta^{13}\text{C}$  values of Procambarus clarkii and Procambarus acutus acutus, Indigo Island Crawfish and Migratory Waterfowl Experimental Station, Iberville Parish, Louisiana, November 1985 through May 1986.

Source	Degree of Freedom	Sum of Square	F Value	Pr > F
Species	1	15.9	3.6	0.07
Pond	2	474.8	53.5	0.01
Season	3	49.6	3.7	0.02
Error	33	146.3		
Total	39	695.5		

Appendix Table 5. Paired-comparison t-test used to determine the differences between  $\delta^{13}\text{C}$  values of Procambarus clarkii and Procambarus acutus acutus determined by mass spectrometry and the values calculated from equation 2.

N	Mean	Standard Error	T	Prob >  T
20	-0.48	0.34	-1.41	0.17

Appendix Table 6. Calculation of  $\delta^{13}\text{C}$  values of Procambarus clarkii and Procambarus acutus acutus and percent contribution of each food to tissue elaboration, using equations 2 and 3.

Abbreviation of terms used in the Appendix Table 6

Ri	=	Rice
De	=	Detritus
Pe	=	Periphyton
Pa	=	<u>Panicum</u> spp.
Leaflit	=	Leaf litter (in wooded pond only)
Cyperus	=	<u>Cyperus</u> spp.
Echino	=	<u>Echinochloa</u> spp.
Polygonum	=	<u>Polygonum</u> spp.
Algae	=	Unidentified algae
Zoopl	=	Zooplankton
Chirono	=	Chironomid larvae
Insect	=	Unidentified insect
Bait	=	Menhaden bait

Species : Procambarus clarkii

Pond : Rice

Date : November 1985

$\delta^{13}\text{C}$  value of crawfish by mass spectrometry = -20.69

Food i	$P_i$	$D_i$	$P_i \cdot D_i$	$\delta^{13}\text{C}_i$	$\left[ \frac{P_i \cdot D_i}{\sum P_i \cdot D_i} * \delta^{13}\text{C}_i \right]$	$F_i$ (%)
Ri+De+Pe	0.869	0.6	0.521	-21.79	-19.78	93
Cyperus	0.092	0.3	0.028	-12.40	-0.60	3
Echino	0.015	0.3	0.004	-13.80	-0.11	0
Zoopl	0.008	0.8	0.006	-20.19	-0.22	1
Chirono	0.016	0.9	0.014	-23.55	-0.59	3
Sum	1.000		0.574		-21.30	100

Species : Procambarus clarkii

Pond : Rice

Date : January 1986

$\delta^{13}\text{C}$  value of crawfish by mass spectrometry = -26.26

Food i	$P_i$	$D_i$	$P_i \cdot D_i$	$\delta^{13}\text{C}_i$	$\left[ \frac{P_i \cdot D_i}{\sum P_i \cdot D_i} * \delta^{13}\text{C}_i \right]$	$F_i$ (%)
Ri+De+Pe	0.923	0.6	0.554	-24.83	-22.66	91
Cyperus	0.003	0.3	0.001	-12.40	-0.02	0
Echino	0.004	0.3	0.001	-13.80	-0.03	0
Zoopl	0.018	0.8	0.014	-26.86	-0.64	3
Insect	0.052	0.7	0.036	-27.14	-1.63	6
Sum	1.000		0.607		-24.98	100

Species : Procambarus clarkii

Pond : Rice

Date : March 1986

 $\delta^{13}\text{C}$  value of crawfish by mass spectrometry = -24.44

Food i	$P_i$	$D_i$	$P_i \cdot D_i$	$\delta^{13}\text{C}_i$	$\left[ \frac{P_i \cdot D_i}{\sum P_i \cdot D_i} * \delta^{13}\text{C}_i \right]$	$F_i$ (%)
De+Pe	0.412	0.6	0.247	-24.95	-12.94	61
Cyperus	0.487	0.3	0.146	-12.40	-3.80	18
Echino	0.004	0.3	0.001	-13.80	-0.03	0
Algae	0.021	0.8	0.017	-27.89	-0.98	5
Zoopl	0.020	0.8	0.016	-26.45	-0.89	4
Insect	0.005	0.7	0.003	-27.39	-0.20	1
Chirono	0.051	0.9	0.046	-23.37	-2.25	11
Sum	1.000		0.477		-21.10	100

Species : Procambarus clarkii

Pond : Rice

Date : May 1986

 $\delta^{13}\text{C}$  value of crawfish by mass spectrometry = -22.10

Food i	$P_i$	$D_i$	$P_i \cdot D_i$	$\delta^{13}\text{C}_i$	$\left[ \frac{P_i \cdot D_i}{\sum P_i \cdot D_i} * \delta^{13}\text{C}_i \right]$	$F_i$ (%)
De+Pe	0.545	0.6	0.327	-23.48	-10.98	46
Cyperus	0.024	0.3	0.007	-12.40	-0.13	1
Algae	0.015	0.8	0.012	-21.86	-0.37	2
Insect	0.106	0.7	0.074	-27.89	-2.96	13
Bait	0.310	0.9	0.279	-22.12	-8.82	38
Sum	1.000		0.699		-23.26	100



Species : Procambarus clarkii  
 Pond : Open  
 Date : November 1985

$\delta^{13}\text{C}$  value of crawfish by mass spectrometry = -19.34

Food i	P <sub>i</sub>	D <sub>i</sub>	P <sub>i</sub> *D <sub>i</sub>	$\delta^{13}\text{C}_i$	$\left[ \frac{P_i \cdot D_i}{\sum P_i \cdot D_i} * \delta^{13}\text{C}_i \right]$	F <sub>i</sub> (%)
De+Pe	0.941	0.6	0.565	-18.01	-17.27	95
Cyperus	0.002	0.3	0.001	-12.40	-0.01	0
Echino	0.042	0.3	0.013	-13.80	-0.29	2
Zoopl	0.005	0.8	0.004	-21.55	-0.15	1
Insect	0.010	0.7	0.007	-29.15	-0.35	2
Sum	1.000		0.589		-18.07	100

Species : Procambarus clarkii  
 Pond : Open  
 Date : January 1986

$\delta^{13}\text{C}$  value of crawfish by mass spectrometry = -17.60

Food i	P <sub>i</sub>	D <sub>i</sub>	P <sub>i</sub> *D <sub>i</sub>	$\delta^{13}\text{C}_i$	$\left[ \frac{P_i \cdot D_i}{\sum P_i \cdot D_i} * \delta^{13}\text{C}_i \right]$	F <sub>i</sub> (%)
De+Pe	0.815	0.6	0.489	-16.58	-13.48	78
Cyperus	0.005	0.3	0.001	-12.40	-0.03	0
Echino	0.057	0.3	0.017	-13.80	-0.39	2
Chirono	0.013	0.9	0.012	-20.24	-0.39	2
Insect	0.060	0.7	0.042	-24.49	-1.71	10
Zoopl	0.050	0.8	0.040	-22.29	-1.48	8
Sum	1.000		0.601		-17.49	100

Species : Procambarus clarkii

Pond : Open

Date : March 1986

 $\delta^{13}\text{C}$  value of crawfish by mass spectrometry = -16.69

Food i	P <sub>i</sub>	D <sub>i</sub>	P <sub>i</sub> *D <sub>i</sub>	$\delta^{13}\text{C}_i$	$\left[ \frac{P_i * D_i}{\sum P_i * D_i} * \delta^{13}\text{C}_i \right]$	F <sub>i</sub> (%)
De+Pe	0.879	0.6	0.527	-15.01	-13.08	79
Cyperus	0.007	0.3	0.002	-12.40	-0.04	0
Echino	0.014	0.3	0.004	-13.80	-0.10	1
Algae	0.006	0.8	0.005	-19.81	-0.16	1
Zoopl	0.010	0.8	0.008	-22.16	-0.29	2
Insect	0.084	0.7	0.059	-28.51	-2.77	17
Sum	1.000		0.605		-16.44	100

Species : Procambarus clarkii

Pond : Open

Date : May 1986

 $\delta^{13}\text{C}$  value of crawfish by mass spectrometry = -16.88

Food i	P <sub>i</sub>	D <sub>i</sub>	P <sub>i</sub> *D <sub>i</sub>	$\delta^{13}\text{C}_i$	$\left[ \frac{P_i * D_i}{\sum P_i * D_i} * \delta^{13}\text{C}_i \right]$	F <sub>i</sub> (%)
De+Pe	0.690	0.6	0.414	-14.94	-9.24	52
Echino	0.006	0.3	0.002	-13.80	-0.04	0
Algae	0.001	0.8	0.001	-15.53	-0.02	0
Chirono	0.202	0.9	0.182	-20.24	-5.50	31
Insect	0.101	0.7	0.071	-28.51	-3.01	17
Sum	1.000		0.669		-17.81	100

Species : Procambarus clarkii

Pond : Wooded

Date : November 1985

 $\delta^{13}\text{C}$  value of crawfish by mass spectrometry = -20.92

Food i	$P_i$	$D_i$	$P_i \cdot D_i$	$\delta^{13}\text{C}_i$	$\left[ \frac{P_i \cdot D_i}{\sum P_i \cdot D_i} * \delta^{13}\text{C}_i \right]$	$F_i$ (%)
Pa+De+Pe	0.686	0.6	0.412	-22.03	-17.20	78
Echino	0.144	0.3	0.043	-13.80	-1.13	5
Polygonum	0.119	0.3	0.036	-27.18	-1.84	8
Zoopl	0.011	0.8	0.009	-25.86	-0.43	2
Insect	0.040	0.7	0.028	-31.37	-1.67	7
Sum	1.000		0.527		-22.26	100

Species : Procambarus clarkii

Pond : Wooded

Date : January 1986

 $\delta^{13}\text{C}$  value of crawfish by mass spectrometry = -27.86

Food i	$P_i$	$D_i$	$P_i \cdot D_i$	$\delta^{13}\text{C}_i$	$\left[ \frac{P_i \cdot D_i}{\sum P_i \cdot D_i} * \delta^{13}\text{C}_i \right]$	$F_i$ (%)
De+Pe	0.438	0.6	0.263	-28.02	-14.05	52
Leaflit	0.239	0.6	0.143	-30.27	-8.29	30
Echino	0.180	0.3	0.054	-13.80	-1.42	5
Polygonum	0.091	0.3	0.027	-27.18	-1.42	5
Insect	0.052	0.7	0.036	-32.70	-2.27	8
Sum	1.000		0.524		-27.45	100

Species : Procambarus clarkii

Pond : Wooded

Date : March 1986

 $\delta^{13}\text{C}$  value of crawfish by mass spectrometry = -29.40

Food i	P <sub>i</sub>	D <sub>i</sub>	P <sub>i</sub> *D <sub>i</sub>	$\delta^{13}\text{C}_i$	$\left[ \frac{P_i * D_i}{\sum P_i * D_i} * \delta^{13}\text{C}_i \right]$	F <sub>i</sub> (%)
De+Pe	0.432	0.6	0.259	-27.84	-11.71	41
Leaflit	0.396	0.6	0.238	-29.57	-11.40	40
Echino	0.024	0.3	0.007	-13.80	-0.16	1
Zoopl	0.008	0.8	0.006	-25.16	-0.26	1
Insect	0.100	0.7	0.070	-30.96	-3.52	12
Chirono	0.040	0.9	0.036	-25.33	-1.48	5
Sum	1.000		0.616		-28.52	100

Species : Procambarus clarkii

Pond : Wooded

Date : May 1986

 $\delta^{13}\text{C}$  value of crawfish by mass spectrometry = -28.50

Food i	P <sub>i</sub>	D <sub>i</sub>	P <sub>i</sub> *D <sub>i</sub>	$\delta^{13}\text{C}_i$	$\left[ \frac{P_i * D_i}{\sum P_i * D_i} * \delta^{13}\text{C}_i \right]$	F <sub>i</sub> (%)
De+Pe	0.269	0.6	0.161	-28.63	-7.48	26
Leaflit	0.643	0.6	0.386	-28.68	-17.92	61
Zoopl	0.008	0.8	0.006	-26.64	-0.28	1
Insect	0.040	0.7	0.028	-32.31	-1.46	5
Chirono	0.040	0.9	0.036	-33.68	-1.96	7
Sum	1.000		0.618		-29.10	100

Species : Procambarus acutus acutus  
 Pond : Rice  
 Date : November 1985

$\delta^{13}\text{C}$  value of crawfish by mass spectrometry = -21.59

Food i	$P_i$	$D_i$	$P_i \cdot D_i$	$\delta^{13}\text{C}_i$	$\left[ \frac{P_i \cdot D_i}{\sum P_i \cdot D_i} * \delta^{13}\text{C}_i \right]$	$F_i$ (%)
Ri+De+Pe	0.876	0.6	0.526	-21.79	-19.40	90
Cyperus	0.059	0.3	0.018	-12.40	-0.37	2
Echino	0.002	0.3	0.001	-13.80	-0.01	0
Zoopl	0.023	0.8	0.018	-20.19	-0.63	3
Insect	0.040	0.7	0.028	-23.55	-1.12	5
Sum	1.000		0.590		-21.53	100

Species : Procambarus acutus acutus  
 Pond : Rice  
 Date : January 1986

$\delta^{13}\text{C}$  value of crawfish by mass spectrometry = -26.91

Food i	$P_i$	$D_i$	$P_i \cdot D_i$	$\delta^{13}\text{C}_i$	$\left[ \frac{P_i \cdot D_i}{\sum P_i \cdot D_i} * \delta^{13}\text{C}_i \right]$	$F_i$ (%)
Ri+De+Pe	0.824	0.6	0.494	-24.83	-20.45	82
Cyperus	0.043	0.3	0.013	-12.40	-0.27	1
Echino	0.007	0.3	0.002	-13.80	-0.05	0
Zoopl	0.026	0.8	0.021	-26.86	-0.93	4
Insect	0.100	0.7	0.070	-27.14	-3.17	13
Sum	1.000		0.600		-24.86	100

Species : Procambarus acutus acutus

Pond : Rice

Date : March 1986

 $\delta^{13}\text{C}$  value of crawfish by mass spectrometry = -23.97

Food i	P <sub>i</sub>	D <sub>i</sub>	P <sub>i</sub> *D <sub>i</sub>	$\delta^{13}\text{C}_i$	$\left[ \frac{P_i * D_i}{\sum P_i * D_i} * \delta^{13}\text{C}_i \right]$	F <sub>i</sub> (%)
De+Pe	0.246	0.6	0.148	-24.95	-7.94	37
Cyperus	0.560	0.3	0.168	-12.40	-4.49	21
Echino	0.008	0.3	0.002	-13.80	-0.07	0
Algae	0.138	0.8	0.110	-27.89	-6.64	31
Zoopl	0.020	0.8	0.016	-26.45	-0.91	4
Insect	0.028	0.7	0.020	-27.39	-1.16	5
Sum	1.000		0.464		-21.20	100

Species : Procambarus acutus acutus

Pond : Rice

Date : May 1986

 $\delta^{13}\text{C}$  value of crawfish by mass spectrometry = -21.64

Food i	P <sub>i</sub>	D <sub>i</sub>	P <sub>i</sub> *D <sub>i</sub>	$\delta^{13}\text{C}_i$	$\left[ \frac{P_i * D_i}{\sum P_i * D_i} * \delta^{13}\text{C}_i \right]$	F <sub>i</sub> (%)
De+Pe	0.312	0.6	0.187	-23.48	-5.97	26
Cyperus	0.043	0.3	0.013	-12.40	-0.22	1
Algae	0.157	0.8	0.126	-21.86	-3.73	16
Insect	0.144	0.7	0.101	-27.89	-3.82	16
Chirono	0.144	0.9	0.130	-23.37	-4.11	18
Bait	0.200	0.9	0.180	-22.12	-5.41	23
Sum	1.000		0.736		-23.26	100

Species : Procambarus acutus acutus

Pond : Open

Date : November 1985

$\delta^{13}\text{C}$  value of crawfish by mass spectrometry = -20.97

Food i	$P_i$	$D_i$	$P_i * D_i$	$\delta^{13}\text{C}_i$	$\left[ \frac{P_i * D_i}{\sum P_i * D_i} * \delta^{13}\text{C}_i \right]$	$F_i$ (%)
De+Pe	0.953	0.6	0.572	-18.01	-17.21	94
Echino	0.018	0.3	0.005	-12.40	-0.11	1
Zoopl	0.009	0.8	0.007	-21.55	-0.26	1
Insect	0.020	0.7	0.014	-29.15	-0.68	4
Sum	1.000		0.598		-18.26	100

Species : Procambarus acutus acutus

Pond : Open

Date : January 1986

$\delta^{13}\text{C}$  value of crawfish by mass spectrometry = -18.56

Food i	$P_i$	$D_i$	$P_i * D_i$	$\delta^{13}\text{C}_i$	$\left[ \frac{P_i * D_i}{\sum P_i * D_i} * \delta^{13}\text{C}_i \right]$	$F_i$ (%)
De+Pe	0.887	0.6	0.532	-16.58	-15.16	90
Cyperus	0.015	0.3	0.004	-12.40	-0.10	1
Echino	0.058	0.3	0.017	-13.80	-0.41	2
Insect	0.040	0.7	0.028	-24.49	-1.18	7
Sum	1.000		0.582		-16.84	100

Species : Procambarus acutus acutus

Pond : Open

Date : March 1986

 $\delta^{13}\text{C}$  value of crawfish by mass spectrometry = -16.98

Food i	P <sub>i</sub>	D <sub>i</sub>	P <sub>i</sub> *D <sub>i</sub>	$\delta^{13}\text{C}_i$	$\left[ \frac{P_i * D_i}{\sum P_i * D_i} * \delta^{13}\text{C}_i \right]$	F <sub>i</sub> (%)
De+Pe	0.602	0.6	0.361	-15.01	-8.45	45
Cyperus	0.034	0.3	0.010	-12.40	-0.20	1
Echino	0.031	0.3	0.009	-13.80	-0.20	1
Zoopl	0.056	0.8	0.045	-22.16	-1.55	8
Insect	0.167	0.7	0.117	-28.51	-5.20	28
Chirono	0.110	0.9	0.099	-20.24	-3.12	17
Sum	1.000		0.641		-18.72	100

Species : Procambarus acutus acutus

Pond : Open

Date : May 1986

 $\delta^{13}\text{C}$  value of crawfish by mass spectrometry = -17.77

Food i	P <sub>i</sub>	D <sub>i</sub>	P <sub>i</sub> *D <sub>i</sub>	$\delta^{13}\text{C}_i$	$\left[ \frac{P_i * D_i}{\sum P_i * D_i} * \delta^{13}\text{C}_i \right]$	F <sub>i</sub> (%)
De+Pe	0.824	0.6	0.494	-14.94	-11.70	69
Echino	0.001	0.3	0.000	-13.80	-0.01	0
Algae	0.002	0.8	0.002	-15.53	-0.04	0
Chirono	0.070	0.9	0.063	-20.24	-2.02	12
Insect	0.103	0.7	0.072	-28.51	-3.26	19
Sum	1.000		0.631		-17.02	100



Appendix Table 7. Analysis of variance of food consumption (g dry weight food/g wet weight crawfish/day) for Procambarus clarkii and Procambarus acutus acutus in the digestibility study.

Source	Degree of Freedom	Sum of Square	F Value	Pr > F
Diet	6	94.686	17.47	0.01
Species	1	1.752	1.94	0.17
Sex	1	0.033	0.04	0.85
Diet*Species	6	7.141	1.32	0.28
Diet*Sex	6	2.276	0.42	0.86
Species*Sex	1	1.161	1.29	0.27
Block	1	22.982	25.44	0.01
Diet*Spec*Sex*Bloc <sup>1</sup>	33	29.814	5.51	0.01
Day	4	2.639	4.02	0.01
Diet*Day	24	2.793	0.71	0.84
Species*Day	4	0.900	1.37	0.24
Sex*Day	4	0.020	0.03	1.00
Diet*Species*Day	24	5.032	1.28	0.17
Diet*Sex*Day	24	1.034	0.26	1.00
Species*Sex*Day	4	0.482	0.73	0.57
Error b	414	67.944		
Total	557	240.946		

<sup>1</sup> Error a

Appendix Table 8. Analysis of variance of fecal elimination (g dry weight feces/g wet weight crawfish/day) for Procambarus clarkii and Procambarus acutus acutus in the digestibility study.

Source	Degree of Freedom	Sum of Square	F Value	Pr > F
Diet	6	4.319	11.93	0.01
Species	1	0.146	2.41	0.13
Sex	1	0.000	0.00	0.99
Diet*Species	6	0.599	1.66	0.16
Diet*Sex	6	0.161	0.44	0.84
Species*Sex	1	0.034	0.56	0.46
Block	1	0.871	14.44	0.01
Diet*Spec*Sex*Bloc <sup>1</sup>	33	1.991	3.71	0.01
Day	4	0.267	4.11	0.01
Diet*Day	24	0.357	0.91	0.58
Species*Day	4	0.108	1.67	0.16
Sex*Day	4	0.051	0.79	0.53
Diet*Species*Day	24	0.294	0.75	0.79
Diet*Sex*Day	24	0.100	0.26	0.99
Species*Sex*Day	4	0.018	0.27	0.90
Error b	414	6.727		
Total	557	16.047		

<sup>1</sup> Error a

Appendix Table 9. Analysis of variance of dry matter digestibility coefficients for natural foods and formulated diets for Procambarus clarkii and Procambarus acutus in the digestibility study.

Source	Degree of Freedom	Sum of Square	F Value	Pr > F
Diet	6	41021.6	27.71	0.01
Species	1	487.5	1.98	0.17
Sex	1	17.4	0.07	0.79
Diet*Species	6	2448.7	1.65	0.16
Diet*Sex	6	948.6	0.64	0.70
Species*Sex	1	24.7	0.10	0.75
Block	1	288.2	1.17	0.29
Diet*Spec*Sex*Bloc <sup>1</sup>	33	8142.1	1.63	0.02
Day	4	1764.3	2.91	0.02
Diet*Day	24	3954.3	1.09	0.36
Species*Day	4	98.3	0.16	0.96
Sex*Day	4	780.8	1.29	0.27
Diet*Species*Day	24	4041.8	1.11	0.33
Diet*Sex*Day	24	2681.9	0.74	0.81
Species*Sex*Day	4	290.9	0.48	0.75
Error b	414	62770.8		
Total	557	130204.4		

<sup>1</sup> Error a

Appendix Table 10. Analysis of variance of apparent energy digestibility coefficients for natural foods and formulated diets for Procambarus clarkii and Procambarus acutus acutus in the digestibility study.

Source	Degree of Freedom	Sum of Square	F Value	Pr > F
Diet	6	63624.2	62.18	0.01
Species	1	403.7	2.37	0.13
Sex	1	52.6	0.31	0.58
Diet*Species	6	2220.9	2.17	0.07
Diet*Sex	6	643.2	0.63	0.71
Species*Sex	1	6.2	0.04	0.85
Block	1	148.9	0.87	0.36
Diet*Spec*Sex*Bloc <sup>1</sup>	33	5627.4	1.47	0.05
Day	4	845.1	1.82	0.12
Diet*Day	24	2693.7	0.97	0.51
Species*Day	4	120.1	0.26	0.90
Sex*Day	4	599.9	1.29	0.27
Diet*Species*Day	24	2883.5	1.04	0.42
Diet*Sex*Day	24	2306.8	0.83	0.70
Species*Sex*Day	4	263.9	0.57	0.68
Error b	414	47944.3		
Total	557	130854.2		

<sup>1</sup> Error a

Appendix Table 11. Analysis of variance of energy retention (cal/g dry weight crawfish/day) for Procambarus clarkii and Procambarus acutus acutus in 63-day growth study.

Source	Degree of Freedom	Sum of Square	F Value	Pr > F
Diet	6	356994.4	157.82	0.01
Species	1	17724.6	47.01	0.01
Diet*Species	6	10381.9	4.59	0.01
Rep(Diet*Species) <sup>1</sup>	28	10556.4	1.40	0.11
Sex	1	79.4	0.30	0.59
Diet*Sex	6	710.6	0.44	0.85
Species*Sex	1	292.8	1.09	0.30
Diet*Species*Sex	6	937.8	0.58	0.74
Rep*Sex(Diet*Species)	27	3862.5	0.53	0.97
Error b	117	31442.9		
Total	199	443606.6		

<sup>1</sup> Error a

Appendix Table 12. Analysis of variance of ecdysis energy (cal/g dry weight crawfish/day) for Procambarus clarkii and Procambarus acutus acutus in 63-day growth study.

Source	Degree of Freedom	Sum of Square	F Value	Pr > F
Diet	6	3160.6	75.95	0.01
Species	1	78.5	11.32	0.01
Diet*Species	6	79.3	1.91	0.12
Rep(Diet*Species) <sup>1</sup>	28	194.2	1.20	0.25
Sex	1	1.0	0.17	0.68
Diet*Sex	6	11.3	0.33	0.92
Species*Sex	1	1.2	0.20	0.66
Diet*Species*Sex	6	30.3	0.87	0.52
Rep*Sex(Diet*Species)	27	185.6	1.19	0.26
Error b	117	676.5		
Total	199	4518.9		

<sup>1</sup> Error a

## VITA

Mattana Sanguanruang was born 17 August 1959, in Bangkok, Thailand. She attended Sribunyanon High School, Nonthaburi, Thailand and was graduated in February 1975.

In May 1975, she entered Prince of Songkla University, Songkla, Thailand and was graduated 2<sup>nd</sup> class honor with the Bachelor degree in Biology in March 1979.

In August 1979, she entered the Graduate School at Louisiana State University in Baton Rouge and earned the Master of Science degree in Animal Physiology in December 1981.

In January 1982, she was accepted into the master program in Department of Experimental Statistics, Louisiana State University and was graduated with the Master of Applied Statistics degree in August 1983.

Upon completion of the Master degree in Applied Statistics, she was accepted into the doctoral program of the School of Forestry, Wildlife, and Fisheries, Louisiana State University.

Since May 1987, she has been employed by the Department of Experimental Statistics, Louisiana State University, and Louisiana Department of Wildlife and Fisheries as a statistical consultant on Louisiana Offshore Oil Port Project. She is now candidate for the degree of Doctor of Philosophy in Wildlife and Fisheries Science from the School of Forestry, Wildlife, and Fisheries.

# DOCTORAL EXAMINATION AND DISSERTATION REPORT

Candidate: Mattana Sanguanruang

Major Field: Wildlife and Fisheries Science

Title of Dissertation: Bioenergetics of Red Swamp Crawfish (Procambarus clarkii) and White River Crawfish (Procambarus acutus acutus) in Cultivated, Noncultivated and Wooded Ponds in South Louisiana.

Approved:

Robert P. Romaine  
Major Professor and Chairman

J. Glen Hembrey  
Dean of the Graduate School

## EXAMINING COMMITTEE:

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Date of Examination:

December 2, 1988