# Effectiveness of Synthetic Molecules, and Animal and Vegetable Extracts as Baits for Harvesting Red Swamp Crayfish, *Procambarus clarkii*

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**ABSTRACT.** The present study was designed to evaluate the efficiency of different biogenic amines (putrescine, cadaverine, tyramine, spermine and spermidine) and their precursory amino acids (arginine, histidine, lysine and tyrosine), as well as animal extracts (from fish, crustaceans and mollusks) and vegetable extracts (*Chara* sp., coconut and alfalfa) as baits in traps for red swamp crayfish, *P. clarkii*. The methodology was conceived as a logical sequence to proceed from rapid screening of a large number of treatments and was divided into three phases: (1) chemodetection bioassays, (2) chemattraction bioassays, and (3) field bioassays carried out under natural conditions. The attractants that showed the best performance as baits for *P. clarkii*, were: the fish soluble extract (included at 2.96%), the putrescine (included at 0.30%) and the red crab soluble extract (included at 2.69%). The benefits of using these attractants, when added to common pelleted feed, come from their low cost, availability and enhanced handling properties. 

**KEYWORDS.** Harvest, red swamp crayfish, *Procambarus clarkii*, baits

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## INTRODUCTION

Red swamp crayfish, *Procambarus clarkii*, is distributed naturally and has been introduced in many of the states of northern Mexico, as well as in the southern United States, specifically in Texas, Alabama, Louisiana, Mississipi, Florida, Arkansas, Tennessee, Missouri, Illinois, New Mexico and Oklahoma (Hobbs et al. 1989). Red swamp crayfish is an attractive species for aquaculture because of its great capacity to adapt to different hábitats. Besides, it has fast growth and a high reproductive potential (LaCaze 1981; Huner and Barr 1991). The largest commercial production of this species is carried out mainly in the state of Louisiana, where 95% of the total production is harvested in an area of more than 44,000 ha, producing nearly 19 million kg (McClain 2001). The existing natural and introduced populations in northern México provide an opportunity for their exploitation, considering the increasing acceptance of the species.

Red swamp crayfish are particularly active in the evening and continue their feeding activity until dawn. Several food items have been suggested as being important, including periphyton, plankton, bacteria, detritus and benthonic organisms (Avault et al. 1974). However, some aquatic plants are also part of the diet of this species and recently it has been demonstrated that they also consume animals like copepods, waterfleas and amphipods (Huner and Barr 1991).

Red swamp crayfish harvest takes place 3-4 month per year, representing 50-70% of the production costs (McClain and Romaire 1999); but with the rapid expansion of this industry in the US, the demand for effective baits of low cost has increased (Cange et al. 1982).

Among the baits more commonly used are fish like gizzard shad (D'Abramo and Niquette 1991), but significant quantities of menhaden, (*Brevoortia tyrannus*), carp (*Cyprinus carpio*), suckers (*Catostomus* sp.), and the head and viscera of processed fish are also used (Avery and Lorio 1999). Beef melt (spleen) is considered a good bait, but is generally too expensive for commercial use (Huner and Bar 1991). Beef liver has been used with good results (Huner and Barr 1991). This was confirmed by Rodríguez (1993) who, while comparing the effectiveness of baits, found that beef liver offered better results than chicken viscera, fish muscle or shrimp heads. Fish and meat are cut and dropped into the trap, but are messy, often having a strong unpleasant odor, and they are inconvenient to store, especially on a farm (Hunter and Pfister 1990). They are seasonal in supply and may be expensive (Kawamura et al. 1995). As an alternative, studies have demostrated that channel catfish, *Ictalurus punctatus*, oil (Avault et al. 1985) and sugarcane-fish combinations (Kawamura et al. 1995) are effective as baits in red swamp and crab traps, respectively. This has led to the development of grain-based, manufactured crayfish baits similar in formula to low-quality fish feed (Huner 1990). These baits are easily stored and may last longer in the traps.

The objectives of this study were to evaluate the effectiveness of synthetic biogenic amines and their precursory amino acids, as well as of extracts of animals and vegetables sprayed on low-quality fish feed as baits for trapping red swamp crayfish.

## *MATERIALS AND METHODS*

Adults and juveniles of red swamp crayfish were collected from streams near Santiago and Cadereyta Jiménez, Nuevo Leon. Additionally, some of the experimental organisms were obtained by means of reproduction of the collected adults. After they reached an average weight of 7±1 g, 391 crayfish in intermolt, form II (nonbreeding) were used in laboratory trials.

Intact red swamp crayfish with all appendages were utilized, including antennaes and antennules, as the receptor cells are located in these organs. The animals were kept with a photoperiod of 12 hours light and 12 hours darkness. They were carefully conditioned to the basal diet for 7 days before beginning the tests. The bioassays were carried out with crayfish individuals previously maintained without food during 24 hours. Single animals were used in each trial to avoid possible group response and were not reused after testing to avoid any preference for a particular treatment.

The experimental series employed in this study was planned to evaluate the following treatments: amino acids (arginine, histidine, lysine and tyrosine), biogenic amines (putrescine, histamine, cadaverine, tyramine, spermine, spermidine), animal extracts (fish, *Mugil cephalus*; crustaceans: blue crab, *Callinectes sapidus*; red crab, *Pleurocodes planipes*; molluskans: apple snail, *Pomacea bridgesi*, and squid, *Loligo* sp.); and vegetal extracts (coconut, *Cocus nucifera*; *Chara* sp., alfalfa, *Medicago sativa*). A commercial attractant (*Langobuds*®, Quali Tech, Chaska, Minnesota, USA[1]) and a basal diet designed to be non-attractive were used as positive and negative controls, respectively.

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1. Use of trade or manufacturer's names does not imply endorsement.

The amino acids and the biogenic amines were obtained from Sigma Chemical Co. (St Louis, Missouri, USA), with a minimum purity of 98%. The preparation of the extracts was carried out by mixing the samples with similar parts of water. These mixtures were homogenized and centrifuged (2-4°C) for 10 minutes at 10,000 g. An exception was made with the red crab extracts, the different fractions (ether extract, red crab solubles, freeze-dried red crab extract, and red crab oil) of which were obtained as industrial by-products of a fish meal processing plant. Each of these extracts was used as a separate treatment.

A hieriachical methodology was developed for screening the treatments in order to identify the best bait for red swamp crayfish. This methodology was conceived as a logical sequence to proceed from a rapid screening of a total of 24 treatment as potential excitants (chemodetection bioassay), progressively through more discriminatory procedures for assessing chemotaxis (chemoattraction bioassay), to a final selection of the most potent chemostimulants for use in the field as bait for red swamp crawfish capture.

### *Chemodetection Bioassay*

This bioassay was carried out following the methodology proposed by Pittet et al. (1996), which is based on the qualitative investigation of the degree of excitment of the organism in presence of the test molecules.

The equipment consists of a glass test chamber, $15 \times 10 \times 15$ cm$^3$ (L $\times$ W $\times$ H), containing the test animal in tapwater. The test chamber is surrounded by a black wood box with a window on the opposite extreme for observation with a video camera. Throughout the trial (3 minutes), the movements of antennules, antennae, mandibules, maxillipeds, and dactyls were noted together with gross movements of the animal about the test chamber. A response value using the ordinal scale of Pittet et al. (1996) was adopted to reflect increasing levels of activity:

0: no apparent reaction
1: maxillipeds beating sporadically; no antennular activity
2: maxillipeds beating regularly; no antennular activity
3: maxillipeds beating regularly; sporadic antennular activity
4: maxillipeds beating continually; sporadic antennular activity
5: maxillipeds beating continually; extreme antennular activity

The treatments were added as a fine spray, to 1 gram of the basal diet, by aspersion. Considering the values registered for each one of the 24 treatments, five doses and three replicates were carried out. A dose-response curve was obtained for each one of the treatments by means of a second order polynomial regression analysis.

### *Chemoattraction Bioassay*

The methodology adopted for the chemoattraction bioassay was similar to that described by Costero and Meyers (1993), which entails the use of tanks of 120 × 30 × 40 $cm^3$ without water flow. These tanks have a movable division on one end to hold the animals while the treatment was placed at the other end of the tank. Prior to the testing sessions, the aeration tubes were removed from the aquaria. The water temperature was maintained constant at 26±1°C by means of automatic aquarium heaters and the pH during the experimental period was 7.5-8.

Five critical behavioral response descriptors (perception, orientation, displacement, arrival and ingestion), as described Costero and Meyers (1993), were considered (Table 1).

*Treatments:* Eleven treatments were compared: putrescine, coconut extract, red crab solubles, cadaverine, mullet extract, histidine, *Chara* sp. extract, lysine, a commercial diet, the commercial attractant Langobuds®, and the basal diet.

*Addition of treatments:* The treatments were added as a fine spray to 5 g of the basal diet by aspersion at the optimal dose determined in the previous phase. The basal diet was designed to be non-attractive; the formulation was characterized by its high content of soybean and wheat meal (300 g/kg and 500 g/kg, respectively), known to have an anti-palatable effect (Mendoza 1993).

TABLE 1. Sequential phases of the feeding behavior of crustacean.

| Phase | Behavior |
|---|---|
| Perception | Characterized by intermittent flicking movement of the antennae. |
| Orientation | The animal increases the searching activity and orientates itself towards the food source direction. |
| Movement | The animal walks towards the food source, often following a zig zag path, but if the stimulation is powerful it swims. |
| Arrival | The animal arrives at the food source and spends some time exploring it. |
| Ingestion | The animal eats or refuses the food. |

*Recording the test:* A remote-controlled video camera was used to avoid influencing the behaviors of the red swamp crayfish. Sequences of behaviors were video-recorded until diet intake was quantified. A stopwatch was used to record the actual time, simultaneously with movie recording. Time zero was set at the moment that the movable partition of the aquarium was raised. Video recordings were registered by two independent evaluators.

*Experimental design:* Three replicates were conducted for each treatment. The results were evaluated by a one way Analysis of Variance (ANOVA) to determine differences between treatments. Tukey's test (Zar 1996) was used to identify differences among the mean values at $P = 0.05$ level.

### *Field Bioassay*

To confirm the results obtained in the laboratory, a bioassay with traps in natural conditions was carried out in the San Bartolo river, located in Cadereyta, Nuevo León, México. This was particularly important to confirm the performance of the attractants as baits for crayfish. In this trial, red swamp crayfish were not stocked because a natural population of red swamp crayfish was already present.

The bioassays consisted of placing traps using the treatments as baits. The traps, with two entrance funnels, were placed at a distance of 10 meters and the treatment was tested.

The test times were of 20, 40 and 80 minutes, after which was registered the number of crayfish captured by treatment in each time. All trapped animals were released, the baits were renewed, and the disposition of the baited traps changed for the next set. Three replicates of each treatment were carried out to validate the results. These were evaluated by a one-way Analysis of Variance (ANOVA) to determine if there were differences among treatments. Tukey's test (Zar 1996) was used to identify differences among the mean values at $P = 0.05$ level.

## *RESULTS AND DISCUSSION*

### *Chemodetection Bioassay*

The observations allowed the construction of dose-response curves. Results showed that the highest degree of excitation among amino acids was produced with histidine (3.25) and lysine (3.05), while in the case of biogenic amines, putrescine (3.84) and cadaverine (3.3) treatments offered the best response. In the case of animal extracts, red crab solubles and fish extracts showed the best results with 3.42 and 3.27, respectively. In the case of vegetable extracts, the coconut extract showed the highest excitement degree (3.47), followed by the *Chara* sp. extract (3.15). Finally, the commercial attractant, used as positive control, evoked an important behavioral excitaton (3.83), while the basal diet and the commercial diet did not elicit any degree of excitation.

Table 2 shows the response degrees and the optimal doses reached for each one of the treatments.

### *Chemoattraction Bioassay*

The ANOVA revealed the existence of significant differences among the treatments during the different phases of the feeding behavior ($P < 0.05$). Comparisons of means by the Tukey's test revealed that those treatments that elicited the shortest time of response were the commercial attractant, putrescine, *Chara* sp., mullet extract, red crab solubles, lysine, cadaverine and coconut extract. By contrast, the longest time lapsed, as an animal presented the ingestion stage, was observed when individuals were exposed to histidine, the commercial diet, and the basal diet (Figure 1). The average results of all the different phases of feeding behavior are listed in Table 3.

The different behaviors related to diet intake used to quantify chemoattraction were similar to those identified for other crustacea such as pacific white shrimp, *Litopenaeus vannamei* (Costero and Meyers 1993) and freshwater prawn, *Macrobrachium rosenbergii* (Harpaz et al. 1987; Mendoza et al. 1997).

During all the experimental phases special care was taken to use only organisms with complete appendages. Integrality of appendages was observed immediately before using the organisms in the different experimental phases, as suggested by Devine and Atema (1982). In this regard, Cowan (1991) observed that American lobster, *Homarus americanus*, individuals that didn't possess lateral antennules lost all detection ability. Similarly, Dunham and Oh (1992) mentioned that the total or partial absence of appendages modifies significantly the capacity of detection of stimuli.

### *Field Bioassay (Natural Conditions)*

For this phase, the selected treatments were: putrescine, *Chara* sp. extract, mullet extract and red crab solubles, since they showed the best

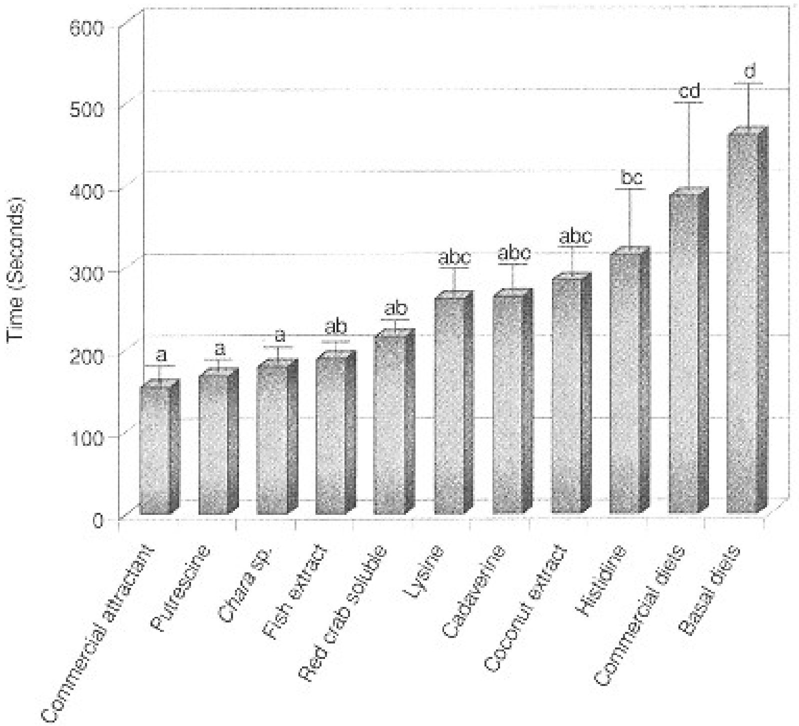
TABLE 2. Optimal doses and response degrees in the chemodetection bioassays.

| Treatment | Optimal doses % | Response degrees |
|---|---|---|
| Alfalfa sp. extract | 3.41 | 0.11 |
| Arginine | 0.37 | 1.9 |
| Basal diet (−) | 4.6 | 0.91 |
| Blue crab extract | 3.72 | 2.06 |
| Cadaverine | 0.33 | 3.3 |
| *Chara* sp. extract | 3.03 | 3.15 |
| Coconut extract | 2.48 | 3.47 |
| Commercial attractant | 0.26 | 3.83 |
| Commercial diet (+) | 0.29 | 1.65 |
| Fish extract | 2.96 | 3.27 |
| Freeze-dried red crab | 0.36 | 1.68 |
| Freshwater snail extract | 2.85 | 2.3 |
| Histamine | 0.26 | 1.77 |
| Histidine | 0.29 | 3.25 |
| Lysine | 0.29 | 3.05 |
| Putrescine | 0.3 | 3.84 |
| Red crab solubles | 2.69 | 3.42 |
| Red crab oil | 2.7 | 1.52 |
| Red crab ether extract | 3.92 | 0.86 |
| Spermidine | 0.36 | 1.78 |
| Spermine | 0.31 | 1.88 |
| Squid extract | 2.85 | 2.43 |
| Tyramine | 0.34 | 1.53 |
| Tyrosine | 0.29 | 0.91 |

results in the chemoattraction bioassays. A commercial attractant (Langobuds®) and beef liver were used as positive controls, while the basal diet was used as a negative control.

The results revealed the existence of significant differences among the treatments in relation to the number of red swamp crayfish captured at the different times. The number of individuals captured with the mullet extracts was higher than with the rest of the treatments, followed by

FIGURE 1. Mean time elapsed in seconds, from the perception to the ingestion phase ($P < 0.05$). Means with the same letter(s) belong to homogeneous groups.



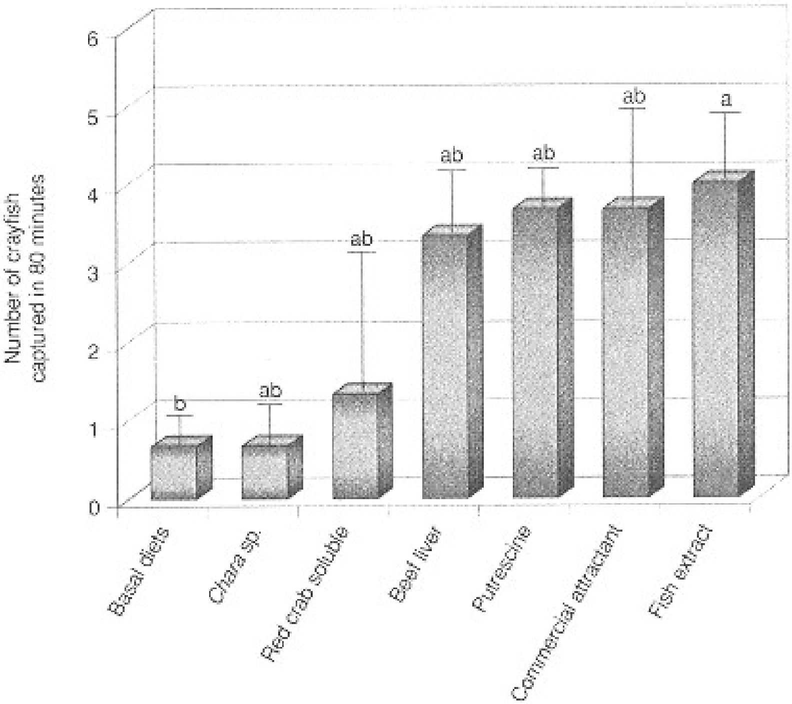

the commercial attractant (Langobuds®), putrescine, beef liver, and red crab soluble, whereas the lower number of individuals was captured with the basal diet and the *Chara* sp. extract (Figure 2).

In this test, beef liver was used as an alternative positive control since Rodríguez (1993) mentioned its use offering better results that a series of extracts and viscera of different species. Here the mullet extract gave the best results (4 captures), coinciding with the observations of Huner and Bar (1984) who mentioned that among the most effective baits are different fish species (they promoted fish such as sardines, carps and catfish) and that fish-feed baits are superior to livestock diets in colder waters but similar in warm weather. Contrary to that observed in the laboratory bioassays, crayfish were not attracted by the *Chara* sp. extract (1.33 average captures), probably because of its natural abundance in the habitat where the study was carried out.

TABLE 3. Mean responses±SD (time in seconds) obtained during the different phases of feeding behavior for each treatment.

| Treatment | Perception | Orientation | Movement | Arrival | Ingestion |
|---|---|---|---|---|---|
| Commercial attractant | 30.3±5.03a | 40.0±7.21a | 51.0±11.13a | 143.3±17.24a | 155.0±10.81a |
| Putrescine | 41.0±9.00ab | 46.6±10.09ab | 68.3±9.50ab | 139.0±8.18a | 168.6±12.66a |
| *Chara* sp | 45.3±8.50ab | 59.3±16.29abc | 91.0±17.69ab | 168.0±20.42a | 179.3±16.20a |
| Fish extract | 60.0±19.67abc | 85.0±38.59abc | 106.3±49.52abc | 156.6±31.53a | 188.6±14.29ab |
| Red crab soluble | 87.3±7.09bcd | 98.3±17.39abcd | 144.3±9.29bc | 193.0±9.85abc | 215.3±6.43ab |
| Lysine | 70.3±15.57abcd | 88.0±14.80abc | 117.6±32.25abc | 188.3±28.75ab | 260.6±35.02abc |
| Cadaverine | 57.3±8.02abc | 108.3±9.07bcd | 137.0±7.57bc | 211.0±19.52abc | 263.6±34.82abc |
| Coconut extract | 82.3±13.05abcd | 107.3±11.72bcd | 123.0±15.13abc | 209.3±14.01abc | 283.3±32.25abc |
| Histidine | 118.3±18.77de | 157.3±18.58de | 194.0±26.85c | 274.6±68.65cd | 314.33±72.86bc |
| Commercial diets | 101.6±17.39cde | 121.6±26.50cde | 142.3±24.00bc | 262.0±48.54bc | 386.3±119.78cd |
| Basal diets | 152.6±38.13e | 173.6±44.77e | 196.3±57.07c | 453.3±28.50d | 460.6±68.13d |

FIGURE 2. Number of red swamp crayfish captured in 80 minutes ($P < 0.05$). Means with the same letter(s) belong to homogeneous groups.




The mean total length of the captured individuals was 10.5±3 cm, which is higher than the minimal commercial size (Momot and Romaire 1981). Considering the results observed in the last phase, this suggests that there are no differences between juveniles and adults concerning their alimentary preferences.

Mean water temperature during this experimental phase was 18±2°C, which is in the range of that reported by Mazlum and Eversole (2001) who signaled that there were no differences in capturing white river crayfish, *Procambarus acutus*, during cool-harvest (≥15°C) and warm-harvest ponds (≥20°C). Avery and Lorio (1999) suggest the utilization of fish/manufactured bait combinations at these temperatures.

Bait costs vary, depending on the type of bait used and seasonal supply, but generally range from $14 to $27 per 100-pound units (Avery and Lorio 1999). In the case of putrescine, its use costs $15.4 per

100-pound units, plus the cost of a low quality pelleted diet ($10.9 or less per 100-pounds); however, its use offers the advantage of a shelf stable product that is easily available, thus lowering the overall costs when compared to the utilization of baits like fishes.

As expected, red swamp crayfish responded to the feeding stimulant, suggesting that chemoreception is used in the foraging strategy of this species. Similar observations have been reported by Steele et al. (1999).

The relevance of this study is reflected in the fact that the addition of feeding stimulants as attractants, on food of low quality to be used as baits, increases red swamp crayfish capture. Furthermore, these baits are economical and easy to handle.

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