Molting and Mortality of Red Swamp and White River Crawfish Subjected to Eyestalk Ablation: A Preliminary Study for Commercial Soft-Shell Crawfish Production

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Abstract

Eyestalk ablation may reduce the cost of soft-shell crawfish production by reducing the molt interval. In this study, both immature and mature red swamp crawfish _Procambarus clarkii_ and white river crawfish _Procambarus zonangulus_, formally _Procambarus actus actus_ (Hobbs and Hobbs 1990), were ablated using a pair of modified pliers and placed in a recirculating system. Molting percentages, mortalities and mean molt intervals of the ablated crawfish were analyzed. Eyestalk ablation resulted in dramatic reduction of molt intervals and mortalities comparable with the current commercial (non-ablation) soft-shell crawfish production systems. The mean molt intervals of the ablated red swamp crawfish ranged from 6.7 to 7.8 days for immature and 9.2 days for mature animals; whereas, the molt interval of ablated white river crawfish was 8.9 and 11.2 days for immature and mature animals, respectively. Mortalities obtained in this study ranged from 20 to 48% and 32 to 66% for immature and mature crawfish, respectively. During the experiments, molting percentages and mortalities were not consistent. Secondary treatments such as air clotting and cauterization did not alter mortality significantly.

The production of soft-shell crustaceans has been recognized as having a great potential (Redmayne 1989) and is considered a new horizon for aquaculture (Wear 1990). Such an industry has been developed for soft-shell crawfish in the gourmet food market since the mid-1980s in the southeastern USA, primarily in Louisiana (Culley and Duobinis-Gray 1990).

Soft-shell crawfish production is based on the molt cycle in which a crawfish periodically sheds its old exoskeleton, permitting expansion of the soft tissues and increases in body size and volume (Aiken and Waddy 1987). A newly molted crawfish has a soft exoskeleton and is called a soft-shell crawfish. In nature, a mature red swamp or white river crawfish usually does not molt until the reproductive cycle has been completed (Huner 1990). However, juvenile crawfish molt more frequently, normally at least every 30 days depending on temperature. However, adverse conditions such as crowding, lack of food, extreme temperature, etc., can cause a delay in the molt for up to six months or more (Huner and Avault 1977). In commercial soft-shell crawfish operations, an extended molting cycle is often experienced, thereby, increasing the turnover time and unit production costs.

There are several ways to accelerate the crawfish molting cycle, including injection of molting hormones (ecdysones), limb removal and bilateral eyestalk ablation (Aiken and Waddy 1987). Bilateral eyestalk ablation was suggested as a method for producing soft-shell crawfish for fish bait (Huner and Avault 1977).

The molting process of crustaceans, including crawfish, is hormonally regulated (Fingerman 1987). There are two main organs involved in regulating the process, the Y-organ which produces molting hormones and the X-organ which produces molt-in-
hibiting hormones. The X-organs lie in the proximoventral edge of the medulla terminalis in the eyestalks while the Y-organs are located in the maxillary segments (Fingerman 1987). If the X-organs are removed by eyestalk ablation, the molt-inhibiting hormone is no longer produced, stimulating molting.

Hormonal imbalances may result from eyestalk ablation and cause high mortality (Brown and Cunningham 1939; Smith 1940; Bittner and Kopanda 1973; Huner and Lindqvist 1984), especially in mature animals (Huner and Lindqvist 1984). However, high mortality is not always a problem with immature animals as reported by some investigators (Huner and Avault 1977; Nakatani and Otsu 1979).

Due to the lack of production-oriented research, ablation technology is still not readily available for commercial application. Successful commercial implementation of ablation technology for soft-shell crawfish production demands several prerequisites. First, the ablated crawfish must be able to molt significantly quicker than non-ablated ones. Second, a convenient ablation technique, instead of individual surgery, is warranted to increase the efficiency of the operation to reduce labor costs. Third, the ablation technique must be such that the mortality levels are economically acceptable. This study was designed: 1) to document molting success and mortality of red swamp and white river crawfish subjected to bilateral eyestalk ablation; 2) to determine the mean molt interval (the average interval from the time the crawfish were ablated to the time crawfish molted) for bilaterally eyestalk-ablated, harvestable sized immature and mature crawfish; and 3) to evaluate the effects of different bilateral eyestalk ablation techniques.

Materials and Methods

Experimental Systems

Four experiments were conducted within two different recirculating systems during 1989 and 1990 in the Civil Engineering Aquatic System Laboratory (CEASL) at Louisiana State University, Baton Rouge, Louisiana. The first three experiments were conducted in a controlled environmental chamber (ablation chamber) and the fourth in an open holding system.

The controlled environmental chamber was set up within a wooden enclosure (Fig. 1). The chamber contained nine acrylic trays (74 cm L × 38 cm W × 10 cm D) separated into three sub-units, each having a sump and a fluidized bed reactor for biofiltration. The three units were interconnected to an upflow sand filter for solids removal and supplemental biofiltration. The design of the water treatment units was based on the parameters recommended by Malone and Burden (1988).

The temperature and photoperiod of the ablation chamber were controlled by a Kaypro-2X computer through an ADC-1 data acquisition and control device (Remote Measurement System, Inc.). The photoperiod was set to a 12:12 hour light, dark cycle. The water was heated by immersed heaters and cooled by the air conditioning system of the room.

External to the environmentally controlled chamber was a fiberglass holding tray (90 cm L × 183 cm W × 15 cm D) used
in the fourth experiment. This tray was subdivided into six equally sized sections (90 cm L x 30 cm W) and partitioned with gray PVC sheeting, with slotted bottoms, allowing for free water circulation. The holding tray was connected to an upflow sand filter for biofiltration and solids removal (Malone and Burden 1988). The upflow sand filters of both the ablation chamber and the holding system were backwashed daily. The photoperiod and temperature of the holding tray were not specifically controlled however, the temperature mimicked that of the room temperature which varied from 20 to 23 C.

**Experimental Procedure**

Ablation was performed bilaterally with a pair of modified pliers (Fig. 2). The pliers proved to be much more efficient and just as effective as using scissors, forceps and/or scalpels. The pliers were fabricated from a pair of common pliers (Stanley, 84-092) by shaping and bending the two tips. The length of the pliers after modification was approximately 14 cm, and the width of the modified tips was approximately 2 mm. As indicated in Fig. 2, this pair of pliers can easily be used to hold the two eyestalks of a crawfish and remove them simultaneously. The ablated crawfish were put back into water immediately, unless otherwise noted. The control groups were processed through the same procedure but were not ablated. After ablation, the crawfish were observed for 15 days; and during this period, all crawfish were fed approximately 1% of their body weight daily with formulated ration (Burris Aquaculture Feeds, Franklinton, Louisiana).

Experiments 1 and 2 contained three treatments while Experiments 3 and 4 consisted of four treatments. Each treatment was replicated twice with 25 crawfish each. Experiment 1 (16-30 May 1989) was performed to determine whether a 5 min air clotting period after eyestalk removal would reduce mortalities of immature red swamp crawfish. For the first treatment group, the crawfish were immediately returned to the water after the eyestalks were ablated. For the second treatment group, a 5 min air clotting period was allowed before returning the crawfish to the water. Experiment 2 (8-22 June 1989) was conducted to determine if heat cauterization following ablation would reduce mortality. For the first treatment group, the tips of the modified pliers were held in the blue portion of a torch flame for five seconds before being used to cut the eyestalks. For the second treatment group, the eyestalks were removed with the same pliers, without heating. Experiment 3 (2-16 June 1990) was designed to investigate the effects of ablation on molting and mortality.
FIGURE 2. The modified pliers and its operation in the ablation process. Above: the modified pliers; below: ablation operation with the modified pliers.
Table 1. Total molting percentages, mortalities and their corresponding 95% confidence intervals of different treatments (N = 50).

<table>
<thead>
<tr>
<th>No.</th>
<th>Treatment ablation technique</th>
<th>Molt (%)</th>
<th>Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean CI95%</td>
<td>Mean CI95%</td>
</tr>
<tr>
<td>1</td>
<td>Air clot 5 minutes</td>
<td>54 39-68</td>
<td>46 32-61</td>
</tr>
<tr>
<td>2</td>
<td>Without air clot</td>
<td>52 37-66</td>
<td>48 34-63</td>
</tr>
<tr>
<td>3</td>
<td>Control</td>
<td>56 41-70</td>
<td>25 13-38</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Experiment 2</td>
<td></td>
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<tr>
<td>4</td>
<td>With cold pliers</td>
<td>75 62-87</td>
<td>25 13-38</td>
</tr>
<tr>
<td>5</td>
<td>Cauterization</td>
<td>68 53-81</td>
<td>32 20-47</td>
</tr>
<tr>
<td>6</td>
<td>Control</td>
<td>40 25-66</td>
<td>10 3-22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Experiment 3</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Immature red swamp</td>
<td>54 39-68</td>
<td>46 32-61</td>
</tr>
<tr>
<td>8</td>
<td>Immature red swamp control</td>
<td>18 9-31</td>
<td>32 20-47</td>
</tr>
<tr>
<td>9</td>
<td>Mature red swamp</td>
<td>34 21-49</td>
<td>66 51-76</td>
</tr>
<tr>
<td>10</td>
<td>Mature red swamp controlb</td>
<td>0 —</td>
<td>34 21-49</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Experiment 4</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Immature white river</td>
<td>80 66-90</td>
<td>20 3-22</td>
</tr>
<tr>
<td>12</td>
<td>Immature white river control</td>
<td>4 0-20</td>
<td>4 0-20</td>
</tr>
<tr>
<td>13</td>
<td>Mature white river</td>
<td>68 53-81</td>
<td>32 20-47</td>
</tr>
<tr>
<td>14</td>
<td>Mature white river controlb</td>
<td>0 —</td>
<td>8 1-26</td>
</tr>
</tbody>
</table>

a Confidence intervals (CI) as binomial variables (Dowdy and Wearden 1983).
b \(N = 25\).

of mature red swamp crawfish by comparing them with the immature crawfish, and Experiment 4 (16–30 May 1990) was to evaluate the molting and mortality of mature and immature white river crawfish.

**Data Collection and Analysis**

The numbers of live, dead, and molted crawfish were recorded daily. In situ pH and dissolved oxygen were measured daily. In addition, water samples were collected on an average of four times weekly for total ammonia-nitrogen and nitrite-nitrogen analysis according to "Standard Methods" (APHA 1985).

By treating the event of whether a crawfish molted or died as a binomial variable, the means of the total molting percentages, mortalities, and their 95% confidence intervals (CIs) were calculated according to the method recommended by Dowdy and Wearden (1983). Statistical differences between means were evaluated using a t-test \((P = 0.05)\). The mean molt intervals after ablation and the standard deviation were calculated based on the method for grouped frequency distributions (Thorndike 1982). In addition, statistical differences within mean molt intervals were further analyzed using Duncan’s multiple range test (Bruning and Kintz 1987).

**Results**

**Water Quality**

The water treatment system for the ablation chamber provided acceptable water quality during most of the experimental periods. Total ammonia nitrogen concentrations in Experiments 1 and 2 were relatively stable throughout the 15 d experimental runs \((0.17 \pm 0.12, 0.16 \pm 0.04 \text{ mg-N/L, respectively})\), while higher concentrations were observed in Experiment 3 \((0.36 \pm 0.27 \text{ mg-N/L})\). Nitrite concentrations in Experiments 1, 2, and 3 were relatively stable \((0.09 \pm 0.12; 0.07 \pm 0.04, 0.15 \pm 0.09 \text{ mg-N/L})\).
Under these water quality conditions, the mortalities of the control animals ranged from 10 to 32% in Experiments 1 to 3.

Water temperature was maintained at 25 ± 1°C, minimum dissolved oxygen concentration averaged 5.4 mg/L and pH ranged from 6.9 to 7.6. These water quality parameters were comparable with the standards for soft-shell crawfish production (Culley and Duobinis-Gray 1990).

Molting Percentage and Mortality

The molting percentages and mortalities for the experiments are summarized in Table 1. As a general observation, the ablated crawfish either molted or died within the 15 day experiment time frame. The higher the mortality, the lower the total molting percentage or vice versa. Ablation induced both immature and mature crawfish to molt. The mean molting percentages of the ablated immature crawfish ranged from 52 to 80%, while the mature crawfish ranged from 34 to 68%.

The molting percentages resulting from the same ablation method for immature crawfish were not consistent between different experiments. Using the same pliers without secondary treatment resulted in statistically equivalent mean molting percentages in Experiments 1 and 3 (treatments 2 and 7). The molting percentage in Experiment 2 (treatment 4), however, was significantly higher ($P < 0.05$) than that in Experiments 1 and 2. Similarly, the same treatment for mature crawfish in Experiments 3 and 4 (treatments 9 and 13) resulted in different ($P < 0.05$) molting rates between the red swamp and white river crawfish.

Additionally, Table 1 indicates that: 1) secondary treatments such as air clotting and heat cauterization did not significantly increase ($P < 0.05$) total molting percentage when compared to simple eyestalk removal; 2) the molting rates of immature ablated crawfish were higher than those of the mature ablated crawfish. This difference was only significant ($P < 0.05$) for the red swamp
crawfish (in Experiment 3) but not the white river crawfish (in Experiment 4); and 3) mortality of the ablated crawfish was significantly greater ($P < 0.05$) than that of the controls.

Molt Interval

The molt intervals for ablated animals were shorter in all experiments (Table 2). Almost all the surviving ablated crawfish molted by the end of 15 days; whereas the control crawfish did not (experiments were terminated at the end of 15 days). The mean molt interval of ablated crawfish in all experiments ranged from 6.7 to 11.3 days, varying less than five days despite differences in species, maturity and experimental conditions.

The ablated immature crawfish molted significantly ($P < 0.05$) sooner than mature ablated crawfish for both species. Control animals did not complete molting by the end of the experiments; thus, comparisons between the ablated and control crawfish are not available.

Molt Pattern

The molt pattern of the ablated crawfish in Experiment 3 (Fig. 3) demonstrates that ablated crawfish not only molted sooner, but also molted synchronously. For example, the ablated immature crawfish started molting on day 5 and finished molting on day 11. The mature ablated crawfish followed the same pattern; although they started molting two days later than the ablated
immature crawfish, both groups completed molting within 11 days. The immature control animals had a broader molting period. Molting started on day 2, with some unmolted animals remaining on day 15. No mature control animals molted.

Mortality Pattern

Although mortality varied among experiments, the majority of it generally occurred within the first several days after ablation. Fig. 4 illustrates the daily mortality rate of the control and ablated immature red swamp crawfish based on pooled data from Experiments 1 and 2 (treatments 2 and 4 for ablated animals and 3 and 6 for control animals). Fig. 4 also depicts a trend in which the ablated immature crawfish died between days 1 and 3 after ablation or between days 5 and 9, when most crawfish were molting. In fact, 45% of the total mortality of the ablated crawfish occurred in the first three days.

Discussion

Molt Interval

The mean molt interval of the ablated immature crawfish ranged from 6.7 to 8.9 days (Table 2). The shortening of the molt interval is accomplished by the rapid increase in the release of the molting hormone following eyestalk ablation (Chang 1989). This results in the immediate entry of the animals into the premolt stage and a dramatic reduction in the time required to complete premolt (Aiken and Waddy 1987). The mean molt intervals from this study compare favorably to other studies of crawfish endocrinology where actual intermolt period from one molt to the next was reported. In a study using younger immature red swamp crawfish (initial total length 8–12 mm) which underwent a maximum of five molts, Nakatani and Otsu (1979) reported that the mean molt intervals were 6.5, 7.1, 7.5, 8.8 and 9.2 days at 22–23 C. Huner and Avault (1977) reported that the mean molt interval for immature red swamp crawfish (5–6 cm total length) was 11.1 days at 26.7 ± 1 C.

The longest molt interval was reported by Bittner and Kopanda (1973). In their study, ablated red swamp crawfish (mean carapace length 8.5 cm) had a mean molt interval (±1 SD) of 18.4 ± 4.5 days, while the control group molted at 88 ± 31 days at 18.5–20 C.

The intermolt period of crawfish can be affected by several factors such as temperature, maturity, species and food availability. These effects are more pronounced in
natural conditions (Bittner and Kopanda 1973; Aiken and Waddy 1987; Huner 1990), but can be greatly reduced after ablation. For example, a mature crawfish in its natural environment will molt only after completing its reproductive cycle (Huner 1990). However, after ablation mature crawfish molted, on the average, in approximately 10 days in this study, only two days later than the ablated immature crawfish. Another example is the variation among species. It was reported that non-ablated immature white river crawfish molt much slower than the red swamp crawfish in soft-shell crawfish systems (Culley and Duobinis-Gray 1990). However, in this experiment, the mean molt interval for ablated white river crawfish was only 1.1 to 2.2 days longer than that of the ablated immature red swamp crawfish.

Non-ablated immature red swamp crawfish molt every 10.9–23.6 d in ponds during certain times of the year (Huner and Avault 1977). After they are brought into the soft-shell crawfish culture systems, molting is often delayed, due perhaps to a combination of overcrowding and other factors yet to be identified. Calculations based on the research conducted by Duobinis-Gray and Culley (1989) for commercial scale applications indicated that the mean molt interval of non-ablated immature crawfish ranged from 44.5 to 51.5 days with an overall mean of 48 days. Ablation, however, shortens the mean molt interval as shown in Table 2.

The reduction in molt interval and the “synchronizing show” of molting can provide advantages in commercial production. First, shortening of molt interval increases production and decreases unit production costs. Second, the production of a facility is more predictable. The producer can control the supply more easily to meet market demand. Finally, all crawfish harvested from the field can be directly used for soft-shell crawfish production. Although desirable, it is not necessary to separate the mature from the immature crawfish since they will both molt after ablation.

**Mortality**

It has been suggested that the ablation process can induce mortality through the production of a lethal hormonal imbalance (Brown and Cunningham 1939). Eyestalk removal affects at least five neurosecretory hormones (besides those produced by the X-organs) which control many physiological processes (Aiken and Waddy 1987). Yet, low mortalities after ablation are often reported. For example, working on immature red swamp crawfish, Huner and Avault (1977) obtained 9% mortality, while Nakatani and Otsu (1979) obtained no mortality at all. For Experiments 1 and 3 of this study, ablated treatments displayed high mortality (almost 50%, Table 1) for reasons which were not clearly identified. However, the authors believe that with selection of healthy crawfish and proper management after ablation, the mortality can be reduced.

The mortalities observed in the present experiments are comparable with those observed in commercial systems. In most non-ablation commercial soft-shell crawfish production units using red swamp crawfish, daily mortality runs 1–3%, while daily molting rates averaged between 1.4–3.8% (Culley et al. 1985; Culley and Duobinis-Gray 1990). The total mortality of a batch operation\(^1\) with the above stated molting and mortality rates was estimated to range from 19 to 61% (Table 3); whereas, the 95% CI for mortality of the ablated immature red swamp crawfish was 13–63% (Table 1). Theoretically, the mortality of ablated crawfish can approach the lower limit of that of a commercial production system using non-ablated crawfish. Mortalities less than 50% are usually obtained with ablation according to the results of this and other research studies (Bittner and Kopanda 1973).

\(^1\) In a batch operation, crawfish are loaded into the system as a batch, dead and molted crawfish are removed without adding new animals until all the initially loaded crawfish molt or die.
Table 3. Estimation of total mortality, molt interval and soft crawfish production of non-ablated immature red swamp crawfish under batch operation conditions.

<table>
<thead>
<tr>
<th></th>
<th>With ablation</th>
<th>Without ablationa</th>
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<tbody>
<tr>
<td>Mean molt interval (day)</td>
<td>7.0</td>
<td>48</td>
</tr>
<tr>
<td>Total mortality (%)</td>
<td>13–63b</td>
<td>19–61</td>
</tr>
<tr>
<td>Daily productionc (kg/day)</td>
<td>5.3–12.4</td>
<td>1.1–1.7</td>
</tr>
</tbody>
</table>

a Based on reported daily molting and mortality data (1–3% and 1.4–3.8%, respectively, Culley et al. 1985; Culley and Duobinis-Gray 1990).
b Based on the 95% interval indicated in Table 1.
c Calculated based on 100 kg crawfish initially loaded in a system.

Mortality caused by the ablation process is compensated for by an increase in average daily production of soft-shell animals. For a batch operation, non-ablation technology will produce 1.1–1.7 kg of soft-shell crawfish daily from each 100 kg of crawfish initially loaded into a facility (Table 3). Ablation technology should increase production to 5.3–12.4 kg per day. Daily production should further increase if dead and molted crawfish are replaced immediately in a continuous loading operation, perhaps by using partitions to separate groups. In either case, the ablation technology will produce several times more soft-shell crawfish than that of the non-ablation technology, provided mortality is maintained at less than 63%.

Ablation Techniques and Their Applications

Based on the experimental results and the preceding discussion, ablation using the modified pliers is feasible for enhancing soft-shell crawfish production. Using this tool, an operator can ablate a crawfish as quickly as he/she can pick it up, little additional effort is required. Therefore, it is much more efficient than other tools previously utilized to perform ablation. The main disadvantage of this technique is the complete removal of the eyestalks without preferential removal of only the X-organs. The commercial implementation of the ablation technology is possible only if consumers are willing to accept the ablated product. In addition, the success of the ablation technology is determined by mortality. The application of ablation technology is recommended only if: 1) the mortality in commercial scale operations (not just in laboratories) can be controlled to a desirable level; and 2) field trials in commercial facilities prove economical. Accordingly, additional research directed at determining the factors which contribute to mortality in ablated crawfish, along with commercial demonstration of ablation technology is clearly warranted.

Acknowledgments

This research was supported by the Louisiana Sea Grant College Program, an element of the National Sea Grant Program, under the direction of NOAA, U.S. Department of Commerce. The authors would like to thank Armant Aquaculture, Inc, of Vacherie, Louisiana for their assistance in constructing the experimental apparatus used in this study. The authors also thank Dr. D. D. Culley for reviewing this manuscript and providing the red swamp crawfish used in the experiments. The authors appreciate the assistance provided by Ms. JoAnn Kurts for data collection.

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