

Reproduction of Eastern White River Crayfish, *Procambarus acutus acutus* (Girard, 1852), in Two Different Habitats

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Abstract: The specific objectives, in the present study, were to compare the reproductive performance such as egg diameter, number of pleopod egg and relative physiological conditions of female white river crayfish in different habitats. Fecundity of adult female *Procambarus acutus acutus* were examined in simulated burrows and excavated pond burrow for three months. Pleopod egg numbers per female increased linearly with crayfish TL. Pleopod egg numbers varied from 106 to 556 for 81 and 127 mm Total Length (TL) female in simulated burrow and from 32 to 330 eggs for 65 mm and 125 TL female in excavated pond burrows, respectively. Similar sized female crayfish in simulated burrow oviposited significantly more eggs than the crayfish species in excavated pond burrows. The slopes and intercept of the TL regression for pleopod eggs for female were significantly different between those individuals held in simulated burrows and excavated pond burrows ($p < 0.05$). HM content of *P.a. acutus* held in simulated burrow (47.1%) was less than that of the excavated pond burrows (62.2%) ($p < 0.05$). The diameters extruded eggs for the two burrow types were similar during the study period.

Key words: Freshwater crayfish, reproduction, habitats

INTRODUCTION

White river crayfish was formally known as *Procambarus acutus acutus* (Girard, 1852). Hobbs and Hobbs^[1] revised taxonomy by separating the previous complex in two different species. The accepted common name for *Procambarus zonangulus* became white river crayfish while eastern white river crayfish is used for *P. a. acutus* still resides in the eastern United States, whereas *P. zonangulus* is found in southern states along the Gulf of Mexico.

Procambarid crayfish culture cycle usually involves a wet-culture and dry-culture phases. Mature crayfish excavate burrows which range from short, simple tubes associated with debris and rubble in permanent water bodies to extended, often elaborate tubes in the clay soils associated with seasonally flooded wetlands^[2,9]. *P. a. acutus* retire to burrows in late spring before the culture ponds are drained for planting forage^[10]. Crayfish remain in the burrows until the ponds are flooded in fall. Ovarian development and ovipositioning occurs within the burrows, hatching and early-instar development within the burrow^[9-10].

Flooding or rainfall is usually necessary to encourage emergence of crayfish from burrow. When ponds fill with water in fall, young-of-the-year exit the burrows and grow through the warm fall weather. Fecundity is a major and valuable parameter to evaluate the production

characteristics of the crayfish population such as age structure, sex ratio and population abundance. The hepatopancreas is the key site of energy store in crayfish^[8]. In a healthy crayfish, the hepatopancreas may account for 10-15% of the wet wet body biomass. The specific objectives, in the present study, were to compare the reproductive performance such as egg diameter, number of pleopod egg and relative physiological conditions of female white river crayfish in different habitats.

MATERIALS AND METHODS

Reproduction performance and relative physiological condition of female crayfish were evaluated in simulated burrows (in laboratory) and pond burrows (in earthen pond). In simulated burrow, fecundity was estimated using 48 adult females with complete sets of appendages collected from the harvest on May 10, 2002 and transported at the Aquatic Animal Research Facility. Female Total Length (TL) of crayfish (from tip of rostrum to tip of telson) were measured to the nearest millimeter and Wet Weight (WWT) was measured to the nearest 0.01 g prior to placing in 1L plastic cups held in raceways receiving dechlorinated aerated water at rate about of 59 Lh^{-1} . Crayfish ranged from 81 to 127 mm in TL and from 16.7 to 63.4 in weight.

The simulated burrows were suspended in restaurant glass racks held in three raceways.

Each cup (simulated burrow) had seven 5-mm holes to allow water exchanged. Crayfish were held in simulated burrows at ambient laboratory temperature (22° C) for 3 months without food. Crayfish were checked daily basis until the experiment end. Ovigerous females were removed from the simulated burrows and then pleopod eggs were removed with a forceps and fixed in 10% neutral formalin within the 24 h of ovipositing. After then female crayfish were frozen and stored for analysis.

Random burrows distributed around the pond levees after ponds drained were identified (n=48) and marked with a flag between 5 May and 12 May, 2002. A sample of marked burrows was excavated by using a shovel to determine relative physiological condition, reproduction of eastern white river crayfish between 28 and 30 August, 2002. Burrow residents were sexed and counted. Ovigerous females were removed from the pond burrows and pleopod eggs were removed with a forceps and fixed 10% neutral formalin. Females from burrows were frozen and store for analysis.

Relative physiological conditions of female were by determining moisture content of hepatopancreas^[3]. Frozen female crayfish from (simulated burrow, n=30 and pond burrow, n=30) with complete sets of appendages for condition analysis were thawed, measured for TL and WWT, then hepatopancreas excised and weighted to the nearest 0.01 g after blotting (HM) and dried in a convection oven (80°C) to a constant weight. Percentage of moisture content of the hepatopancreas (HM) was determined from the differences between wet and dry weight of tissue divided by wet weight and multiplied by 100.

Subsamples of 15 eggs of both habitats from each ovigerous females were taken and placed on moistened filter paper in a petri dish and the individual diameters were measured with a dissecting microscope equipped with a calibrated ocular micrometer.

Statistical analysis: One-way ANOVA was used to compare differences between female crayfish egg numbers for simulate and pond burrow. Linear regression was used to determine the relationship between egg sizes, hepatopancreas wet weight, crayfish TL and the number of extruded eggs per female crayfish. In addition, the Analysis of Co Variance (ANCOVA) procedure was used to compare the slopes and intercepts of the regressions equations and to compare eggs per female. ANOVA, ANCOVA and Linear Regression of data were conducted

by using Micro-SAS Statistical Software System Version 8^[11]. Differences in means were detected with Least Significant Differences (LSD) test^[12]. Level of significance was set at $p < 0.05$.

RESULTS

Total Length (TL mm) of female crayfish varied from 65 to 125 mm in excavated pond burrows and 81 to 127 mm in simulated burrow, respectively (Table 1). The average TL of females from simulated burrows was significantly larger than the average TL for females from the excavated pond burrows (Table 1). Pleopod egg numbers varied from 106 to 556 eggs for 81 mm and 127 mm TL females in simulated burrow, respectively. Whereas, the females in 65 and 125 mm TL in excavated pond burrows produce 32 to 330 eggs, respectively.

Table 1: Mean \pm SD and range (in parentheses) size of female eastern white river crayfish in simulated and excavated pond burrows

Parameter	Simulate Burrow	Pond Burrow
Total length (TL mm)	108.6 \pm 8.7 ^a (81-127)	99.4 \pm 24.1 ^b (65-125)
Wet Weight (WWT g)	31.6 \pm 12.9 ^a (16.7-63.4)	29.3 \pm 16.2 ^a (6.4-46.9)
Hepatopancreas Moisture (HM %)	47.1 \pm 10.9 ^a (29.3-69.4)	62.2 \pm 11.9 ^b (56.8-81.6)
Egg Diameter (mm)	2.2 \pm 0.1 ^a (1.8-2.6)	2.0 \pm 0.01 ^a (1.6-2.3)
Egg No/Female	352.7 \pm 101.4 ^a (106-556)	182.2 \pm 100.0 ^b (32-330)

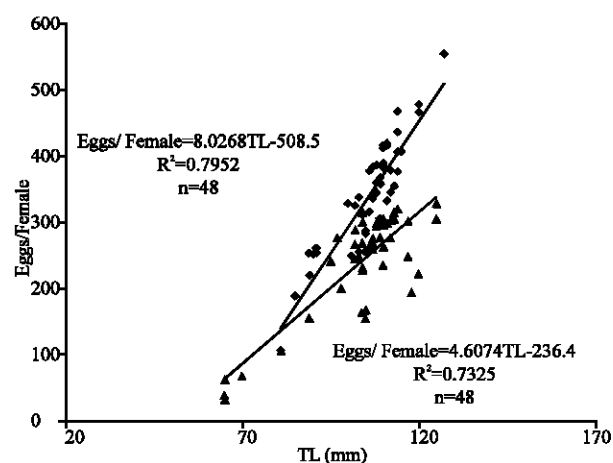


Fig. 1: Relationship between Total Length (TL) and number of pleopod eggs for female in simulated burrow (square) and excavated pond burrows(triangles)

Mean total number of eggs per female in simulated burrow (352.7) was significantly higher than in pond burrows (182.2) (Table 1). Pleopod egg numbers linearly increased with Total Length (TL) of the female crayfish (Fig. 1). The slopes and intercept of the regression lines for pleopod eggs and TL for female were significantly different between those individuals held in simulated burrows and excavated pond burrows (Fig. 1).

The diameters of the extruded eggs were similar in simulated burrows and excavated pond burrows (Table 1). *P. a. acutus* had significantly less hepatopancreas moisture in simulated burrow (47.1%) compared to crayfish held in excavated pond burrows (62.2%) (Table 1).

DISCUSSION

Crayfish must survive in burrows for up to 3-4 months or more until ponds are flooded in fall. Factors that potentially affect the survival and reproduction of females in burrowing include the physiological condition of the crayfish before burrowing, maturity and ovarian development of females prior to draining and the availability of water in burrows^[4]. Humidity in burrows is near 100% when water is present^[13] and because of dissolved oxygen levels in burrow water is near 0 mg/L⁻¹^[13]. *Procambarus* use atmospheric oxygen for metabolism provided gill tissues remain moist^[14]. Postponing the flooding of ponds until water temperatures have cooled may reduce yields due to starvation and cannibalism in burrows and slowed crayfish growth^[5,15].

The production of crayfish eggs depends on abiotic factors such as supply food, water quality, habitats and fertile eggs^[16-20]. For example, *O. virilis* has adapted to the climates where nutrition abundance is high. These environments stimulate conditions of *O. virilis* to select a strategy in which large number of eggs is produced^[21]. In the present study, *P. a. acutus* extruded and attached 95% of the ovarian eggs in simulated burrow. However, female crayfish did not show the same pattern in excavated pond burrows. The change for egg loss, failure to achieve reproductive potential is greater in the natural environment. For example, a 117 mm TL *P. a. acutus* excavated burrows and laid 250 eggs, however a similar sized female crayfish in simulated burrow had 384 eggs. In addition, our observations indicated that some females in excavated pond burrows had the eggs in their stomach, while the other females do not extrude all of their eggs at the spawning and some eggs fail to attach to the pleopods. Thus, many crayfish lose a considerable number of pleopod eggs. This could affect the number of

new recruit to join into the population negatively.

The physiological condition of females prior to burrowing is important to reproductive success. Crayfish store large amount of energy in their hepatopancreas for the egg development and survival to live in the burrow^[6]. For this reason, moisture content of the hepatopancreas has been used a good indicator of crayfish condition^[3,10,14,19-22]. Generally, as organic reserves are depleted from the hepatopancreas tissues, moisture level in the tissues increases. This situation was also observed in our study clearly. For example, a moisture level in the hepatopancreas in simulated burrow was 47.1% compared to the moisture content of hepatopancreas of 62.2% in excavated pond burrows. In a healthy crayfish, the hepatopancreas can account 10-15% of the wet body mass^[1].

If ponds are severely depleted of food resources prior to the end of the production season, it has been hypothesized that potential broodstock may enter burrows lacking sufficient organic reserves in the hepatopancreas to maintain adequate physiological condition during the summer^[23] and this could be manifested by a decrease in fecundity.

Egg size within a population and possibly within broods from a single individual may be an advantage for the offspring to adapt to the environmental variations. Such variation would include environments where food availability is pulsed, energy input is low and conditions are not suitable such as the case in cool water habitats and pond conditions. It is not always that larger crayfish tended to produce larger eggs but individual differences might be considerably high and reflect the nutritional status of female during both simulated and excavated burrow period. Such a phenomenon is common in other animal groups^[24], including the crayfish *Pacifastacus leniusculus*.

CONCLUSIONS

Eastern white river crayfish life cycle traits limit the harvest to one recruitment and culture protocol should permit better management and improved eastern white river crayfish production within their native region.

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