

## Reproductive aspects of the spider crab *Libinia dubia* under laboratory conditions

### Aspectos reproductivos del cangrejo araña *Libinia dubia* bajo condiciones de laboratorio

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#### ABSTRACT

The present study documents some aspects of the reproductive biology of the spider crab *Libinia dubia* (H. Milne Edwards, 1834) in conditions of captivity. Forty males and 43 ovigerous females were collected with traps and transported to the laboratory, where they were kept in seawater tanks. Average egg count per female was 20,637 ( $\pm$ SD 12,683.33), with an 81% ( $\pm$ SD 4.62) viability until hatching. Males were larger and heavier than females. On average, females measured 69 mm in length  $\times$  59 mm in width. The equation for the linear relationship between female weight (W) and number of eggs produced per female was:  $Y = -10,290.03 + 242.89 X$  ( $r^2 = 0.866$ ). Egg development at a temperature of  $24 \pm 1$  °C occurred in five stages based on coloration: stage 1 (day 1-10), bright orange; stage 2 (day 11-24), orange; stage 3 (day 25-29), gray; stage 4 (day 30-34), light gray; and stage 5 (35-37), white, visible larvae. Females spawned at least twice and were receptive to males after each spawning event, although no spermatophore transfer was observed. The adaptive capacity of *L. dubia* to captivity makes its use feasible in breeding under controlled conditions, suggesting that it has potential for aquaculture.

**Key words:** *Libinia dubia*, spider crab, aquaculture, octopus bait.

#### RESUMEN

El presente estudio documenta algunos aspectos acerca de la biología reproductiva del cangrejo araña *Libinia dubia* (H. Milne Edwards, 1834) en condiciones de cautiverio. Cuarenta machos y 43 hembras ovígeras fueron recolectados con trampas y transportados al laboratorio, donde se les mantuvo en estanques de agua marina. El promedio de huevos por hembra fue de 20,637 ( $\pm$ SD 12,683.33), con un 81% ( $\pm$ SD 4.62%) de viabilidad hasta el momento de la eclosión. Los machos fueron más grandes y pesados que las hembras. Estas registraron una talla promedio de 69 mm de longitud  $\times$  59 mm de ancho. La ecuación para la regresión lineal entre el peso de las hembras y el número de huevos producido por hembra fue:  $Y = -10,290.03 + 242.89 X$  ( $r^2 = 0.866$ ). El desarrollo de los huevos a una temperatura promedio de  $24 \pm 1$  °C presentó cinco estadios basados en el cambio de la coloración del huevo: estadio 1 (días 1 a 10), naranja brillante; estadio 2 (días 11 a 24), naranja; estadio 3 (días 25 al 29), gris; estadio 4 (días 30 al 34) gris tenue y estadio 5 (días 35 al 37), blanco, larva visible. Cada hembra desovó dos veces y posterior a cada evento de desove se mostraron receptivas a los machos, aunque no se observó ninguna transferencia del espermatóforo. La capacidad de adaptación de *L. dubia* a condiciones de cautiverio hace factible su reproducción bajo condiciones controladas y sugiere que tiene potencial para la acuicultura.

**Palabras clave:** *Libinia dubia*, cangrejo araña, acuicultura, carnada para pulpo.

## INTRODUCTION

The spider crab *Libinia dubia* (H. Milne Edwards, 1834) is an epialtid member of majoids distributed from Massachusetts, United States, to Cuba (Williams *et al.*, 1977) and the Yucatan Peninsula, Mexico. In the Yucatan region it is locally known in Maya as “maxkil”, which means “dirty crab”, and is in high demand as bait for the small-scale octopus fishery (Espinoza-Méndez *et al.*, 2003). As bait, it is very attractive to octopus and highly resistant, allowing fishers to use it up to four times longer than common blue crab *Callinectes sapidus* (Rathbun, 1896) or other local crab species. *Libinia dubia* has been used traditionally as octopus bait due to its previous relative abundance and its presence in shallow marine waters and coastal wetlands, which facilitated capture and kept capture costs low. However, in recent years, the collapse of African octopus fisheries led to an opening of international markets since 2000. As a consequence, local populations of *L. dubia* have declined due to overexploitation (CONABIO, 2010). That is why the demand for spider crab (*L. dubia*) as bait increased, so its price is equal to and sometimes above the market price of the octopus. This phenomenon directly affects economic viability of the octopus fishery and may threaten local *L. dubia* populations if current levels of exploitation continue.

The reproductive biology of *L. dubia* under laboratory conditions has never been studied. The present work is the first step for the culture development of *L. dubia* as a potential alternative to reduce fishing pressure on natural populations, to produce enough quantities to lower its cost as bait, and to increase the economic viability of the octopus fishery in Yucatan. As part of this effort, the aim of the present study was to document the reproductive biology of this species under experimental conditions, including adaptation to a culture system, controlled reproduction, and viability of production.

## MATERIALS AND METHODS

Using folding Dutch traps, 83 adult *L. dubia* (43 ovigerous females and 40 males) were captured in July 2007 from waters near Isla Arenas, Campeche, Mexico. This period was chosen because the reproductive period of the closely related species *Libinia emarginata* Leach 1815 is from June to December (Hinsch, 1968). Species identification was confirmed based on taxonomic keys (Williams *et al.*, 1977). Organisms were transported to the Telchac Puerto Marine Laboratory Unit of the Centro de Investigación y de Estudios Avanzados (CINVESTAV, Merida). The experimental culture system consisted of a recirculation water system using a Boyu (EFU-1500) filter with capacity of 8000-15,000 L and 24-Watt ultraviolet lamps. Nine oval fiberglass tanks (210 cm long × 140 cm wide × 60 cm deep) were used. The tanks had a sand and organic matter bottom and were under conditions of constant aeration and total darkness (Carmona-Osalde *et al.*, 2002). The water was kept at a temperature of 24 °C ± 1 °C, salinity 34 ± 1.19 ppt, and

dissolved oxygen 5 ± 0.14 mg L<sup>-1</sup> on average. At least four crab couples (with a sex ratio of 1:1) were introduced into each tank. All crabs were fed with a commercial balanced shrimp feed with 25% protein and 10% lipids (Camaronina®) provided daily in two rations. The organisms were measured for total carapace length and width using a vernier caliper and their weight was measured on a scale (Ohaus, 0.01 g accuracy) after blotting excess moisture. The eggs were removed manually from females and counted under a stereoscopic microscope (4X). These data were used to calculate a simple linear regression between fecundity (defined as the number of eggs in Stage I, see below) and female body weight, and ANOVA was used to compare weight between sexes (Zar, 1984; Rodríguez-Serna *et al.*, 2000). Females spawned for a second time 25 to 30 days after their first spawning. These females were isolated in individual fiberglass aquaria (70 × 40 × 35 cm) and were daily monitored to observe and describe egg development. After their eggs hatched, the females were returned to the larger tanks where they quickly attracted males. Color change and incubation time was recorded (Espinoza-Méndez *et al.*, 2003; Yeh & Rouse, 1994). Egg viability was calculated according to the larval hatching rate (Total egg number/Total larval number × 100).

## RESULTS

There were significant differences in weight, length and width between males and females of *L. dubia*, with males being heavier and larger than females (Table 1). On average, the dimensions of females were 84.3 g in weight, 69.0 mm in length, and 59.0 mm in width, whereas the dimensions of males were 105.22 g in weight, 73.3 mm in length and 63.3 mm in width.

Female total length and width showed them to be full-size adults, because sexual maturity in *L. dubia* females occurs after a terminal change (last molt) (Hinsch, 1972). Survival of adult crabs subjected to experimental conditions was high (90%), with an overall lower mortality among females (5%) than among males (22%). Average egg count of females was 20,637 ± 12,683, with a minimum of 4,900 and a maximum of 75,000. On average, 80.1% (±SD 4.62; ±SE 0.92; Min 68%; Max 91%; N = 43) of the eggs remained viable until hatching occurred. The biggest females measured 70 mm long × 60 mm wide and exhibited 80% egg viability (Fig. 1). A linear regression explained 87% of the variability in the number of eggs produced by females of increasing weight (Fig. 2).

Table 1. Mean ± Standard Deviation morphometric measurements for females (N = 43) and males (N = 40) of *Libinia dubia*.

	Weight (g)	Length (mm)	Width (mm)
Females	84.3 <sup>a</sup> ± 7.91	69 <sup>a</sup> ± 0.18	59 <sup>a</sup> ± 0.18
Males	105.2 <sup>b</sup> ± 14.86	73 <sup>b</sup> ± 0.74	63 <sup>b</sup> ± 0.74

In each column, different superscript letters indicate significant differences ( $p < 0.05$ ).

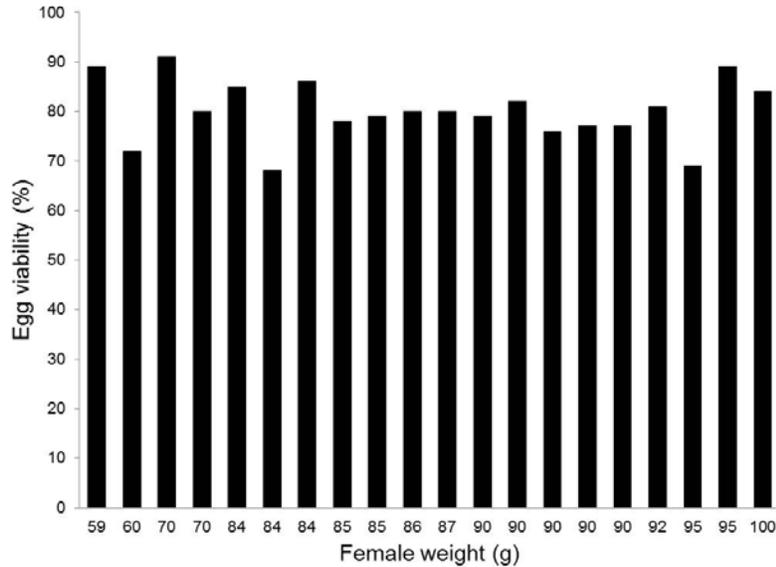


Figure 1. Egg viability (%) according to weight of females of *Libinia dubia*.

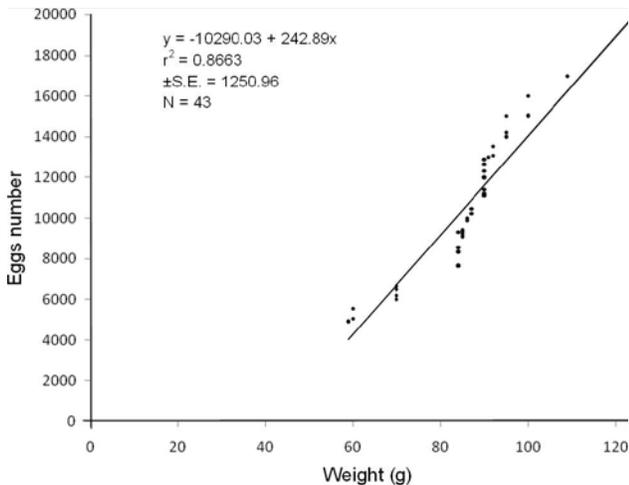


Figure 2. Egg number *versus* body weight in females of *Libinia dubia*.

The equation for this relationship is:  $Y = -10290.03 + 242.89 X$  ( $r^2 = 0.866$ ;  $\pm SE = 1250$ ;  $N = 43$ ). Based on color change, egg development occurred in five stages (Table 2): Stage 1, bright orange immediately after spawning; Stage 2 (11 to 24 days after spawning), light orange; Stage 3 (25 to 29 days), gray; Stage 4 (30 to 34 days), light gray; Stage 5 (35 to 37 days), white, almost transparent, with visible larvae. Larval release was asynchronous and occurred in two batches over a 48 h period: phase one began at dusk of day 1 and stopped at dawn of day 2, and phase two began at dusk of day 2 and lasted until larval release was completed. Total survival of hatched larvae did not exceed 5%.

Table 2. Changes in egg color during embryonic development of *Libinia dubia* at an average temperature of 22 °C.

Egg stage	Duration (days after spawning)	Egg color
1	1-10	Bright orange
2	11-24	Light orange
3	25-29	Gray
4	30-34	Light gray
5	35-37	White (larvae visible)

## DISCUSSION

In a previous study, Espinoza-Méndez *et al.* (2003) found that 87% of ovigerous females of *L. dubia* occurred in the 45 to 65 mm carapace width interval. In our study, all crabs were larger than 45 mm total length and all females were ovigerous, indicating that all crabs were adults.

It appears that males were more sensitive to confinement because of their aggressive behavior. Antagonistic behavior has not been previously reported for *L. dubia*, but the males clearly exhibited aggressive actions toward each other, even in the absence of females. In the controlled system used here, this behavior resulted in substantial damage to smaller male individuals and even in death of some individuals. Ovigerous females were isolated in individual aquaria and hence were undisturbed until the larvae hatched. Afterwards, they were returned to the larger tanks, where the non-ovigerous females quickly attracted males. The females were caught and held by the males with the posterior

limbs in what is known as an "obstetric posture" (Hinsch, 1968). During this behavior, males isolated the female from other males in what was probably only a protective response since no spermatophore transfer was observed. The obstetric posture continued for a number of hours, even while crabs fed and moved. DeGoursey and Auster (1992) observed this behavior in natural *L. emarginata* populations and called it "protective behavior". Ovigerous females remained buried in the sand bottom most of the time, reacting only to the presence of food, which they identified and consumed quickly. The females frequently cleaned the eggs by aerating them several times daily. On average, females spawned again after 35 days, and released larvae by first raising the abdomen as high as possible with the first pair of pereopods, and then by opening and closing the abdomen intermittently. Larvae were released at irregular intervals with delicate movements of the pleopods and once released were left to drift in the water column. Release always occurred during the afternoon hours. Only 5% of the larvae that hatched in the aquaria survived. However, this result is considered as positive given that this was the first attempt for controlled production of this species.

The ability shown by *Libinia dubia* in the present study to adapt to confinement and to reproduce in the laboratory, suggests that controlled production of this species is feasible and potentially viable.

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