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EVALUATION OF MANGROVE CRAB (*Ucides occidentalis*) LARVICULTURE IN THE LABORATORY, REPOPULATION OF THE MANGROVES OF TUMBES NATIONAL SANCTUARY

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Abstract: *Ucides occidentalis* (Ocypodidae) It is one of the species of great ecological-commercial impact of the mangrove area in the Tumbes region - Peru; its population shows a significant decrease due to extractive activity and the incidence of effluents from shrimp companies located near the mangrove area; Research projects have been initiated for the development of methodologies aimed at the production of crab larvae for repopulation purposes. However, there is very little knowledge about the embryonic and larval development of this species at a local and Latin American level; The present investigation seeks to study and produce larvae free of specific pathogens in laboratory conditions. In our research we worked with 41 ovigerous females, under laboratory conditions, a hatching rate of 93.76% was obtained; the embryonic period covered eight stages with characteristics similar to U. *cordatus* (Ocypodidae), the duration of each stage was on average 2.5 ± 0.5 days and its total incubation period of 20 days. The larval period was 6 larval instars, one megalopa instar and one considered prejuvenile instar; with a survival up to megalopa of 3.75%; larval development was recognized mainly by the number of setae present in the first maxiliped that have a similarity to U. *cordatus* (Ocypodidae) (Amaro and Fiscarelli, 2001; Rodrigues and Hebling, 1989); the transfer time to a new stadium was 3.0 ± 0.5 days.
Keywords: Mangrove crab, embryonic development, larval development, hatching, aquaculture.

INTRODUCTION

Mangrove crabs are both ecologically and economically important; one of the most representative genera in the tropical region of America, both on the Pacific and Atlantic coasts, is the genus Ucides (Solano-Flores et al., 2010).

For Peru, mainly the Tumbes region, *Ucides occidentalis (Ocypodidae)* is one of the species with the greatest ecological impact in mangrove areas; for recycling close to 80% of litter, aerating the soil and stimulating the activity of aerobic bacteria that decompose organic matter (Ordinola-Vieyra et al., 2020; Solano-Flores et al., 2010); Likewise, it is one of the most important marine resources in the Region, due to the magnitude of its commercialization in the local and regional market, extended to other coastal regions of Peru (Ordinola-Montero et al., 2010).

U. occidentalis (Ocypodidae) is also the most exploited mangrove crab in the Tumbes region; which has led to a drastic decline in its population, falling from 120 million in 1996 to 77.06 million in 2007, that is, a decrease of 35.8% of the population in 11 years(Alemán y Ordinola, 2017; Ordinola-Elmer et al., 2010)

For this reason, the IMARPE - Tumbes headquarters determined the need for a new survey of this mangrove crab and update the population assessment, in order to reinforce or implement new measures to conserve this important resource(Ordinola-Elmer et al., 2010).

However, the studies carried out on U. *occidentales* (Ocypodidae) have mainly covered their morphology, biology (Schuiteman-Pozo et al., 2019; Zambrano and Meiners, 2018); genetic diversity and population structure (Ordinola-Zapata et al., 2018; Ordinola-Vieyra et al., 2020); general eating habits in juveniles and adults (Córdova, 2018); but regarding its larval development, no research record has been found for the species U. *occidentales* (Ocypodidae).

But, there are investigations of larval development, larviculture cycle under laboratory conditions as part of a repopulation strategy in U. *cordatus* (Ocypodidae) (Galley-Green et al., 2011; Da Silva-Cottens et al., 2012; Silva -Menezes et al., 2009; Simith-Dieleet al., 2013; De Souza-Simith et al., 2018); its embryological cycle (Rodrigues and Hebling, 1989; Simith-Diele et al., 2013), and even diseases in eggs and larvae (Lavilla-pitogo and De la Peña, 2004).

The objective of this research is to study and produce larvae free of specific pathogens from populations of *U. occidentalis* (Ocypodidae) (Red mangrove crab) under laboratory conditions, based on the closest species studied U. cordatus (Ocypodidae); in order to contribute to the increase of the population and aquaculture in the Mangroves of Tumbes National Sanctuary in the long term.

MATERIALS AND METHODS

OBTAINING FEMALE CRABS

Ovigerous females captured by crab collectors from the mangrove consortium of northwestern Peru were received at the facilities of the Experimental Educational Collective Center for Aquatic and Aquaculture Biology and Biotechnology of Puerto Pizarro (CEBAP) belonging to the research company INCABIOTEC SAC

The females were taken from three main points in the mangrove area of the Tumbes region: Puerto 25, El Bendito and Puerto Pizarro. These captures were made within the crab ban period, December 2019 to March 2020 and January to March 2021; The necessary permission for the investigation was obtained as part of a repopulation measure, with the responsibility of the INCABIOTEC SAC team in returning the females after the hatching period of the eggs.

DISINFECTION PROCESS

We proceeded to disinfect each of the females obtained, by means of a total immersion bath in treated seawater plus iodine (0.2%) for 15min; After time, they

were rinsed with treated seawater. Next, three measurements were made with a high-precision digital Varnier; cephalothorax length, cephalothorax width and thickness of the individual, and the total weight on a 0.01g SF-400D precision balance

Finally, each female is placed in a 20-liter tub, which contained a 4" PVC pipe 20cm long (for shelter) and three to four mangrove leaves; At 5pm, 3 liters of treated seawater plus aeration were added (simulating a tidal rise effect); until the next morning, the water is removed; this process was repeated until the moment of spawning, then they were released.

EVALUATION OF EMBRYO DEVELOPMENT

Once the females were installed, 10% of the ovigerous females, randomly selected, were extracted a sample of the egg mass with weights that varied from 0.0209 g to 0.0755 g for direct observation of the embryonic stage (Pinheiro-Baveloni et al., 2003) on the OLYMPUS CX31 microscope.

HATCHING

A sample of the egg mass (randomly selected females) was extracted to be weighed on an analytical balance of 0.0001 g precision, weight of 25 subsamples of (0.1g) were made and then counted in a stereoscope, obtaining an average number of eggs. On the other hand, weights of the females were taken before and after hatching; this number serves as a reference to be used in the difference in weight of the mass of eggs and to be able to determine approximately the number of eggs per female.

The hatchings were related to the lunar period, beginning two days before and ending three days after the appearance of the full and/or new moon; hatching began in the early hours of the night and ended in some cases until the beginning of the following morning;

The nauplii were filtered through a 100 micron mesh, placed in a 5L bucket, then the number of nauplii/ml/female was evaluated following the volumetric method (number of individuals per ml, taking 5 samples and obtaining an average). determining the number of new ones per female in a final volume of 5 liters of water.

Finally, the distribution was carried out in pools prepared with treated seawater and inoculated with microalgae two to three days before hatching (140,000 - 400,000 cells/ml).

EVALUATION OF CROP PARAMETERS

Daily parameters were taken twice a day: temperature (T°), dissolved oxygen (DO) and pH (Hanna HI98128 Phmeter and Starter 300D Oximeter); Likewise, parameters were taken once a week to determine the water quality: ammonium, nitrite, nitrate and alkalinity (API kits). Additionally, water exchange was programmed for a day from 50% to 75%, daily feeding with a dosage of 6 times a day.

LARVAL DEVELOPMENT EVALUATION

Daily samples were taken from each pool (2tm) and placed on slides to determine the progress of the larval stage (Rodrigues and Hebling, 1989) by direct observation in an OLYMPUS CX31 microscope.

RESULTS

Of 41 females with follow-up; 24 were in stage VI close to hatching, 7 in stage VII and 10 in stage VIII; from the evaluation of the average number of eggs per weight, an average of 9794 eggs/gram was obtained.

The total number of eggs was 12,853,843, which led to 12,051,799 nauplii of *Ucides occidentalis*, represents a hatching rate of 93.76%; the lowest number of eggs determined per female was 126 636 (female

N°38) and the highest was 600 078 (female N°5). Likewise, the lowest number of nauplii obtained was 118,000 (female N°38) and the highest was 565,000 (female N°5); However, this is not related to the hatching percentage, the lowest hatching percentage was 90.16% and the highest 95.65% for females number 40 and 3 respectively (Table 1), and the general hatching trend is close to 95.08%.

Of the number of eggs, 26.83% of the females were between 100 thousand and 199 thousand eggs, 21.95% between 200 thousand and 299 thousand eggs, 29.27% between 300 thousand and 399 thousand eggs, 9.76% between 400 thousand and 499 thousand, 9.76% between 500 thousand and 599 thousand and 2.44% between 600 thousand and 699 thousand eggs.

Finally, of the number of hatched larvae per female, 31.71% were between 100,000 and 199,000 nauplii, 19.51% between 200,000 and 299,000 nauplii, 26.83% between 300,000 and 399,000 nauplii, the 14.63% between 400 thousand and 499 thousand nauplii; and 7.32% between 500 thousand and 599 thousand nauplii.

On the other hand, the parameters followed showed an average temperature of 26.7 °C in the morning and 27.4 °C in the afternoon. Dissolved oxygen 6.30 mg/l in the morning and 6.2 mg/l in the afternoon. The Ph was 7.34 in the morning and 7.21 in the afternoon; salinity of 28 o/oo – 30 o/oo (due to spare parts).

The embryonic period observed in the species U. *occidentalis* covered eight instars with an average of 2.5 ± 0.5 days between each instar and its total incubation period of 20 days. The larval period was 6 instars, one megalopa instar and one instar considered prejuvenile (Fig. 3). The passage to a new larval stage was determined to be 3.0 ± 0.5 days, considering the appearance of setae in the abdominal region (Amaro and Fiscarelli, 2001; Rodrigues and Hebling, 1989).

The food supplied was arranged every 2.5 days, according to microscope observation of the stage in which the larva was. For Zoea I and II, their feeding was with microalgae and a liquid diet of zoeas for shrimp larvae. For the Zoea III stage, the feeding was combined between microalgae and a dry diet of 100 microns of food for shrimp larvae; Zoea IV feeding was combined 25 % microalgae and 75 % artemia nauplii; Zoea V and VI fed on artemia nauplii and rotifers (Teixeira, 2007; c; Silva-Menezes et al., 2009; De Souza-Rodrigues et al., 2017).

DISCUSSION

The embryonic period observed in the species U. *occidentalis* coincided with that reported by Pinheiro and Hattori (2003) in their investigation of the embryonic development of U.*cordatus* (Fig. 1); however, the duration of each stage was slightly different, while for the report of U. cordatus it is an average of 2.7 ± 0.9 days; for the evaluation carried out in U. *occidentalis* it was an average of 2.5 ± 0.5 days and its total incubation period of 20 days.

The closest reference to larviculture of the larval period is that of U. *cordatu*s (Ocypodidae), which has reported 6 larval stages, one megalopa stage and one juvenile (Rodrigues and Hebling, 1989; Da Silva-Cottens et al., 2012; Silva-Menezes et al., 2009); which contrasts slightly with that observed for U. *occidentales* (Ocypodidae) in a stage considered prejuvenile (Fig. 3); although this is still not conclusive, opening to more research on the species.

During this period the number of nauplii decreased as they reached a new stage; survival in each stage was low compared to that reported for its closest relative U. cordatus (Ocypodidae) estimated by Da Silva-Cottens et al. (2012) under laboratory conditions,

but high in those reported in the natural environment (Teixeira, 2007); (Table 2).

On the other hand, there are studies on aquaculture feeding that affirm that the main food for Zoea I and II stage is cultivated microalgae (Teixeira, 2007; Araújo-Alves et al., 2002; Silva-Menezes et al., 2009; De Souza -Rodrigues et al., 2017) or sporadic growing microalgae in a mesocosm system (c); agreeing that the environment where the recently hatched larva would be placed would be in a pond with growing microalgal culture; in the case of *western* U. it showed to be viable with the addition of liquid diets every 3 hours.

Although there are no reports that show a consensus on a specific food order for the different stages of larval culture for *U. cordatus* (Ocypodidae); they agree on the use of rotifers, artemia larvae and microalgae (Guilherme, 2008; Da Silva-Cottens et al., 2012; Da Silva-Cottens et al., 2009); Finally, some authors affirm that if only commercial food is given for larvae, it will not be possible to ensure optimal survival of the larva (De Souza-Rodrigues et al., 2017). What was observed for larviculture in *U. occidentalis* does not specify that the use of live and/or commercial food has an impact on their survival; It is believed that further study is needed to draw conclusions.

CONCLUSIONS

The species *Ucides occidentalis* (Ocypodidae) Within the research, it showed a high adaptability to be worked in laboratory conditions for embryonic development; obtaining a hatching rate of 93.76%; however, for larval development there is still a need to optimize protocols that allow a greater survival of 3.75 % in the megalopa stage, a stage prior to the juvenile. There is a high similarity in the embryological and larval characteristics with the species *U. cordatus* (Ocypodidae), varying in the duration for each phase of the stages: embryonic of 2.5 ± 0.5 days on average and larval of 3.0 ± 0.5 days on average. Thus, this research is shown as the first report of larval culture in laboratory conditions for *U. occidentales* (Ocypodidae).

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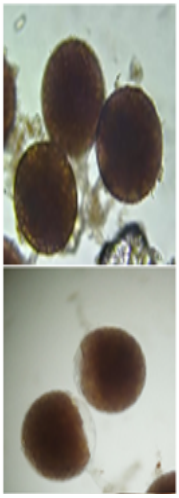
TABLES AND FIGURES

# Female	Weight (g)	Width (cm)	Height (cm)	Length (cm)	Egg mass weight (g)	Number of eggs	Number of larvae	% Hatching
40	122.60	6.4	4.9	5.3	15.25	149359	134667	90.16
3	121.40	6.3	4.5	5.2	28.18	275995	264000	95.65
38	110.05	5.0	6.0	5.2	12.93	126636	118000	93.18
5	120.45	6.0	4.9	5.6	61.27	600078	565000	94.15

Table 1. Details of the ovigerous females of Ucides occidentalis with the lowest and highest number of eggs, larvae and hatching percentage.

Stadium	Survival percentage		
	U. cordatus		*U. Occidentalis*
	Laboratory	natural environment	Laboratory
Zoea I	100	46	93.76
Zoea II	84	40	87.70
Zoea III	80	5.6	75.00
Zoea IV	78	4.2	63.00
Zoea V	70	2.8	49.85
Zoea VI	38	0.9	30.31
Megalopa	26	-	3.75

Table 2. Survival comparison between *Ucides cordatus* (Ocypodidae) under laboratory conditions (Da Silva-Cottens et al., 2012), in a natural environment (Teixeira, 2007) and *Ucides occidentalis* (Ocypodidae).

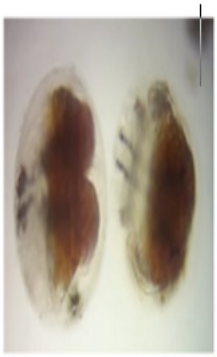


Stage II: From slit to blastula: two days after egg laying, egg fully divided with droplets of yolk of similar size.
Eggs shiny dark color, and the embryo not yet defined in lateral view.

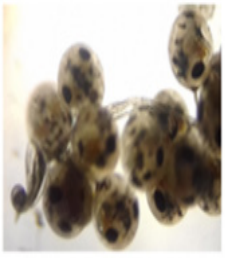
Stage III: Naupliar stage: Five days after egg deposition, yolk fragmentation is more evident and yolk droplets are larger.

Stage IV: Metanupliar stage: eight days after extrusion, the embryo occupied 1/4 to 1/3 of the dark ocher egg. Embryonic ocular region well defined but not yet pigmented.

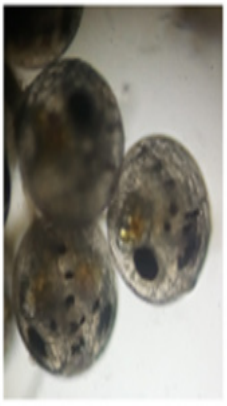
Stage V: Pigmented stage: Ten days after eggs were laid, the yolk now curls up in dorsal view and occupies half the volume of the egg. Dark ocher egg coloration. Eye lobes now more evident, with black pigmentation in the Central area.



Stage VI: Double chromatophoric bridge stage: in the lateral view, 14 days after egg laying, two bilobed yolks occupied 1/3 of the egg volume and V-shaped in the dorsal view. In the ventral view, a pair of chromatophores in each abdominal somite, linked by a double chromatophoric bridge



Stage VII: Pre-hatch stage: sixteen days after egg laying. In lateral view, the embryo occupied 3/4 of the volume of the egg and two subjects small connected plots



Stage VIII: Incubation Stage: Nineteen days after extrusion, the larva is fully formed and fills the egg space. Egg color light ocher. In lateral position, two small drops of yolk (1/8 of the volume of the egg) in the dorsal region of the carapace. These yolk droplets are similar in size and shape to embryonic eyes.

Figure 1. Description of embryonic development of western Ucids (Ocypodidae) with references in description of U. cordatus (Ocypodidae) (Rodríguez and Hebling, 1989).

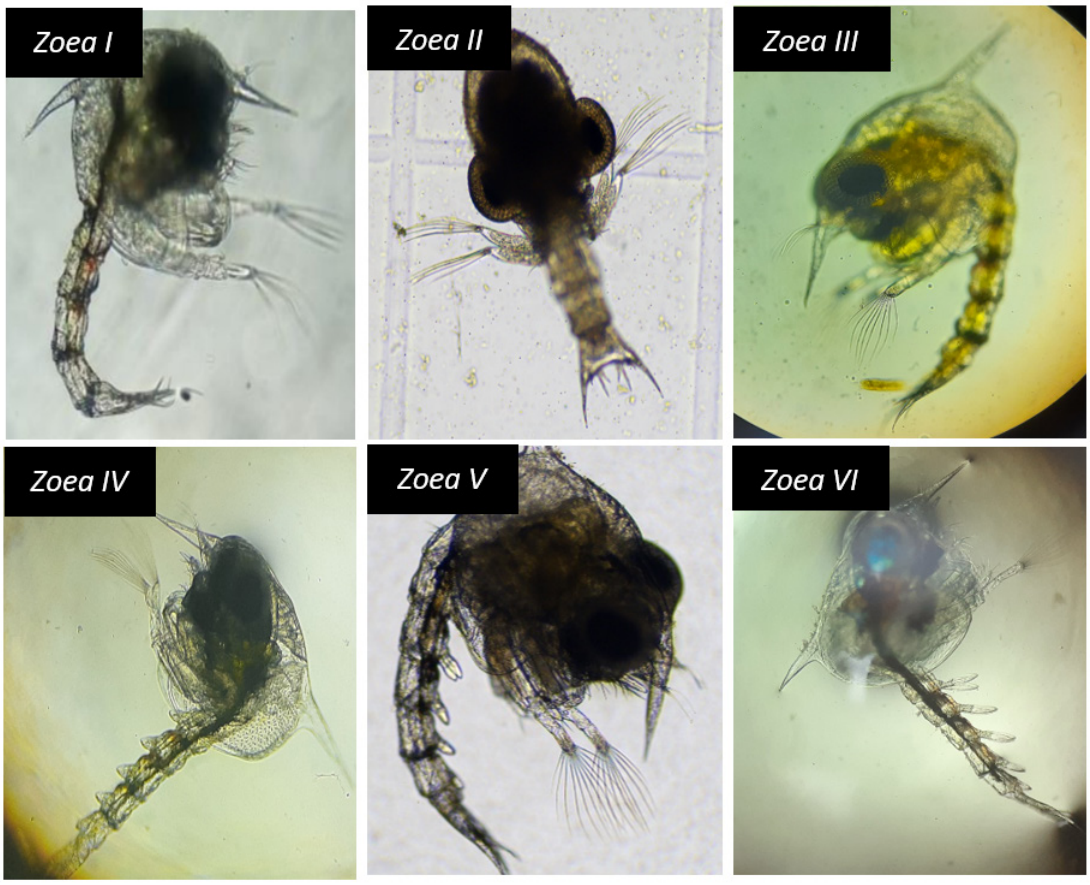


Figure 2. Description of larval development of Western Ucids (Ocypodidae) ; Zoea I: 4 filaments; Zoea II: 6 filaments, Zoea III: 8 filaments; Zoea IV: 10 filaments and setal buds in the abdominal region; Zoea V: 11 filaments and growth of setae in the abdominal region; Zoea VI: 13 filaments and complete development of abdominal setae.

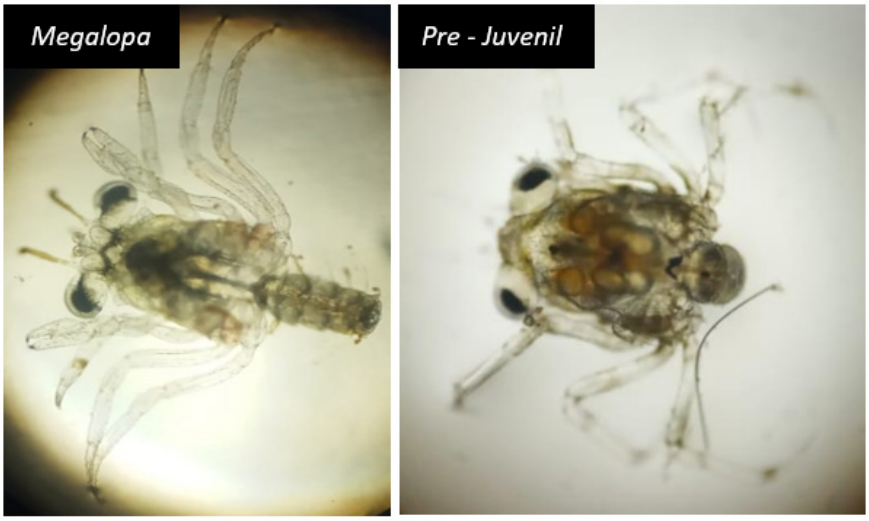


Figure 3. Megalopa and pre-juvenile of Western Ucids (Ocypodidae) cultivated in the facilities of the Centro Colectivo Educativo Experimental de Biología y Biotecnología Acuática y Acuícola de Puerto Pizarro (CEBAP)