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EMBRYONIC AND LARVAL DEVELOPMENT OF THE RED MANGROVE CRAB *UCIDES OCCIDENTALIS* (ORTMANN, 1987): MICROALGAL FEEDING AND ITS EFFECT ON METAMORPHOSIS AND SURVIVAL

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ABSTRACT The global demand for crabs, coupled with increasing threats to natural populations, creates the need to investigate captive breeding programs. To achieve this, it is crucial to acquire a comprehensive understanding of key aspects of their life cycle. This study represents the early comprehensive characterization of the red mangrove crab, *Ucides occidentalis*. Furthermore, leveraging epifluorescent microscopy, we examined the effect of six distinct microalgae diets on the progression of larval stages and their subsequent survival. Embryonic development in *U. occidentalis* unfolded over a period of 14 days, systematically detailed across eight distinct phases, each marked by the progressive emergence of the embryo and its associated appendages. Notably, an increase in the heart rate was registered just prior to spawning. Following spawning, a brief period of 10–15 min elapsed before the embryos successfully ruptured the chorion. Larval development underwent segmentation into five zoeal stages (zI–zV), spanning a 15-day duration, with an equivalent temporal period encompassing the megalopal stage until the attainment of the first juvenile crablet. Each transition between stages was heralded by a molting event. Despite our observations confirming the ingestion and digestion of the assessed microalgae, it became evident that diet supplementation with rotifers and brine shrimp is essential to optimize molting periods and, consequently, enhance survival rates. Specifically, diatoms *Chaetoceros gracilis* and *Chaetoceros muelleri* were ingested and digested up to the zoea V stage. In contrast, the microalgae *Tetraselmis maculata* and *Rhodomonas salina*, while undergoing ingestion and digestion, were only able to sustain larvae until the zoea III stage. The outcomes of our research affirm the viability of *U. occidentalis* crablet production within a laboratory setting, thereby offering the potential inclusion of this species as a valuable aquaculture product. This endeavor holds the promise of contributing to the conservation and enhancement of wild *U. occidentalis* populations.

KEY WORDS: *Ucides occidentalis*, embryonic development, crab feeding, early crab description, epifluorescence, crab production

INTRODUCTION

The species *Ucides occidentalis* (Ortmann, 1987), commonly referred to as the red mangrove crab, exhibits a geographical distribution that encompasses the tropical West Pacific region. Specifically, this species is known to inhabit areas spanning from Espiritu Santo Island, situated in Baja California Sur, Mexico, to Las Vacas, Peru, as documented by Bright (1966). It is noteworthy that recent empirical observations have expanded the recognized distribution of *U. occidentalis* to encompass the San Pedro de Vice estuary, located within the Sechura region of Piura, Peru, as reported by Alemán and Ordinola (2017).

The presence of the crustacean species within the mangrove ecosystem assumes a pivotal ecological role, primarily owing to its herbivorous proclivity (Barragán 1993). This dietary behavior contributes significantly to the modulation of fallen mangrove leaves, effectively channeling them back into the ecosystem as bioavailable organic matter through excretion (Twilley et al. 1997), thereby intensifying the cycling of soil nitrogen (Lindquist et al. 2009). Furthermore, these crustaceans actively participate in soil modification by constructing burrows, an activity that exerts a notable influence on gas exchange dynamics within the mangrove substrate. This excavation activity facilitates the enhancement of aerobic microorganism activity, which, in turn, accelerates the decomposition of organic matter (Schories et al. 2003, Lindquist et al. 2009).

Historically, *Ucides occidentalis* has served as a vital source of economic sustenance for artisanal fishing communities (Oesterling & Petrocci 1995). The extant body of documentation from various regions, including El Salvador (Rivera et al. 2013), Panama (Vega 2018), Peru (Ordinola-Zapata et al. 2018), and Ecuador (Solano et al. 2010, Zambrano 2017), corroborates this historical precedent. It is noteworthy that within the latter two nations, Ecuador and Peru, more extensive traceability measures are in place, affording a nuanced understanding of the present status of this resource. Such enhanced traceability mechanisms even facilitate the implementation of protective measures, including temporal resource closures when deemed necessary (Ministerial Agreement No. 016; Article 2, published in the Official Gazette No. 284, https://srp.produccion.gob.ec/wpcontent/uploads/2021/07/ACUERDO-MPCEIP-SRP-2021-0016-A-VEDA-REPRODUCCION-CANGREJO-ROJO-2021.pdf).

In the context of Ecuador, empirical data illustrate a pronounced pattern of aggressive crab extraction. For instance, in the year 2009, substantial crab captures were documented, amounting to 2,897,707 crabs per year and 3,184,795 crabs per year in the provinces of El Oro and Guayas, respectively (Solano et al. 2010). By the year 2019, scientific bulletins in

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Ecuador had recorded an estimated capture of *Ucides occidentalis* at 40,153,248 individuals, translating to a monthly average of 3,650,295 (https://www.gob.ec/ipiap). These figures imply an annual harvest exceeding 40 million crabs within the mangrove ecosystems of Ecuador. Furthermore, Ecuador crab exports, comprising frozen, peeled, dried, or brine-preserved specimens, were quantified at 29.4 metric tons in 2017, with a Free on Board value amounting to $108.4 thousand (Mendoza Avilés et al. 2019). The scarce and not very traceable information related to the annual extraction rate and the recent increase in crab exports and their added values make it necessary to develop a better quantitative record that allows projecting strategies for the rational exploitation of the resource and its conservation over time. A parallel situation is developing in Peru, where the wild population of *U. occidentalis* has experienced a drastic reduction of approximately 35.8% in the last 11 y (Ordinola-Zapata et al. 2018). In contrast, it is noteworthy that *U. occidentalis* is subject to significantly less exploitation in other regions, such as the Colombian Pacific (Castellanos-Galindo et al. 2017).

Despite the historical backdrop, it is notable that scant scientific literature has been produced concerning the species in question. Research efforts have primarily centered on the examination of attributes pertinent to fisheries, encompassing parameters such as catch sizes (Solano et al. 2010), landings (Moreno & Ruiz 2010), catch per unit effort (Solano et al. 2010), and inquiries of ecological significance (Schuiteman et al. 2019, Pontón-Cevallos et al. 2021), as well as investigations into gonadal maturity (Avila et al., 2022). Recently, a concise account detailing the embryonic development (Ramos-Veliz et al. 2022) has become available, complemented by the morphological characterization of the inaugural larval stage of *Ucides occidentalis* (zoea I) (Schuiteman et al. 2019). It is worth noting that no reports have surfaced regarding the aquacultural aspects of this species. Indeed, there remains an absence of knowledge pertaining to its cultivation practices and dietary preferences under controlled conditions, spanning all stages of its life cycle.

During their initial developmental stages, species cultivated in aquaculture predominantly rely on microalgae as a fundamental food source. This reliance finds robust support in scientific investigations, which have accentuated the indispensable role of microalgae in fostering the early growth of diverse aquatic organisms, including fish (El-Khodary et al. 2021), molluscs (Yang et al. 2022), echinoderms (Castilla-Gavilán et al. 2018), and crustaceans (Abd Rahman et al. 2018, Turcihan et al. 2021). This reliance stems from the noteworthy nutritional attributes of microalgae, encompassing their capacity for synthesizing and amassing significant quantities of essential fatty acids (Patil et al. 2005). Additionally, microalgae serve as a rich reservoir of amino acids (Ansari et al. 2021), vitamins (Brown et al. 1999), and minerals (Shah et al. 2018). Cumulatively, the substantial body of scientific evidence shows the substantial nutritional contributions offered by microalgae and their consequential impact on the performance of cultured aquatic organism larvae. These assertions have been substantiated through a diverse array of methodologies and analytical tools.

Epifluorescence microscopy has been empirically proven as a valuable tool for the judicious selection of microalgae species in numerous studies involving a wide spectrum of aquatic organisms. These encompass gastropod molluscs (Aranda et al. 1991), bivalve molluscs (Le Pennec & Rangel-Davalos 1985), echinoderms (Duy et al. 2015), fish (Tytler et al. 1997), and crustaceans (Factor & Dexter 1993). The present research endeavor aims to contribute to this body of knowledge by (1) documenting the early phases of the life cycle, including embryonic and larval stages, of *Ucides occidentalis* and (2) employing epifluorescent microscopy to assess the efficiency of microalgae ingestion and digestion during larval development. This multifaceted inquiry further explores the consequential effects on metamorphosis and the overall survival of the larvae under scrutiny.

MATERIALS AND METHODS

Live Food Culture: Microalgae

The microalgae used as nutritional sources during the investigation of the early life cycle of *Ucides occidentalis* were sourced from the National Center for Aquaculture and Marine Research (CENAIM) affiliated with the Escuela Superior Politécnica del Litoral (ESPOL). The microalgal species encompassed *Pavlova lutheri*, *Chaetoceros gracilis*, and *Chaetoceros muelleri*. Furthermore, in the context of the ingestion–digestion assessment, the microalgae *Tisochrysis lutea*, *Tetraselmis maculata*, and *Rhodomonas salina* were used.

Microalgae were cultured in a controlled environment, utilizing filtered seawater (0.45 μm) characterized by a salinity of 34 ± 0.1 g/kg. The cultures were exposed to UV light (FSW) and operated under sustained fluorescent lighting conditions, with a photon flux density in the range of 47–67 μmol photons active photosynthetic radiation per square meter per second. Adequate aeration was consistently maintained throughout the cultivation process. The culture medium adhered to the f/2 formulation developed by Guillard (1975). Notably, for diatom cultures, this medium was enriched with 1% sodium metasilicate.

The ambient temperature was maintained at 20.0°C ± 0.5°C. Before deployment as larval feed, the microalgae cultures contained within 6-L containers were harvested during their exponential growth phase. Subsequently, their cell density was accurately estimated using a hematocytometer, which served as the basis for calculating the precise feeding dosages tailored for the larvae.

Lastly, the production of benthic microalgae, specifically *Navicula* sp. and *Amphora* sp., was undertaken to facilitate their utilization during the megalopa phase, leading up to the development of the first juvenile crablet stage.

Rotifer Culture

The rotifer *Brachionus plicatilis* was cultured following a standardized protocol established at the CENAIM facility. In brief, a rotifer strain, derived from the CENAIM repository and maintained under temperate conditions at 20°C, was transferred into 200-L tanks containing FSW, and cultured to attain a target density of 10 rotifers per milliliter. The rotifers were fed *Tetraselmis maculata* at a concentration of 200,000 cells/mL. Prior to each feeding event, the rotifers were harvested during their exponential growth phase, and concentrated in a 10-L container through a 60-μm sieve to estimate population density for dosing purposes. The rotifer culture was maintained under continuous aeration at a water temperature of 25.5°C ± 1.0°C.

Artemia Culture

Artemia cysts (Batch 7122336204, INVE Aquaculture), underwent a hydration process in 1-µm-filtered and autoclaved water (FSWA) for 1 h, at a concentration of 2.5 g/L. Subsequently, the cysts underwent decapsulation, which involved the addition of 660 µL of NaOH (32%) and 10 mL of NaClO (50%). This decapsulation process was terminated after approximately 2 min through the introduction of 14 mL of $Na_2S_2O_3$. Throughout the reaction, aeration was consistently supplied. The decapsulated cysts were rinsed with FSWA and placed into two separate 1-L beakers, each containing FSWA for a 24-h incubation period at 28°C. The incubation protocol was characterized by robust aeration and a constant light intensity of approximately 2,000 lux.

The Artemia nauplii, specifically those at the 24-h stage and devoid of discernible mouth and anus structures, was concentrated within a 1-L beaker. This concentration step was undertaken to accurately estimate their population density and to facilitate precise dosing as required for subsequent feeding.

Red Crab Ucides Occidentalis Females Broodstock

Mature female specimens, displaying clear indicators of gonadal maturation and fecundity as denoted by the presence of eggs in the initial stages of embryonic development, were methodically procured. This acquisition was thoughtfully synchronized with lunar phases, following the methodology outlined by Cuadrado Ortiz et al. (2022). Subsequently, a cohort of 15 mature female specimens was carefully translocated from the mangrove habitats situated in the sector of 6 de Julio, Naranjal, Guayas, Ecuador, to the controlled research facilities at the CENAIM-ESPOL. To minimize potential aggression among the transported individuals, each female was securely housed within separate containers during the relocation process, with notable emphasis on the prudent conservation of water resources, warranting a no-water transport protocol.

Laboratory Conditioning and Spawning Induction

Containers, measuring 58 cm in length, 39 cm in width, and 30 cm in height, were used for the housing of *Ucides occidentalis*. These containers were filled with a total of 15-L of FSW. Within each container, a cylindrical PVC pipe, measuring 20 cm in length and 10.2 cm in diameter, was introduced to serve as a refuge for the crabs, thus mitigating potential stressors in accordance with the methodology elucidated by Ramos-Veliz et al. (2022). A piece of plastic mesh was positioned at the base of each container to prevent inadvertent slippage by the female crabs. These female crabs (15), characterized by carapace dimensions averaging 54.5 ± 2.6 mm in width, 72.7 ± 5.0 mm in length, chelae measuring 29.5 ± 7.0 mm in width and 86.8 ± 13.3 mm in length, and an average weight of 150.4 ± 3.5 g, were individually allocated to each container.

The water in the containers was changed every 24 h (100%). To induce spawning, specifically during days 13–14 (postconditioning), which corresponds to phase VIII of embryonic development, a solution comprising *Pavlova lutheri*, *Chaetoceros gracilis*, and *Chaetoceros muelleri*, each in a 1:1:1 ratio, was introduced into the system. This solution was administered during water change, serving as a potent spawning stimulus for the female crabs. The concentration of this solution was maintained at 100,000 cells/mL.

Environmental conditions during both the conditioning and spawning induction phases were meticulously controlled, with water temperature maintained at 28.5°C ± 1.0°C, regulated using a Marina submersible heater (Hagen, China). Salinity levels were kept at 34 g/kg, and photoperiod conditions were maintained at a 12:12-h cycle (light:dark), supplemented with moderate aeration. Spawning occurred spontaneously, coinciding with the zenith of the full moon phase. Subsequent to spawning, all female crabs were conscientiously repatriated to their original habitat.

Embryo Description

To delineate the embryonic development of *Ucides occidentalis* chronologically, daily sampling endeavors encompassed the collection of 20–25 eggs (per female) carefully extracted from three female specimens. Eight distinct embryonic stages were meticulously characterized based on a comprehensive evaluation encompassing internal and external morphological attributes of the eggs, egg coloration, the relative proportion of yolk, egg size, and cardiac activity. The latter was quantified manually, with five counts conducted within a minute interval and subsequently averaged. These criteria adhered to established protocols outlined by García-Guerrero and Hendrickx (2006), Pinheiro and Hattori (2003), Samuel and Soundarapandian (2009), as well as the more specific guidelines provided by Ramos-Veliz et al. (2022) for the embryonic characterization of *U. occidentalis*, building upon prior work relating to *Panopeus chilensis*, *Portunus sanguinolentus*, and *Ucides cordatus*.

This sampling regime was initiated on the second day of the conditioning phase, coinciding with embryonic phase I, and continued until the fourteenth day of conditioning, corresponding to embryonic phase VIII. The nomenclature used for the description of embryonic development is closely aligned with terminology established in previous investigations focused on *Ucides occidentalis* (Ramos-Veliz et al. 2022).

For the documentation of morphological characteristics, the eggs were meticulously positioned on slides to facilitate measurement and imaging. Digital capture of these morphological attributes was achieved using a high-resolution digital camera (MDX503, Lanoptik) connected to a trinocular microscope (model CX31RTSF, Olympus), complemented by Nahwoo iWorks 2.0 software for data acquisition and analysis.

Larval Description and Larval Culture

Fifteen days subsequent to the initiation of the conditioning phase, a cohort of five mature female crabs successfully spawned. The resultant zoea I larvae, characterized by an average length of 441.0 ± 15.6 µm, were carefully collected using a 300-µm mesh sieve and subsequently relocated to three separate 1,000-L holding tanks, with a stocking density of 0.5 larvae/mL. The larval culture was meticulously conducted under quiescent conditions, entailing a daily complete water exchange regime, maintaining a salinity level of 34 g/kg. The larval nutritional regimen was initiated with a microalgal composite comprising *Pavlova lutheri*, *Chaetoceros gracilis*, and *Chaetoceros muelleri* in a 1:1:1 ratio, with a cell concentration of 100,000 cells/mL

(divided into two doses of 50,000 each, 08:00 and 17:00 h). This algal blend remained consistent throughout the larval culture period. Subsequent to the zoea III stage, zooplanktonic organisms were introduced into the dietary regimen, specifically the rotifer *Brachionus plicatilis* and *Artemia franciscana*, at densities of 20 and 0.6 ind/mL, respectively (see Table 1, for details).

Precise temperature control was maintained throughout the larval culture phase, with the water temperature sustained at 28.5°C ± 0.5°C. This thermal regulation was achieved through the deployment of a submersible heater (Marina, Hagen, China). Additionally, the culture tanks were systematically aerated to ensure optimal oxygenation levels.

Megalopa stage crab larvae, harvested on day 15 postspawning, were carefully translocated into plastic containers, mirroring the specifications of those used during the conditioning of mature female crabs. Within these containers, a 1-cm layer of meticulously filtered sand (<200 μm particle size) was uniformly distributed, accommodating a stocking density of 1 megalopa larva per square centimeter. The nutritional regimen prescribed to these megalopa larvae comprised a daily provision of a composite diet. This diet entailed a blend of benthic microalgae, specifically *Navicula* sp. and *Amphora* sp., at a 1:1 ratio, supplemented with live Artemia, with three individual Artemia organisms allocated for each larval unit. Water replenishment, involving a 2 cm water depth exchange, was performed on alternate days over a duration of 15 days. This meticulous water management regimen persisted until the attainment of juvenile red crabs, commonly referred to as "crablet".

Following each water exchange, a representative sample of 30 larvae was subjected to morphological analysis to elucidate the successive transformations during metamorphosis. This analysis involved the capture of high-resolution images utilizing a digital camera (MDX503, Lanoptik) connected to a trinocular microscope (model CX31RTSF, Olympus) and the utilization of the Nahwoo iWorks 2.0 software. For the characterization of zoeal stages, attention was primarily directed toward the discernible attributes of the cephalothorax, abdomen, antennule, antenna, first maxilliped, and second maxilliped, as these features lent themselves to ready observation. In the case of the megalopa stage and the initial juvenile crab, specific attention was afforded to the antenna, cephalothorax, abdomen, and carapace, respectively. The terminological framework used for describing larval development remained congruent with prior research on *Ucides cordatus*, as articulated in the work of Rodrigues and Hebling (1989). Recorded measurements were anchored in specific anatomical parameters, including larval size (measured from the anterior margin of the eye to the telson), megalopa size (from the tip of the rostral spine to the posterior end of the telson), and the carapace length of the first juvenile crab (from the anterior margin of the forehead to the posterior margin of the carapace), following the conventions delineated by Li et al. (2022).

TABLE 1.

Food provided to *Ucides occidentalis* larvae during their larval culture.

Stage	*Mix of microalgae (cells/mL)	*Brachionus plicatilis* (ind/mL)	*Artemia franciscana* (ind/mL)
Zoea I	100.000	0	0
Zoea II	100.000	0	0
Zoea III	100.000	20	0.6
Zoea IV	100.000	20	0.6
Zoea V	100.000	20	0.6
Megalopa	100.000	40	1

*The microalgal feed consisted of a mixture of *T. lutea*, *C. gracilis*, *T. maculata*, and *P. lutheri* in a 1:1:1:1 ratio.
0: It was not used as food at that stage.

Ingestion and Digestion Trial

In the search for a precise nutritional contribution and to know the performance of red crab larvae when fed only with microalgae, monospecific diets were adopted, specifically *Tetraselmis maculata*, *Rhodomonas salina*, *Tisochrysis lutea*, *Pavlova lutheri*, *Chaetoceros gracilis*, and *Chaetoceros muelleri*. These diets were systematically allocated in triplicate, with daily administrations at a concentration of 100,000 cells/mL. The daily dietary allocation was subdivided into two separate dosages administered at 08:00 and 17:00 h.

Zoea I larvae, sourced from a dedicated larval culture tank (as outlined in section 2.5), were subjected to concentration through a 300-μm sieve. Subsequently, these larvae were evenly distributed into cylindro-conical tanks, each possessing a volumetric capacity of 50-L, with an operational volume of 30-L. The larval stocking density was maintained at 0.05 larvae/mL.

Complete water exchanges, involving the replacement of 100% of the culture water, were executed on a daily basis. Concurrently, during each water replacement event, larvae were concentrated within a 300-mL beaker. Subsequently, three discrete 2-mL samples were collected to record growth, survival (ascertained through the extrapolation of the larvae population density within a known volume to the total volume), and metamorphic transitions (stage changes). Temperature was maintained at 27.5°C ± 0.5°C. To assess microalgal ingestion and digestion by the larvae, individuals from each treatment group were harvested through a 300-μm mesh sieve. These specimens were then subjected to rinsing with FSW before being affixed to glass slides for microscopic analysis. The evaluation took place under an epifluorescence microscope (Eclipse 200 with FICT filter-; Nikon, Tokyo, Japan) on specific postspawning days, namely days 2 (corresponding to the zoea I stage), 4 (zoea II), 7 (zoea III), 10 (zoea IV), 13 (zoea V), and 16 (megalopa larvae). The sample size for each assessment point consisted of n = 15–20 specimens.

The assessment of ingestion and digestion stages was conducted in accordance with the methodology originally proposed by Le Pennec and Rangel-Davalos (1985) for mollusc larvae, albeit with slight procedural modifications. In essence, the categorization of these stages can be summarized as follows:

Stage 1 (I) was defined as the phase characterized by the conspicuous presence of well-defined fluorescence signals within the digestive gland of the larvae. During this ingestion phase, individual fluorescent microalgae cells were readily discernible, appearing intact and singularly distinguishable (displaying a red coloration).

Stage 2 (II), denoting the digestion phase, was characterized by the absence of individual microalgal cells while retaining an observable fluorescence, manifesting as a staining effect that permeated the digestive gland. This fluorescence took on varying shades of pink, orange, or yellow.

Stage 3 (III), termed the nondigestion phase, pertained to larvae whose digestive glands were entirely devoid of content, accompanied by an absence of discernible larval growth. In this stage, fluorescence ceased to be observable.

Statistical Analyses

The evaluation of treatment effects on molting, as indicated by the proportion of larvae successfully transitioning to the immediate superior developmental stage, and survival outcomes at the conclusion of each metamorphic phase, was conducted employing a one-way analysis of variance (ANOVA). Preceding the statistical analysis, assessments were made to ensure data normality and variance homogeneity, employing the Shapiro–Wilk and Bartlett tests, respectively. Post hoc analysis for discerning intertreatment disparities was executed through the utilization of the Tukey *a posteriori* test. The criterion for statistical significance across all tests was established at $P < 0.05$.

RESULTS

Embryo Description

Figure 1 illustrates the successive stages of embryonic development in *Ucides occidentalis*. Figure 1A, corresponding to the initial phase (day 1 postconditioning), reveals distinct characteristics: the eggs exhibit macrolecithic attributes, are centrolecithic in arrangement, and maintain a spherical morphology.

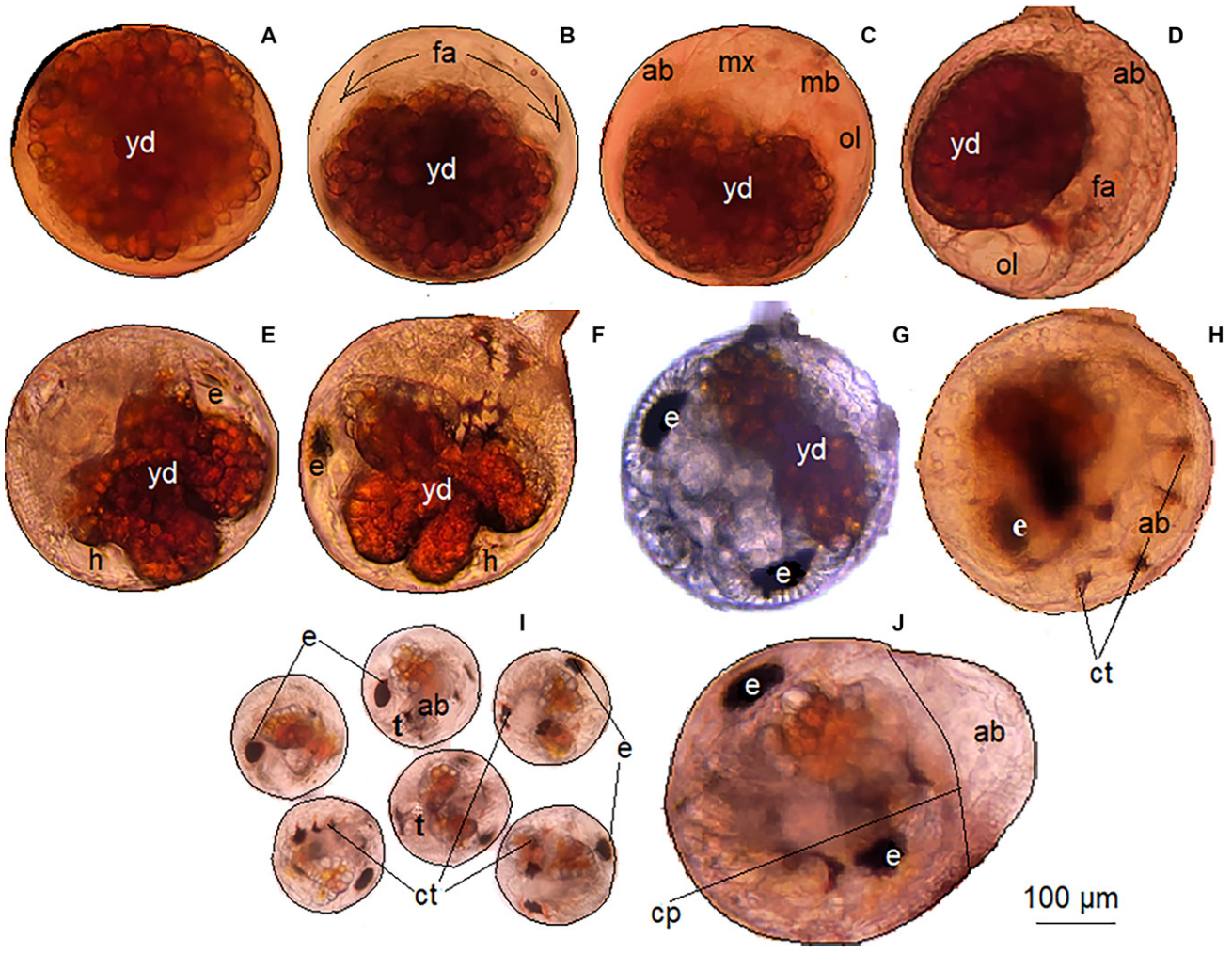

Figure 1. Description of the embryonic development of *Ucides occidentalis*. Stage I (A) egg yolk sectioned into drops, in stage II (B) appearance of primordial tissues: optic lobe, developing cephalic and thoracic-abdominal appendages (fa); (C), differentiated antenna-antennulla, maxilla, and maxilliped primordia; in stage III (D) abdomen in the process of segmentation and slight appearance of abdominal chromatophores; In stages IV (E) an embryo with more complex eyes, more marked chromatophores and a beating heart was observed. In stage VI (F) a noticeable decrease in the yolk is observed. The cephalothorax is now formed, the abdomen is enlarged, and its segmentation is almost complete. Enlarged, densely pigmented eyes that differ in the cornea and retina are seen. All appendages are segmented. At stage VII (G), the embryo occupies all the space inside the egg. Few yolk droplets are still stored dorsally to the cephalothorax. The cephalothorax and abdomen were separate, and the contours were observable. At stage VIII (H–J), all appendages were well developed, and abdominal segmentation was complete. The eyes were fully pigmented and the chromatophores were now more visible right down to the appendages. The abdomen is folded against the front of the cephalothorax. The embryo is about to hatch and stretches frequently to break the chorion that covers the egg (J). yd: yolk drops; fa: formation of appendages; ab: abdomen; mx: maxillipeds; mb: maxilla and maxillule; ol: eye lobe; e: eye; t: telson; h: heart; ct: chromatophores; cp: portion of cephalothorax. The 100-µm bar serves as a reference for all images except (I).

Notably, these eggs are predominantly occupied, approximately 98%, by a fragmented mass of purple yolk, with no discernible indications of internal structural development.

Moving to Stage II (Fig. 1B), which pertains to day 3 postconditioning, a marked transformation unfolds. The yolk material undergoes fragmentation into small oil droplets while concurrently undergoing a reduction in volume, albeit retaining its distinctive coloration. Additionally, the emergence of primordial cells becomes evident during this phase.

Stage III, spanning days 4 and 5 postconditioning, is depicted in Figure 1C. This stage is characterized by several noteworthy features, including the onset of appendage formation, the initiation of tissue differentiation, and the onset of organogenesis. Notably, the primordial cells observed earlier have undergone differentiation, culminating in the development of key embryonic structures. Further observations reveal the nascent formation of the ocular region and the differentiation of appendages, specifically the antenna–antennulla, maxilla, maxillipeds, as well as the thoracic and abdominal regions.

Stage IV, documented on days 6 and 7 postconditioning (as illustrated in Fig. 1D), is marked by discernible developments. The primordial structures corresponding to the abdomen and cephalothorax have undergone considerable expansion, now distinctly separate from each other. Furthermore, the antenna, antennulla, maxilla, and maxillipeds have transitioned into rudimentary bud-like formations, situated posteriorly to the eyestalks. Notably, the yolk content exhibits a shift toward a lighter hue during this stage.

Transitioning to Stage V, which encompasses days 8 and 9 postconditioning and is illustrated in Figure 1E, further advancements in embryonic development become evident. Notably, the primordial structures, including the antenna–antennulla, maxilla, and maxilliped, have undergone substantial growth. At this juncture, the embryo occupies a significant portion of the egg's volume, encompassing at least one-quarter. The abdominal region exhibits ongoing development, albeit with incomplete segmentation (metameres). Distinctively, the anterior section of the embryo manifests the emergence of a slightly darker oval-shaped eye retina. This phase marks the initiation of a more advanced phase of differentiation and growth. The primordial formations of the antenna–antennulla and maxilla, along with the maxilliped, exhibit greater prominence and differentiation.

Stage VI, observed at day 10 postconditioning (Fig. 1F), is typified by several notable transformations. Most prominently, a substantial reduction in the yolk content is evident. The cephalothoracic region has now taken definitive form, whereas the abdomen has undergone significant enlargement, with segmentation nearing completion. Furthermore, the eyes exhibit marked changes, characterized by enlargement and an increased density of pigmentation. Distinct differences are discernible in the corneal and retinal regions of the eye structure. Notably, the heart rate manifests a slow, steady rhythm, averaging between 90 and 100 beats per minute.

Progressing to Stage VII, which corresponds to day 12 postconditioning (as depicted in Fig. 1G), the following features were observed. The embryo now occupies entirely the egg's internal space. Whereas a few residual yolk droplets remain dorsally adjacent to the fully visible cephalothorax, the remaining yolk constituents are distributed among four distinct lobes. Importantly, the cephalothorax and abdomen have undergone a complete separation, manifesting discernible contours. The abdominal region has substantially enlarged and is now distinctly divided into segments, known as somites, marked by the presence of chromatophores. The cardiac structure has also experienced significant growth, resulting in a vigorous heartbeat, typically oscillating within the range of 180–190 beats per minute.

Concluding the developmental sequence at Stage VIII, on day 14 postconditioning (Fig. 1H–J), all appendages have reached advanced stages of development. The segmentation process of the abdominal region is now definitively complete. The eyes exhibit full pigmentation, and chromatophores have become notably pronounced, extending down to the appendicular structures. In this stage, the heart rate registers a further increase in tempo compared with preceding stages, typically attaining rates of approximately 235–245 beats per minute. Notably, the abdomen becomes folded against the anterior cephalothoracic region. The embryo, now poised for hatching, engages in frequent stretching motions to rupture the chorion enveloping the egg. The hatching event ultimately unfolds on day 15 postconditioning.

Throughout the entire developmental progression from Stage I to Stage VIII, the observed increase in egg size amounts to 16.29%, representing a transition from an initial measurement of 327.4 ± 11.5 μm to a final measurement of 391.1 ± 22.4 μm.

Larval Description: Zoeal Stages

The larval development of *Ucides occidentalis* encompasses a sequence of five zoeal stages, followed by a megalopal stage. Following the spawning event, the eggs exhibited hatching within a relatively brief window of 10–15 min, yielding the inaugural zoea larva (as depicted in Fig. 2A). It is noteworthy that each transition between these larval stages was invariably heralded by a molting event.

The temporal intervals characterizing these stage changes were as follows: from zoea I to zoea II, a duration of 50.0 ± 2.0 h postspawning was observed; the transition from zoea II to zoea III transpired within a timeframe of 72.0 ± 1.5 h. Subsequently, the progression from zoea III to zoea IV required 84.0 ± 2.5 h, and the shift to zoea V entailed a duration of 84.0 ± 1.5 h. Finally, the transition from zoea V to the megalopal stage was characterized by an interval of 88.0 ± 2.0 h. Conclusively, the transformation from the megalopal stage to the first juvenile crablet stage encompassed a period of 15 days. The collective duration of larval culture, spanning from the emergence of the first zoea larva to the attainment of the first juvenile crablet stage, spanned a total of 30 days. Below, a comprehensive account of the principal morphological alterations characterizing each of the larval stages is presented, with visual representations available in Figure 2 for reference.

Size

The cumulative body length of the larvae exhibited distinct values throughout the zoeal developmental stages, as follows: 441.1 ± 15.6 μm for zoea I, 501.6 ± 41.5 μm for zoea II, 784.1 ± 79.8 μm for zoea III, 835.9 ± 15.7 μm for zoea IV, and 1155.3 ± 143.0 μm for zoea V. It becomes readily apparent that the observed variations in larval body length were conspicuously associated with the transitions between these zoeal stages.

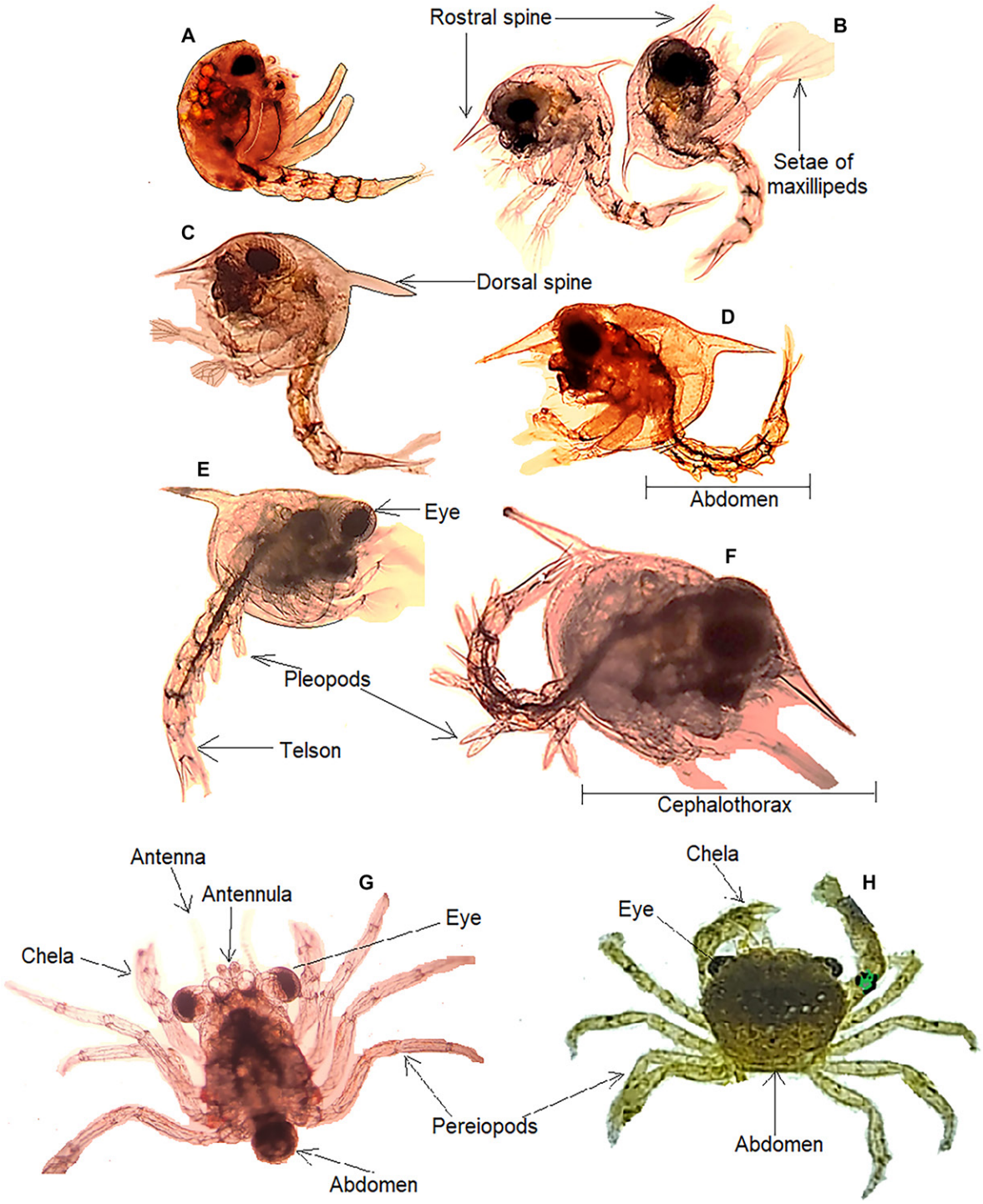

Figure 2. Larval development of *Ucides occidentalis* in the laboratory. (A) Zoea I stage larva 15 min after hatching; (B) zoea I stage larva 6 h posthatching; (C) zoea II stage larva; (D) zoea III stage larvae with the primary appearance of pleopods in the abdomen; (E) zoea IV stage larva with evident pleopod bifurcation; (F) zoea V larva; (G) megalopa larva, with morphology appearance of the first pair of chelated pereiopods; (H) early crablet of red crab with the morphology of an adult and all its differentiated appendages. The images have different magnification scales for a better appreciation of their structures.

Cephalothorax

The anatomical structure of red crab larvae entails a division into two distinct regions: the cephalothorax and the abdomen, as visually depicted in Figure 2D, F. Within the cephalothorax, a total of seven appendages are discernible, comprising the antennulla, antenna, mandible, maxillule, maxilla, first maxilliped, and second maxilliped. Furthermore, the cephalothorax exhibits noteworthy dorsal features in the form of a dorsal spine and a

rostral spine, as illustrated in Figure 2B, C. It is notable that the dorsal spine was marginally longer in comparison with the rostral spine.

Abdomen

During the zoeal stages, specific abdominal configurations were observed. In zoea I and zoea II, the abdomen consisted of six somites, including the telson. Conversely, from zoea III through zoea V, the abdomen comprised seven somites, which also included the telson, as depicted in Figure 3B. Notably, in zoea II, somites 2–3 exhibited two anteriorly directed lateral bulges, whereas somites 4–5 each bore a pair of posterolateral spines. In zoea III, somites 2–3 featured two posteriorly directed hooks, whereas somites 4–5 displayed a pair of posterolateral spines, which elongated in conjunction with larval development. Furthermore, in zoea III, the development of pleopods was initiated. In zoea IV,

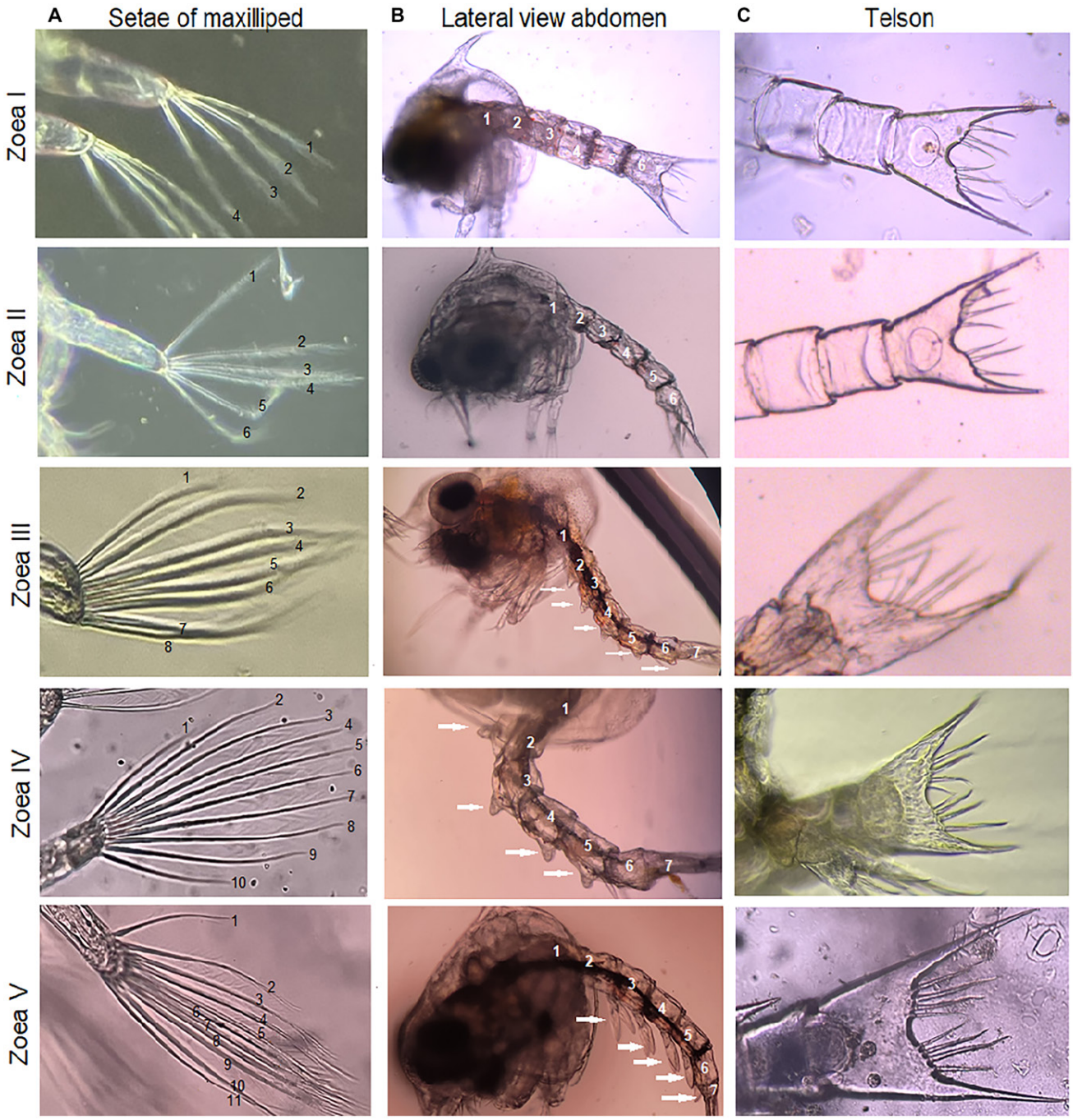

Figure 3. Morphological changes of maxillipeds setae, abdomen, and telson of zoeal stages. (A) Changes in the number of setae of the maxillipeds from zoea I to zoea V; (B) the lateral view of the abdomen from zoea I to zoea V; (C) the telson view from zoea I to zoea V. The arrows indicated the body changes during larval development, and the numbers presented the somites. The images have different magnification scales for a better appreciation of their structures.

the pleopods, comprising five pairs, exhibited continued growth, and somites 4–5 retained a pair of spines. As for zoea V, it was characterized by an increase in the length of five pairs of pleopods and one pair of uropods, as illustrated in Figure 3B.

Telson

During the zoeal stages, specific features of the telson were observed. In zoea I and zoea II, the telson's fork exhibited three pairs of feathery setae along the postanterior margin. Additionally, each furcal axis was adorned with a lateral spine, as depicted in Figure 3C. In zoea III, an additional pair of setae emerged within the median cleft of the posterior margin. Subsequently, in zoea IV and zoea V, there was a noticeable elongation of these setae, as illustrated in Figure 3C.

First and Second Maxilliped

The maxilliped comprised three main parts: the coxa, the endopodium, and the exopodium. The endopodium was composed of five segments, whereas the exopodium consisted of two segments. The count of feathery swimming setae varied across the zoeal stages: in zoea I, there were 4 setae; in zoea II, 6 setae; in zoea III, 8 setae; in zoea IV, 10 setae; and in zoea V, there were 11 setae, as illustrated in Figure 3A. The number of plumose setae remained the same in both maxillipeds.

Megalopa Stage and the First Juvenile Crab

The megalopa stage, being the longest in the larval cycle, underwent significant morphological changes documented at two distinct time points (day 1 and day 13). Megalopa larvae

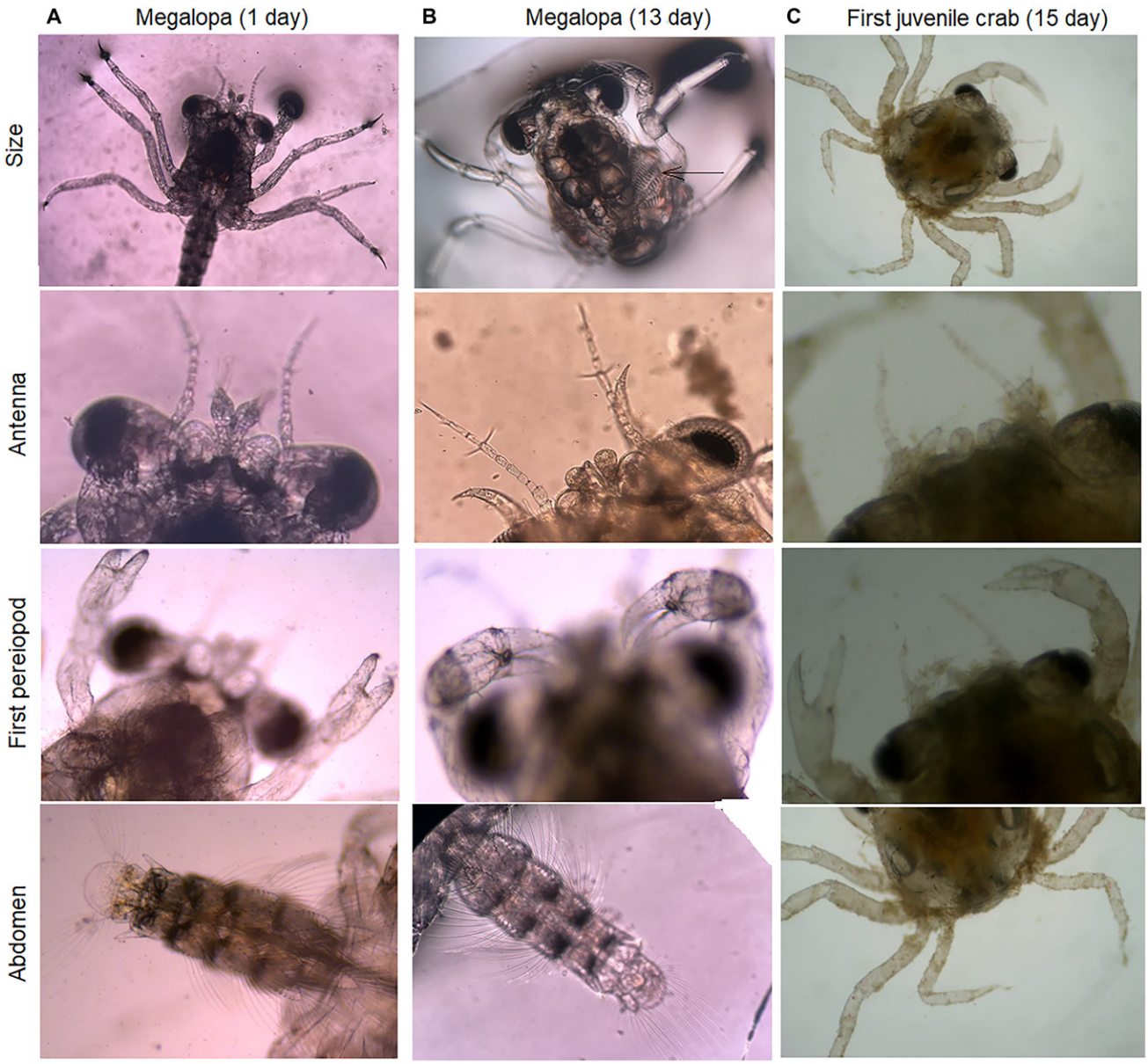

Figure 4. Morphological changes of size, antenna, first pereiopod, and abdomen of *Ucides occidentalis* larvae and early crab. (A) Changes in megalopa of 1-day-old; (B) changes in megalopa of 13-day-old; (C) changes in the first juvenile crablet of 15 days. The arrow indicated the gill tree formation in 13-day-old megalopa larvae. The images have different magnification scales for a better appreciation of their structures.

initial exhibited a flattened body, elongated abdomen, and pereiopods resembling those of juvenile crabs (refer to Fig. 4A). The total length of megalopa larvae at different ages was as follows: 1,171.8 ± 11.6 µm for 1-day-old megalopas, and 1,237.3 ± 32.2 µm for 13-day-old larvae.

In early megalopa stages, the fifth pair of pereiopods, located at the posterior region of the cephalothorax, exhibited some swimming functionality, which diminished by the time the larvae reached 7 days of age. Meanwhile, the chelae of the first pair of pereiopods displayed gradual growth as the culture progressed. During this transition, the dorsal spine regressed, leaving only the rostral spine. Additionally, the eye stalks underwent elongation and became mobile. Notably, the development of gill trees became more apparent after the seventh day of megalopa development. This period also marked an enhanced ability to move outside of water and an increased exoskeleton rigidity.

The first juvenile crab shared morphological characteristics with the adult crab (as depicted in Fig. 4C). The rostral spine disappeared at this stage, and the carapace measured 1,534.1 ± 58.8 µm. Detailed morphological transformations of the antenna, first pair of pereiopods, and abdomen during this final transition are illustrated in Figure 4C.

Ingestion Y Digestion Test

Figure 5 shows *U. occidentalis* larvae in the different categories of ingestion and digestion. Among the tested microalgae species, *Chaetoceros gracilis* and *Chaetoceros muelleri* exhibited superior performance, demonstrating effective ingestion and digestion capabilities through to the zoea V stage. Subsequently, *Pavlova lutheri* and *Tisochrysis lutea*, both flagellates, displayed ingestion and digestion rates extending to the zoea IV stage. Conversely, *Tetraselmis maculata* supported red crab larvae nutritionally only until the zoea stage III. Meanwhile, *Rhodomonas salina* microalgae were ingested and digested by the larvae, albeit resulting in reduced larval condition during molting and compromised physiological well-being, as detailed in Table 2.

Larval Survival and Metamorphosis

Table 3 presents the survival rates observed during each zoeal stage, revealing a progressive reduction in larval population contingent upon the provided dietary sources. To elaborate briefly, in the zoea I stage, the diets consisting of *Tisochrysis lutea*, *Chaetoceros muelleri*, and *Chaetoceros gracilis* exhibited the most favorable survival outcomes, statistically equivalent in performance. For zoea II, *C. gracilis* proved to be the optimal dietary choice with a survival rate of 72.3% ± 4.2%, followed by *C. muelleri* at 62.0% ± 4.6%, and *T. lutea* and *Pavlova lutheri* at 41.0% ± 6.0% and 33.7% ± 4.7%, respectively. The latter two diets demonstrated statistically equal survival rates.

Moving on to zoea III, *Chaetoceros muelleri* and *Chaetoceros gracilis* diets maintained exceptional performance, facilitating the successful molting of 100% of larvae to this stage, and the statistical equality between them is noteworthy. In contrast, the diets of *Tisochrysis lutea* and *Pavlova lutheri*, while exhibiting statistical equality, yielded only a 65% success rate in molting, with the remaining population remaining in the zoea II stage.

In zoea IV, a similar trend persisted, with *Chaetoceros muelleri* and *Chaetoceros gracilis* diets standing out as the most effective, resulting in a 67% molting success rate, whereas the remaining larvae, although capable of ingesting and digesting the microalgae, failed to achieve optimal conditions. Larvae fed with *Tetraselmis maculata* and *Rhodomonas salina* diets experienced complete mortality in the zoea III stage.

In zoea V, survival was limited to the diets of *Chaetoceros muelleri* and *Chaetoceros gracilis,* with survival rates of 26% and 28%, respectively. All other treatments experienced total mortality. In the end, none of the larvae from these two treatments progressed to the megalopa stage.

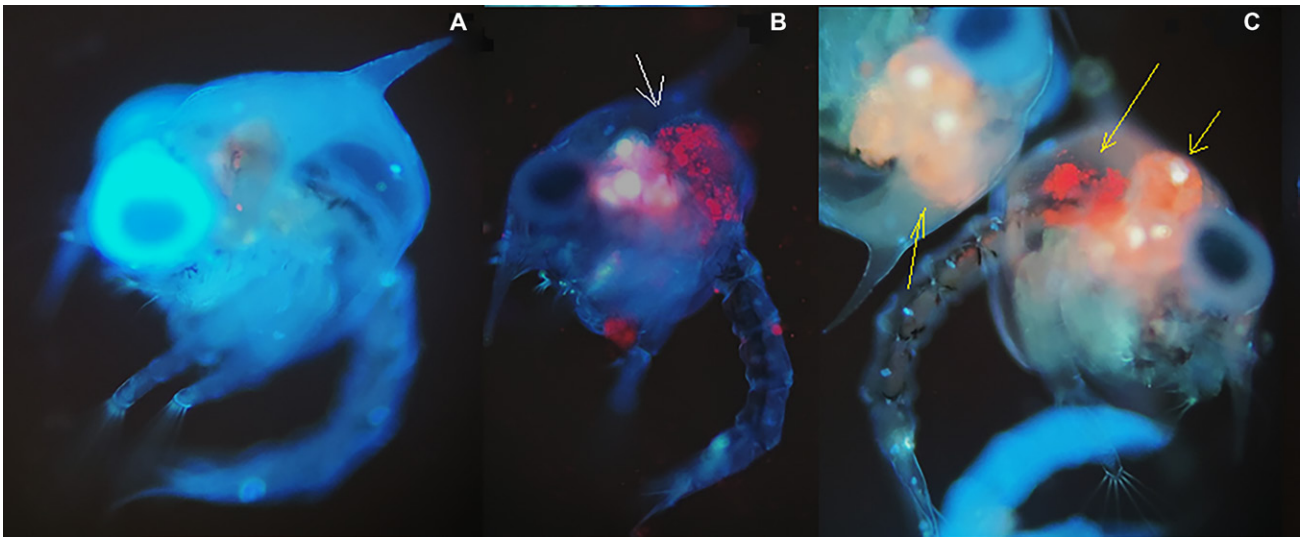

Figure 5. Ingestion and digestion of *Ucides occidentalis* larvae analyzed under an epifluorescence microscope. (A) Larvae without evidence of ingestion, stomach completely empty, (B) larva with clear evidence of ingestion (whole microalgae cells, red color), (C) hepatopancreas with cells of microalgae in the process of digestion (orange and pink colors). The white arrow indicates whole cells (ingestion) and the yellow arrows indicate microalgae cells in digestion processes, according Le Pennec and Rangel-Davalos (1985).

TABLE 2.

Ingestion and digestion stages of the microalgae evaluated as food for *Ucides occidentalis* larvae observed with an epifluorescence microscope.

	Larval stage (postspawning day)					
Microalgal diet	**Zoea I (2)**	**Zoea II (4)**	**Zoea III (7)**	**Zoea IV (10)**	**Zoea V (13)**	**Megalopa (16)**
Tetraselmis maculata	I*	I–II	I–II	M	M	M
Tisochrysis lutea	I–II	I–II	I–II	I–II	M	M
Pavlova lutheri	I–II	I–II	I–II	I–II	M	M
Chaetoceros muelleri	I–II	I–II	I–II	I–II	I–II–III	M
Rhodomonas salina	I–II	I–II	I–II	M	M	M
Chaetoceros gracilis	I–II	I–II	I–II	I–II	I–II–III	M

*Classification modified from Le Pennec and Rangel-Davalos (1985). (I) Ingestion. (II) Digestion. (III) Digestion and empty stomach. (M) Total treatment mortality.

TABLE 3.

Survival (% ± SD) of *Ucides occidentalis* larvae exposed to different species of microalgae.

	Survival					
Microalgal diet	**Zoea I**	**Zoea II**	**Zoea III**	**Zoea IV**	**Zoea V**	**Megalopa**
Tetraselmis maculate	63.0 ± 6.1^{b}	29.3 ± 3.8^{d}	7.7 ± 4.0^{c}	0	0	0
Tisochrysis lutea	81.7 ± 5.5^{a}	41.0 ± 6.1^{c}	19.3 ± 2.5^{b}	7.7 ± 3.5^{c}	0	0
Pavlova lutheri	55.0 ± 6.0^{b}	33.7 ± 4.7^{c}	19.7 ± 3.2^{b}	12.0 ± 1.0^{b}	0	0
Chaetoceros muelleri	83.0 ± 5.3^{a}	62.0 ± 4.6^{b}	55.3 ± 4.0^{a}	47.7 ± 2.5^{a}	26.3 ± 6.0^{a}	0
Rhodomonas salina	40.7 ± 4.5^{c}	19.7 ± 3.2^{e}	3.3 ± 1.5^{c}	0	0	0
Chaetoceros gracilis	83.7 ± 4.0^{a}	72.3 ± 4.2^{a}	59.3 ± 2.5^{a}	51.0 ± 1.7^{a}	28.0 ± 5.6^{a}	0

Different letters indicate significant differences among treatments ($P < 0.05$).

DISCUSSION

This study provides a comprehensive chronological account of the early developmental stages of *Ucides occidentalis*, offering valuable insights into the species unique characteristics. These insights serve as a foundational resource for the enhancement of captive cultivation practices. Moreover, our investigation confirms the critical role of microalgae as a dietary source for sustaining the initial larval stages of the red mangrove crab. The nutritional significance of microalgae has been documented in the larvae of various crustacean species, including *Artemia franciscana* (Pacheco-Vega et al. 2015), *Penaeus vannamei* (Sivakumar et al. 2011, Dinesh Kumar et al. 2017), as well as in crab larvae such as *Portunus pelagicus* (Taufik et al. 2016), *Callinectes danae* (Guarizo et al. 2020), and *Scylla tranquebarica* (Syafaat et al. 2019). These findings underscore the broader ecological relevance and dietary preferences shared among crustacean larvae, thereby contributing to our understanding of their nutritional requirements and ecological roles.

The embryonic development of *Ucides occidentalis* exhibited a duration of 14 days, a timeline consistent with previous findings in related crab species. García-Guerrero and Hendrickx (2006) reported a similar 14-day incubation period for *Goniopsis pulchra* and *Aratus pisonii*. Likewise, Zeng (2007) observed a 14-day duration for *Scylla paramamosain*, whereas Yamaguchi (2001) documented a slightly longer period of 15.4 days for *Uca lactea*. Notably, this duration aligns with the recent description of *U. occidentalis* by Ramos-Veliz et al. (2022), who reported a 15-day incubation period. In contrast, certain crab species have been noted for shorter embryo incubation periods. For instance, *Portunus pelagicus* demonstrated a relatively brief range of 6–7 days (Soundarapandian & Tamizhazhagan 2009), whereas *Scylla olivacea* exhibited an 8-day incubation period (Ikhwanuddin et al. 2015). *Portunus sanguinolentus* displayed a range of 8–11 days (Samuel & Soundarapandian 2009), whereas *Callinectes sapidus* exhibited incubation periods spanning from 10 to 13 days (Walker et al. 2006). On the other end of the spectrum, *Ucides cordatus* reported a longer duration of 19.0 ± 1.0 days (Pinheiro & Hattori 2003). In contrast, the cold-water crab *Chionoecetes opilio* stands out with an exceptionally protracted incubation period ranging from 365 to 410 days (Moriyasu & Lanteigne 1998).

The prehatching egg diameter of *Ucides occidentalis* measured 391.1 ± 22.4 µm, a size comparable to that of other crab species. For instance, *Scylla olivacea* exhibited a prehatching egg diameter of 377.3 ± 11.5 µm (Ikhwanuddin et al. 2015), whereas *Chasmagnathus granulata* ranged from 369 to 386 µm (Gimenez & Anger 2001). In contrast, certain crab species have larger eggs. For instance, the eggs of *Portunus sanguinolentus* were notably larger, measuring approximately 730 µm (Samuel & Soundarapandian 2009). Furthermore, *Ucides cordatus* displayed a prehatching egg size of 526.7 ± 11.1 µm (Pinheiro & Hattori 2003).

The larval development of *Ucides occidentalis*, up to the megalopa larval stage, was accomplished within a 15-day timeframe, which aligns with the duration observed in *Ucides cordatus* (16 days) as reported by da Silva et al. (2012). This developmental period is significantly shorter compared with

certain other crab species, such as *Callinectes sapidus*, where the culture time required to obtain megalopa larvae ranges from 49 to 52 days, as documented by Ospina-Salazar et al. (2023).

To enhance and optimize the culture duration, it is imperative to introduce a variety of food sources with diverse nutritional compositions. In this study, concentrations of 20 rotifers per milliliter beginning at the zoea III stage were used. It was evident that the larvae encountered challenges in capturing this food source. Therefore, it is advisable to add rotifers with reduced activity levels to facilitate more efficient exploitation. It's worth noting that other crab species, such as *Thalamita crenata* and *Panopeus herbstii*, have exhibited a pronounced preference for rotifers, particularly during the initial larval stages (Harvey & Epifanio 1997)

For this preliminary description, artemia was included as a component of the diet starting from the zoea III stage, providing it at a concentration of 0.6 individuals per milliliter. A favorable response to this dietary inclusion was observed, with an increasing trend in consumption observed as the larvae progressed toward the megalopa stage. This pattern of behavior bears similarity to observations made in the crab species *Scylla paramamosain* (Anh et al. 2011). In a distinct context, investigations on *Scylla serrata* have documented an elevated intake of artemia by the larvae before molting (Suprayudi et al. 2002). Likewise, the larvae of *Panopeus herbstii*, during their later larval stages, exhibited a pronounced preference for artemia (Harvey & Epifanio 1997). These findings collectively suggest that Artemia nauplii serves as a crucial nutritional source for the synthesis of essential tissues required for the subsequent developmental stages (Harvey & Epifanio 1997, Baylon et al. 2004).

In the megalopa stage, which occurs around day 15–16 postspawning. Instances of cannibalistic behavior were observed. This behavior was further exacerbated by the emergence of the first pair of chelated pereiopods, leading to a sharp decline in survival rates. This phenomenon aligns with previous observations made in various crab species during the same life phase (Kim & Hwang 1995, Gardner & Maguire 1998, Moksnes et al. 1998, Zmora et al. 2005, Ventura et al. 2008, Romano & Zeng 2017). It is worth noting that even factors such as light intensity can exert an influence on this behavior, as evidenced by studies on *Pseudocarcinus gigas* (Gardner & Maguire 1998). In light of these findings, the implementation of certain strategies to mitigate cannibalism during this critical phase is recommended. These strategies include segregating larval stages through the use of sieves, enhancing turbidity within the culture tank by introducing microalgae, increasing the supply of live artemia, and potentially reducing larval densities.

The duration of the megalopa stage, leading up to the first juvenile crablet molt in *Ucides occidentalis*, was recorded as 15 days. This timeframe is notably consistent with the findings in *U. cordatus*, where the megalopa stage lasted approximately 15.8 days (Simith et al. 2013). In contrast, certain crab species have exhibited more protracted megalopa stages, as observed in *Scylla olivacea*, which ranged from 29.1 to 43.5 days (Jantrarotai et al. 2002). Conversely, some crabs undergo significantly shorter megalopa stages; for instance, *Uca pugnax* achieved the transition from megalopa to the first juvenile crablet in a mere 6 days (O'Connor & Gregg 1998).

In addition to the present early description of *Ucides occidentalis*, this assessment of microalgae diets reveals that, in the case of the red crab, microalgae morphology and size do not appear to be decisive factors governing efficient intake and nutritional utilization. This contrasts with findings in other cultivable marine organisms such as molluscs, fish, and echinoderms, where the influence of these characteristics has been documented (Kaparapu 2018, Hegab et al. 2019, Brown 2002). Notably, even early zoea larvae of crabs exhibit the ability to discriminate between food particles (Perez & Sulkin 2005). Instead, in the case of red crab larvae, the pivotal factor lies in the nutritional value of the microalgae, which significantly impacts growth performance, survival rates, and metamorphosis, particularly in the context of the condition index. Consequently, further research is warranted to explore the temporal intervals between molting events and their correlation with dietary factors, aiming to optimize the molting process and mitigate delays associated with molting issues, as previously reported in both larval and adult crustaceans (Lemos & Weissman 2021).

Utilizing epifluorescent microscopy, an assessment of microalgae ingestion and digestion as a component of the larval diet was conducted. Notably, among the diatoms evaluated, *Chaetoceros gracilis* and *Chaetoceros muelleri* exhibited superior performance, with evidence of ingestion and successful digestion persisting until the zoea V developmental stage. Subsequently, flagellates, specifically *Pavlova lutheri* and *Tisochrysis lutea*, were found to be ingested and digested up to the zoea IV stage, albeit with a gradual decline in their population. Conversely, the microalgae *Tetraselmis maculata* and *Rhodomonas salina* displayed a limited utility as nutrition for red crab larvae, being effectively used only until the zoea III stage, beyond which larval culture experienced elevated mortality rates. Despite exhibiting ingestion and digestion, these microalgae were evidently insufficient in meeting the nutritional demands of the larvae.

Therefore, it is necessary to evaluate other species of microalgae and other microalgal densities in the search to optimize the amount necessary for each specific larval stage. Consequently, there is a compelling imperative to incorporate zooplanktonic diets into the larval regimen to supplement their nutritional prerequisites. It is noteworthy that prior research underscores the crucial role of supplementing culture water with microalgae, not only in enhancing the fatty acid composition of rotifers and artemia, both of which serve as essential components of crab larval diets, but also in ameliorating their efficiency in reducing concentrations of soluble nitrogen and phosphate (Taufik et al. 2016).

The outcomes of this investigation offer valuable insights for the formulation of initial aquaculture methodologies aimed at the cultivation of red mangrove crab *Ucides occidentalis*. Also, these observations indicate that spawning predominantly transpires during the maximum lunar apogee phase. During this critical phase, the utilization of microalgae as both a spawning inducer and a primary nutritional source for the early larval stages is imperative. Furthermore, this research highlights the potential indispensability of employing particulate substrates, such as sand, to facilitate the metamorphic transition from megalopa to the first juvenile crablet stage. Nevertheless, it is worth noting that further research endeavors are warranted to fine-tune and optimize these critical biological processes integral to the cultivation of *U. occidentalis*, encompassing the entire spectrum of its life cycle.

In conclusion, it is imperative to underscore several noteworthy attributes of *Ucides occidentalis* that warrant careful

consideration. Firstly, the species exhibits a remarkable fecundity, with some individuals capable of producing as many as 385,792 eggs per female, as documented by Zambrano and Meiners (2018). Additionally, *U. occidentalis* boasts an extended reproductive season in the Ecuadorian context, spanning from December to May, as corroborated by Avila et al. (2022), Moreno and Solano (2009), and Zambrano (2016). Given these factors, coupled with the substantial market demand for the species, it is imperative to harness this potential as a catalyst for advancing aquaculture practices. By doing so, we can effectively mitigate the strain on natural populations, safeguarding their ecological integrity, while simultaneously diversifying and expanding the aquaculture sector.

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DECLARATION OF CONFLICTS OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

AUTHOR CONTRIBUTIONS

D.R.-P. contributed to the original draft, conceptualization, methodology, writing, and research. J.R. contributed to conceptualization, methodology, and review. N.T. contributed to conceptualization and methodology. S.S. contributed to experimental conception, methodology, revision, and editing of the manuscript. J.N.-W. contributed revision of the manuscript and project administration.

DATA AVAILABILITY DECLARATION

The data that support the findings of this study are available from the corresponding author upon reasonable request.

STATEMENT OF ETHICS

The Ministry of the Environment of Water and Ecuador (MEWE), through authorization 002-18-IC-FAU-DPSE-MA, supervised this investigation. All procedures followed bioethical and responsible research guidelines using *in vivo* animals for experiments following the MEWE and Kilkenny et al. (2010) recommendations.

LITERATURE CITED

Abd Rahman, A. R., Z. C. Cob, Z. Jamari, A. M. Mohamed, T. Toda & O. H. Ross. 2018. The effects of microalgae as live food for *Brachionus plicatilis* (Rotifer) in intensive culture system. *Trop. Life Sci. Res.* 29:127–138.

Alemán, S. & E. Ordinola. 2017. Ampliación de la distribución sur de *Ucides occidentalis* (Decapoda: Ucididae) y *Cardisoma crassum* (Decapoda: Gecarcinidae). *Rev. Peru. biol.* 24:107–110.

Anh, N. T. N., V. N. Ut, M. Wille, N. V. Hoa & P. Sorgeloos. 2011. Effect of different forms of Artemia biomass as a food source on survival, molting and growth rate of mud crab (*Scylla paramamosain*). *Aquac. Nutr.* 17:e549–e558.

Ansari, F. A., A. Guldhe, S. K. Gupta, I. Rawat & F. Bux. 2021. Improving the feasibility of aquaculture feed by using microalgae. *Environ. Sci. Pollut. Res. Int.* 28:43234–43257.

Aranda, D. A., A. Lucas, T. Brulé, M. Andrade, E. Garcia, N. Maginot & M. Le Pennec. 1991. Observations on ingestion and digestion of unicellular algae by *Strombus gigas* larvae (Mollusca, Gastropoda) using epifluorescence microscopy. *Aquaculture.* 92:359–366.

Avila Aguirre, F. A., Yagual Herrera, P. E., & Calles, A. 2022. Análisis histológico de *Ucides occidentalis* en dos estadios de madurez sexual (inicial y maduro) en la zona de Puerto Roma, Golfo de Guayaquil (Doctoral dissertation, ESPOL. FIMCM: Acuicultura).

Barragán, J. 1993. Biología del cangrejo de manglar, *Ucides occidentalis*, ORTMANN, (Crustacea: Decapoda: Gecarcinidae). *Rev. Cienc. Mar Limnol.* 3:135–149.

Baylon, J. C., M. E. A. Bravo & N. C. Maningo. 2004. Ingestion of *Brachionus plicatilis* and *Artemia salina* nauplii by mud crab *Scylla serrata* larvae. *Aquac. Res.* 35:62–70.

Bright, D. B. 1966. The land crabs of Costa Rica. *Rev. Biol. Trop.* 14:183–203.

Brown, M. R., M. Mular, I. Miller, C. Farmer & C. Trenerry. 1999. The vitamin content of microalgae used in aquaculture. *J. Appl. Phycol.* 11:247–255.

Brown, M. R. 2002. Nutritional value and use of microalgae in aquaculture. In: Cruz-Suárez, L. E., Ricque-Marie, D., Tapia-Salazar, M., Gaxiola-Cortés, M. G. & N. Simoes, editors. Advances in aquaculture nutrition VI. Cancún, Quintana Roo, México. pp. 281–292: Proceedings of the VI International Aquaculture Nutrition Symposium. http://hdl.handle.net/102.100.100/199007?index=1.

Castellanos-Galindo, G. A., J. Cantera, N. Valencia, S. Giraldo, E. Peña, L. C. Kluger & M. Wolff. 2017. Modeling trophic flows in the wettest mangroves of the world: the case of Bahía Málaga in the Colombian Pacific coast. *Hydrobiologia.* 803:13–27.

Castilla-Gavilán, M., F. Buzin, B. Cognie, J. Dumay, V. Turpin & P. Decottignies. 2018. Optimising microalgae diets in sea urchin *Paracentrotus lividus* larviculture to promote aquaculture diversification. *Aquaculture.* 490:251–259.

Cuadrado Ortiz, J. E., L. J. Parrales Choez & A. Calles. 2022. Protocolo para desove de hembras ovadas *Ucides occidentalis* en condiciones de laboratorio. Doctoral diss., ESPOL, FIMCM: Acuicultura.

da Silva, U. A., K. Cottens, R. Ventura, W. A. Boeger & A. Ostrensky. 2012. Different pathways in the larval development of the crab *Ucides cordatus* (Decapoda, Ocypodidae) and their relation with high mortality rates by the end of massive larvicultures. *Pesq. Vet. Bras.* 32:284–288.

Dinesh Kumar, S., P. Santhanam, S. Ananth, M. Kaviyarasan, P. Nithya, B. Dhanalakshmi, M. Park & M. K. Kim. 2017. Evaluation of suitability of wastewater-grown microalgae (*Picochlorum maculatum*) and copepod (*Oithona rigida*) as live feed for white leg shrimp *Litopenaeus vannamei* post-larvae. *Aquacult. Int.* 25:393–411.

Duy, N. D. Q., I. Pirozzi & P. C. Southgate. 2015. Ingestion and digestion of live microalgae and microalgae concentrates by sandfish, *Holothuria scabra*, larvae. *Aquaculture.* 448:256–261.

El-Khodary, G. M., H. S. El-Sayed, H. M. Khairy, M. A. El-Sheikh, X. Qi & M. E. Elshobary. 2021. Comparative study on growth, survival and pigmentation of *Solea aegyptiaca* larvae by using four different microalgal species with emphasize on water quality and nutritional value. *Aquacult. Nutr.* 27:615–629.

Factor, J. R. & B. L. Dexter. 1993. Suspension feeding in larval crabs (*Carcinus maenas*). *J. Mar. Biol. Assoc. U. K.* 73:207–211.

García-Guerrero, M. & M. E. Hendrickx. 2006. Embryology of decapod crustaceans III: embryonic development of *Europanopeus canalensis* Abele & Kim, 1989, and *Panopeus chilensis* H. Milne Edwards & Lucas, 1844 (Decapoda, Brachyurar, Panopeidae). *Belg. J. Zool.* 136:249.

Gardner, C. & G. B. Maguire. 1998. Effect of photoperiod and light intensity on survival, development and cannibalism of larvae of the Australian giant crab *Pseudocarcinus gigas* (Lamarck). *Aquaculture.* 165:51–63.

Guarizo, M., T. M. Costa & M. Z. Marochi. 2020. Effect of diet during larval development of *Menippe nodifrons* (Stimpson, 1859) and *Callinectes danae* (Smith, 1869). *Aquacult. Int.* 28:1969–1980.

Guillard, R. R. 1975. Culture of phytoplankton for feeding marine invertebrates. In: Smith, W. L. & M. H. Chanley, editors. Culture of marine invertebrate animals: proceedings—1st conference on culture of marine invertebrate animals greenport. Boston, MA; Springer US. pp. 29–60.

Gimenez, L. & K. Anger. 2001. Relationships among salinity, egg size, embryonic development, and larval biomass in the estuarine crab *Chasmagnathus granulata* (Dana, 1851). *J. Exp. Mar. Biol. Ecol.* 260:241–257.

Harvey, E. A. & C. E. Epifanio. 1997. Prey selection by larvae of the common mud crab *Panopeus herbstii* Milne-Edwards. *J. Exp. Mar. Biol. Ecol.* 217:79–91.

Hegab, M., S. Zaher & H. Ra Mola. 2019. The consumption rate of three zooplankton species of different size fed on the green microalgae *Chlorella vulgaris*. *Egypt. J. Aquat. Biol. Fish.* 23:575–583.

Ikhwanuddin, M., S. S. Lan, N. Abdul Hamid, S. N. Fatihah Zakaria, M. N. Azra, A. Siti Aisah & A. B. Abol-Munafi. 2015. The embryonic development of orange mud crab, *Scylla olivacea* (Herbst, 1796) held in the captivity. *Iran. J. Fish. Sci.* 14:885–895.

Jantrarotai, P., K. Taweechuer & S. Pripanapong. 2002. Salinity levels on survival rate and development of mud crab (*Scylla olivacea*) from zoea to megalopa and from megalopa to crab stage. *Agric. Nat. Resour.* 36:278–284.

Kaparapu, J. 2018. Application of microalgae in aquaculture. *Phykos.* 48:21–26.

Kilkenny, C., Browne, W. J., Cuthill, I. C., Emerson, M., Altman, D. G. 2010. Improving bioscience research reporting: the Arribe guidelines for reporting animal research. *PLoS Biol.* 8:1–5.

Kim, C. H. & S. G. Hwang. 1995. The complete larval development of the mitten crab *Eriocheir sinensis* H. Milne Edwards, 1853 (Decapoda, Brachyura, Grapsidae) reared in the laboratory and a key to the known zoeae of the Varuninae. *Crustaceana.* 68:793–812.

Le Pennec, M. & C. Rangel-Davalos. 1985. Observations en microscopie à épifluorescence de l'ingestion et de la digestion d'algues unicellulaires chez des jeunes larves de *Pecten maximus* (Pectinidae, Bivalvia). *Aquaculture.* 47:39–51.

Lemos, D. & D. Weissman. 2021. Moulting in the grow-out of farmed shrimp: a review. *Rev. Aquacul.* 13:5–17.

Li, J., W. Qiu, H. Hao, F. Chen & K. J. Wang. 2022. Morphology of the complete embryonic and larval development of commercially important mud crab *Scylla paramamosain*. *Aquac. Res.* 53:2298–2316.

Lindquist, E. S., K. W. Krauss, P. T. Green, D. J. O'Dowd, P. M. Sherman & T. J. Smith. 2009. Land crabs as key drivers in tropical coastal forest recruitment. *Biol. Rev. Camb. Philos. Soc.* 84:203–223.

Mendoza Avilés, H. E., K. G. Aranda de la Torre & C. O. Romero Aguirre. 2019. Análisis de los canales de comercialización que inciden en la captura del cangrejo rojo (*Ucides Occidentalis*) de la "Asociación de cangrejeros 6 de julio" del Golfo de Guayaquil. *Rev. Univ. Soc.* 11:93–100.

Moreno, J. & F. Solano. 2009. Cangrejo rojo *Ucides occidentalis* un análisis durante el periodo de veda reproductiva, 2009. *Inst. Nac. Pesca Bol. Cient. Téc.* 20:37–45.

Moreno, J. & W. Ruiz. 2010. Situación actual del guariche *Ucides occidentalis* (Ortmann, 1897) en el estuario del río Chone, Manabí durante abril 2009–2010. *Bol. Cient. Téc.* 20:16–35.

Moksnes, P. O., L. Pihl & J. van Montfrans. 1998. Predation on postlarvae and juveniles of the shore crab *Carcinus maenas*: importance of shelter, size and cannibalism. *Mar. Ecol. Prog. Ser.* 166: 211–225.

Moriyasu, M. & C. Lanteigne. 1998. Embryo development and reproductive cycle in the snow crab, *Chionoecetes opilio* (Crustacea: Majidae), in the southern Gulf of St. Lawrence, Canada. *Can. J. Zool.* 76:2040–2048.

Oesterling, M. J & C. Petrocci. 1995. The crab industry in Venezuela, Ecuador, and Mexico implications for the Chesapeake Bay Blue crab industry. Marine Resource Advisory No. 56; VSG-95-01. Gloucester Point, VA: Virginia Institute of Marine Science, College of William and Mary. http://dx.doi.org/doi:10.21220/m2-dh0k-x313.

Ordinola-Zapata, A., E. Vieyra-Peña, B. Ramírez-Segura & K. Saavedra-Olivos. 2018. Genetic diversity of *Ucides occidentalis* (Ortmann 1897) (Crustacea: Decapoda: Brachiura) based on 16S rRNA gene in Tumbes, Peru. *Sciagropec.* 9:259–267.

Ospina-Salazar, G. H., A. Miranda-Baeza & S. Zea. 2023. Larval development of the blue crab *Callinectes sapidus*: effect of enriched rotifers as live food. *Front. Aquac.* 2:1189325.

O'Connor, N. J. & A. S. Gregg. 1998. Influence of potential habitat cues on duration of the megalopal stage of the fiddler crab *Uca pugnax*. *J. Crustac. Biol.* 18:700–709.

Pacheco-Vega, J. M., M. A. Cadena-Roa, F. Ascencio, C. Rangel-Davalos & M. Rojas-Contreras. 2015. Assessment of endemic microalgae as potential food for *Artemia franciscana* culture. Lat. Am. J. Aquat. Res. 43:23–32.

Perez, M. F. & S. D. Sulkin. 2005. Palatability of autotrophic dinoflagellates to newly hatched larval crabs. *Mar. Biol.* 146:771–780.

Patil, V., K. I. Reitan, G. Knutsen, L. M. Mortensen, T. Källqvist, E. Olsen, G. Vogt & H. R. Gislerød. 2005. Microalgae as source of polyunsaturated fatty acids for aquaculture. *Plant Biol.* 6:57–65.

Pinheiro, M. A. A. & G. Y. Hattori. 2003. Embryology of the mangrove crab *Ucides cordatus* (Brachyura: Ocypodidae). *J. Crustac. Biol.* 23:729–737.

Pontón-Cevallos, J., J. R. M. Jarrín, A. M. Rosado-Moncayo, M. J. Bonifaz, M. del Mar Quiroga, M. E. Espinoza, M. Borbor-Córdova, M. Pozo-Cajas, P. Goethals & L. E. Domínguez-Granda. 2021. Spatio-temporal variability of Brachyura larval assemblages in mangroves of the Gulf of Guayaquil's inner estuary. *Reg. Stud. Mar. Sci.* 41:101601.

Ramos-Veliz, J., O. G. Roche, D. C. Ortega & M. Pozo-Cajas. 2022. Red mangrove crab *Ucides occidentalis* (Ortmann, 1987) (Brachyura: Ocypodidae): complete embryonic development under laboratory conditions. *Lat. Am. J. Aquat. Res.* 50:60–67.

Rivera, C. G., J. E. Barraza-Sandoval, L. F. Castaneda-Romero & E. A. Rodríguez-Urrutia. 2013. Manejo Pesquero Sostenible de *Ucides occidentalis* ("punche"), recurso hidrobiológico de la Cuenca Baja del Río Lempa, Bahía de Jiquilisco, departamento de Usulután. Doctoral diss., Universidad de El Salvador.

Rodrigues, M. D. & N. J. Hebling. 1989. *Ucides cordatus* (Linnaeus, 1763) (Crustacea, Decapoda): complete larval development under laboratory conditions and its systematic position. *Rev. Bras. Zool.* 6:147–166.

Romano, N. & C. Zeng. 2017. Cannibalism of decapod crustaceans and implications for their aquaculture: a review of its prevalence, influencing factors, and mitigating methods. *Rev. Fish. Sci. Aquac.* 25:42–69.

Samuel, N. J. & P. Soundarapandian. 2009. Embryonic development of commercially important portunid crab *Portunus sanguinolentus* (Herbst). *Int. J. Anim. Vet. Adv.* 1:32–38.

Schories, D., A. Barletta Bergan, M. Barletta, U. Krumme, U. Mehlig & V. Rademaker. 2003. The keystone role of leaf-removing crabs in mangrove forests of North Brazil. *Wetl. Ecol. Manag.* 11:243–255.

Schuiteman, M., M. Pozo-Cajas, L. Dominguez-Granda & J. Marin Jarrin. 2019. A description of *Ucides occidentalis* Ortmann, 1897 (Crustacea: Decapoda: Ocypodidae) zoea I. *J. Nat. Hist.* 53:1255–1261.

Shah, M. R., G. A. Lutzu, A. Alam, P. Sarker, M. A. Kabir Chowdhury, A. Parsaeimehr, Y. Liang & M. Daroch. 2018. Microalgae in aquafeeds for a sustainable aquaculture industry. *J. Appl. Phycol.* 30:197–213.

Simith, D. D. J. D. B., F. A. Abrunhosa & K. Diele. 2013. Chemical induction in mangrove crab megalopae, *Ucides cordatus* (Ucididae): do young recruits emit metamorphosis-triggering odours as do conspecific adults? *Est. Coast. Shelf Sci.* 131:264–270.

Sivakumar, N., M. Sundararaman & G. Selvakumar. 2011. Efficacy of micro algae and cyanobacteria as a live feed for juveniles of shrimp *Penaeus monodon*. *Afr. J. Biotechnol.* 10:11594–11599.

Solano, F., L. Flores & W. Ruiz. 2010. Capturas de cangrejo rojo *Ucides occidentalis* en los puertos de la provincia del Guayas y El Oro, Ecuador, durante 2009. *Bol. Cient. Téc.* 20:1–15. http://hdl.handle.net/1834/4793.

Soundarapandian, P. & T. Tamizhazhagan. 2009. Embryonic development of commercially important swimming crab *Portunus pelagicus* (Linnaeus). *Curr. Res. J. Biol. Sci.* 1:106–108.

Suprayudi, M. A., T. Takeuchi, K. Hamasaki & J. Hirokawa. 2002. Effect of Artemia feeding schedule and density on the survival and development of larval mud crab *Scylla serrata*. *Fish. Sci.* 68:1295–1303.

Syafaat, M. N., H. Ma, M. Ikhwanuddin, Gunarto, & Sulaeman, Herlinah. 2019. Effects of different feeding regimes on larvae and crablets of purple mud crab, *Scylla tranquebarica* (Fabricius, 1798). *Aquac. Rep.* 15:100231.

Taufik, M., Z. Bachok, M. N. Azra & M. Ikhwanuddin. 2016. Effects of various microalgae on fatty acid composition and survival rate of the blue swimming crab *Portunus pelagicus* larvae. *Indian J. Geo Mar. Sci.* 45:1512–1521. November 2016

Tytler, P., J. Ireland & L. Murray. 1997. A study of the assimilation of fluorescent pigments of microalgae *Isochrysis galbana* by the early larval stages of turbot and herring. *J. Fish Biol.* 50:999–1009.

Turcihan, G., E. Turgay, R. E. Yardımcı & K. M. Eryalçın. 2021. The effect of feeding with different microalgae on survival, growth, and fatty acid composition of *Artemia franciscana* metanauplii and on predominant bacterial species of the rearing water. *Aquacult. Int.* 29:2223–2241.

Twilley, R. R., M. Pozo, V. H. Garcia, V. H. Rivera-Monroy, R. Zambrano & A. Bodero. 1997. Litter dynamics in riverine mangrove forests in the Guayas River estuary. *Ecuad. Oecol.* 111:109–122. http://dx.doi.org/10.1007/s004420050214.

Vega, Á. J. 2018. Pesca artesanal de *Cardisoma crassum* (cangrejo) y *Ucides occidentalis* (mangote) en el sector nororiental del Golfo de Montijo, Pacífico de Panamá. *Tecnociencia.* 20:5–22.

Ventura, R., U. A. Da Silva, G. Perbiche-Neves, A. Ostrensky, W. A. Boeger & M. R. Pie. 2008. Larval cannibalism rates in the mangrove crab *Ucides cordatus* (Decapoda: Ocypodidae) under laboratory conditions. *Aquac. Res.* 39:263–267.

Walker, A., S. Ando, G. D. Smith & R. F. Lee. 2006. The utilization of lipovitellin during blue crab (*Callinectes sapidus*) embryogenesis. *Comp. Biochem. Physiol. B: Biochem. Mol. Biol.* 143:201–208.

Yang, S., X. Li, Z. Zang, J. Li, A. Wang, Y. Shi, X. Zhang, Z. Gu, X. Zheng & H. E. Vasquez. 2022. Effect of fresh and spray-dried microalgal diets on the growth, digestive enzymatic activity, and gut microbiota of juvenile winged pearl oyster *Pteria penguin*. *Aquac. Rep.* 25:101251.

Yamaguchi, T. 2001. Incubation of eggs and embryonic development of the fiddler crab, *Uca lactea* (Decapoda, Brachyura, Ocypodidae). *Crustac.* 74:449–458.

Zambrano, R. 2016. Período reproductivo de *Ucides occidentalis* en el Golfo de Guayaquil, Ecuador. *Rev. Científica Cienc. Nat. Ambient.* 10:102–106.

Zambrano, R. 2017. First record of malformations in males of *Ucides occidentalis* (Brachyura, Ocypodidae) in the Gulf of Guayaquil, Ecuador. *Crustac.* 90:631–638.

Zambrano, R. & C. Meiners. 2018. Notas sobre taxonomía, biología y pesquería de *Ucides occidentalis* (Brachyura: Ocypodidae) con énfasis en el Golfo de Guayaquil, Ecuador. *Rev. Peru. Biol.* 25:055–066.

Zeng, C. 2007. Induced out-of-season spawning of the mud crab, *Scylla paramamosain* (Estampador) and effects of temperature on embryo development. *Aquac. Res.* 38:1478–1485.

Zmora, O., A. Findiesen, J. Stubblefield, V. Frenkel & Y. Zohar. 2005. Large-scale juvenile production of the blue crab *Callinectes sapidus*. *Aquaculture.* 244:129–139.