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Emergence Rhythms and First Larval Stage Description of the Crab Chiromantes Boulengeri (Calman) from Shatt Al-Arab, Basrah, Iraq

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ABSTRACT: The rhythmic behavior of emergence (locomotion) activity outside the burrows of the crab *Chiromantes boulengeri* (Calman) collected from the intertidal habitat of Shatt Al-Arab River, Basrah, Iraq was studied in the laboratory, with three light regimes (using 10 adult crabs, for 5 consecutive days for each light regime). Under natural illumination (daytime: night-time) the study revealed that the crabs exhibited high locomotor activity (M= 7.1 ind.) during the daytime that was significantly higher than the night-time (M= 4.1 ind.). The result showed no relationship between the locomotor activity of the crabs and the two daily tidal cycles as the two emergence rates during the expected tidal time, the HT & LT (5.6 & 4.6 ind.) were not statistically different from the overall average. When crabs were placed under constant darkness (DD), they showed rhythmic emergence activity during the expected daytime period (3.8 ind.), compared with the activity during the night-time (1.5 ind.), indicating an endogenous agent controlling the circadian rhythmic behavior. Under constant illumination (LL), the crabs showed high emergence activity during both, the expected daytime (6.17 ind.) and expected night-time (6.32 ind.), which shows the control of the exogenous light factor over the locomotion of the crabs. The second aim of this study was to describe the first larval stage of *C. boulengeri* for the first time through the breeding of egg-carrying females. The distinguishing characteristics of the crab from the other species close to it are described.

Keywords: *Chiromantes boulengeri*; Emergence rhythms; Intertidal zone; Shatt Al-Arab River; Basrah.

تناغم الظهور ووصف مرحلة اليرقات الأولى للسرطان *Chiromantes boulengeri* (Calman) من شط العرب، البصرة، العراق

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الملخص: تمت دراسة النشاط الرتيب للظهور (الحركة) خارج الجحور للسرطان النهري *Chiromantes boulengeri* في المختبر ، اذ جمع السرطان من الموائل بين المد والجزر في نهر شط العرب، البصرة، العراق، خلال ثلاثة أنظمة ضوئية (باستخدام 10 سرطانات بالغة لفترة 5 أيام متتالية لكل نظام ضوئي). تحت الإضاءة الطبيعية (النهار: الليل)، كشف تسجيل الفاصل الزمني لمدة ساعتين، ان السرطانات أظهرت نشاطا حركيا عاليا بمعدل يساوي 7.1 فرد خلال النهار. وبفرق معنوي عن الليل بمعدل بلغ 4.1 فرد. لم تظهر النتائج وجود ارتباط بين النشاط الحركي للسرطانات ودورتي المد والجزر اليومية. حيث ان معدلي الخروج اثناء وقتي المد المتوقع والجزرالمتوقع (5.6 و 4.6 فرد) لم يكونا مختلفين معنويا احصائيا. عندما وضعت السرطانات تحت الظلام المستمر (DD)، أظهرت السرطانات نشاط ظهور رتيب خلال فترة النهار المتوقعة مما يشير الى عامل داخلي يتحكم في السلوك الرتيب اليومي، تحت الإضاءة المستمرة (LL) أظهرت السرطانات نشاط ظهور عالي خلال كل من وقتي النهار المتوقع (6.17)، والليل المتوقع (6.32 فرد) مما يدلل على سيطرة عامل الضوء الخارجي على حركة السرطانات. في هذه الدراسة جرى تربية الاناث الحاملة للبيض مختبريا وتفقيس ووصف ورسم الطور اليرقي الأول لأول مرة للسرطان *C. boulengeri*، من خلال تربية الاناث الحاملة للبيض، وحددت الصفات التميزية لها عن بقية الأنواع القريبة منها تصنيفا.

الكلمات المفتاحية: *Chiromantes boulengeri*، رتابة الحركة، منطقة المد والجزر، نهر شط العرب، بصرة.

1. Introduction

The grapsid crab *Chiromantes boulengeri* (Calman) is a permanent species of the macro-benthic invertebrates in the muddy intertidal zone of the estuarine brackish waters of Basrah. The seasonal changes of the abundance of the crab and population production were studied in Shatt Al-Arab River during the periods 1977- 1978 and 1985-1986 [1, 2]. It is well known that the intertidal zone is a rhythmically changing environment which is subjected to a daily fluctuation of the water levels, normally with two high water phases and two low water phases. What is remarkable in a habitat in which the environmental conditions change so dramatically with the ebb and flow of tide is that the rate of many physiological processes continue fluctuating in approximate synchrony with the tide when the organisms were removed to non-tidal constant conditions in the laboratory [3, 4]. Many studies on the importance of the role of burrows for crabs and the other macro-benthic crustaceans in various habitats such as the intertidal zones, mudflats, salt marshes, mangrove, confirm the existence of two main functions: the first is to provide refuges from the sub-tidal predators during the immersion period and the shorebirds during exposure. The second is to mitigate the impact of extreme temperatures and desiccation [5, 6]. In addition, studies on the emergency rhythms of various marine and estuarine Crustacea [7, 4] indicate that they are partly under endogenous control, in which the animals possess a biological clock synchronizing their emergence activity with the natural regime of light and darkness (light intensity) component and/or with the tidal component.

Iraq, especially Basrah including the delta of Shatt Al-Arab River, faces serious challenges and risks due to global climate changes, which will lead to a severe lack of fresh water flow from the two main rivers, Tigris and Euphrates, resulting in a rise of sea salt tide upstream and increased temperature changes. Moreover, many of human activities harmful to the environment occur daily, all of which have led to noticeable effects on the biodiversity of the region [8, 9].

According to previous studies [1, 2], grapsid crabs were found in high densities along the Shatt Al-Arab River and the many small creeks that empty into it. The density of the crabs ranged from 33 to 330 crabs/m^2. Their numbers declined sharply during the period 2012-2016, where the density was estimated to be less than 1/ m^2 [10].

The rhythmic behavior provides a number of ecological advantages: The regulation of locomotory activity can reduce energy in search and food collection as well as avoiding predation [11, 12]. On the other hand, it is noted that the timing of hatching and the release of larvae, whether in the river or at sea, usually corresponds to the lunar and day-night cycles [13, 14].

Our study had two aims, the first was to investigate the relationship between the locomotor activities of the crabs and both the daily cycles (i.e., the dial rhythm), and the semi-daily cycles of the tides. The second aim was to obtain the larvae of the first stage of *C. boulengeri* that has never been described and classified anywhere, from laboratory hatching and releasing larvae.

2. Materials and methods

Adult specimens of the crabs *C. boulengeri* were collected by hand during April-July 2019 from the intertidal zone of Shatt Al-Arab River, Al-Salihea site (N: 30˚50.896'; E: 47˚85.736') during the low tide times. For emergence rhythmic behavior experiments, numbers of male and female crabs were caught and transferred to the laboratory tanks. Ovigerous females were caught more carefully to avoid any damage to the eggs that they carried in their abdomens. Two samples, unpaired t-test (SPSS program) were used to determine the difference between means.

Thirty adult crabs (males and females), 10 crabs for each one light regime experiment were placed in plastic tanks of a size 90 x 30 x 30 cm. In each aquarium, a small amount of sterilized river water was maintained in one half and in the other half a thick layer of mud collected from the intertidal area, in a way that formed a gradual increase of mud height from the water to the end of the aquarium similar to what is there on the river bank. The emergence activity of *C. boulengeri* were recorded under different light regimes in laboratory: natural daily light regime, in complete darkness and with continuous bright light [15, 11]. Round the clock visual observations were made on the crabs in the aquariums counting the number of animals out of side the burrows at 2 hours intervals for five consecutive days. The times of sun rises, sun sets and that of high tides and low tides, throughout the periods of the experiments were taken from the local meteorological (https://tides4fishing.com/iq/iraq/) and the tidal table of the general port company of Iraq.

2.1 Larval release experiment

Three gravid females were collected from Shatt Al-Arab River, during June 2019. Each was reared singly in a glass cylinder filled with sterilized river water of 2ppt. salinity and supplied with constant aeration under natural daily light/night cycle for 1-2 weeks, at a temperature ranging from 27-29 C°, until the larval hatching occurred. The females were fed on small pieces of fresh water fish during the period of the experiments. After hatching first stage, crab Zoeae were fixed with alcohol for microscopic examinations and taxonomic drawing with camera Lucida.

3. Results and Discussion

3.1 Circadian rhythmicity

The results of 5 consecutive days with a normal light-dark cycle are shown in Figure1. The graphs of

counted numbers of crabs outside their burrows, each two hours related to local times and the supposed tidal phase periods, indicate clear high locomotory (emergence) activity during the daytime. The statistical analysis (unpaired t-test, SPSS program, Table 1) shows higher and significance mean number of crabs number (7.1 ind.) during daytime compared with the mean number during the nighttime (4.1 ind.; t: 4.957 P<0.05). On the other hand, no evidence was found on the relationship between the emergence activity and the tidal components, where the difference between the means of emergence crabs during the expected high tide (5.6 ind.) and the expected low tide (4.6 ind.) was not statistically significant, (t: 1.5 P>0.05).

*Upward arrows: time of expected high tide and. Downward arrows: time of expected low tide.

Table 1. Rhythmic activities of *Chiromantes boulengeri* outside the burrows in the daytime "expected" daytime, night time "expected" night time and "expected" high and low tides in different light regimes and the results of "t" test, (n. s. not significant).

Light regime	Mean no. of crabs out of burrows	X	T	P
Natural light-dark cycle	In daytime	7.1		
	In night time	4.4	7.03	0.001
	In "expected" high tide	5.6		
	In "expected" low tide	4.6	1.5	n. s.
Complete darkness	In "expected" daytime	3.8	4.56	0.001
	In "expected" night time	1.52		
Continuous bright light	In " expected" daytime	6.17	0.417	n. s.
	In "expected" night time	6.32		

3.1a Endogenous rhythmic behavior

In order to determine whether the light/dark emergence rhythm is controlled endogenously, another experiment was conducted on the crab activities at a condition of continuous darkness and the results are summarized and presented in 24 h period (Figure 2 b). The emergence activity was significantly higher during the period of expected daytime (3.8 ind.) than the mean number during the expected period of night time (1.53 ind.), (t: -5.578, P<0.05; table 1).

The results of testing whether emergence activity is exogenously stimulated by light, the crabs were subjected to continuous illumination for 5 consecutive days, is shown in Figure 2 c. The t-test statistics revealed no significant difference between the means of numbers during the expected daytime activities and the nighttime activities (6.17 ind. and 6.32 ind. respectively; t: 0.417, P >0.05).

However, the activities of the crab's emergence during the expected high tide of nighttime (4.07 ind.) and the expected low tide of nighttime (5.2 ind.) was not statistically different (t: 1.586, P>0.05) whereas, there is significant difference between the activity during periods of expected daytime's high tide (8.12 ind.) and the expected daytime's low tide (5.6 ind.), (t: 3.394, P<0.05).

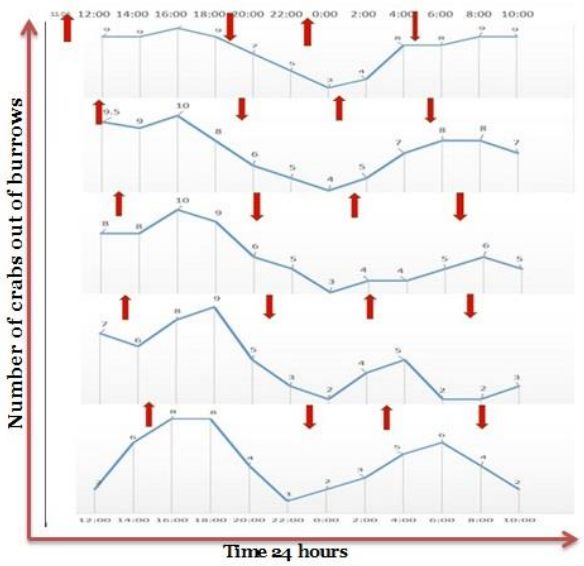

Figure 1. Emergence activity of the crab *Chiromantes boulengeri*, no. of crabs counted every 2 hrs. out of their burrows for 5 consecutive days for 10 crabs in natural day/night cycle.
*Black bars: the dark period from sunset to sunrise was from 19:00 o'clock to 06:00 o'clock.
*Upward arrows: time of expected high tide and. Downward arrows: time of expected low tide.

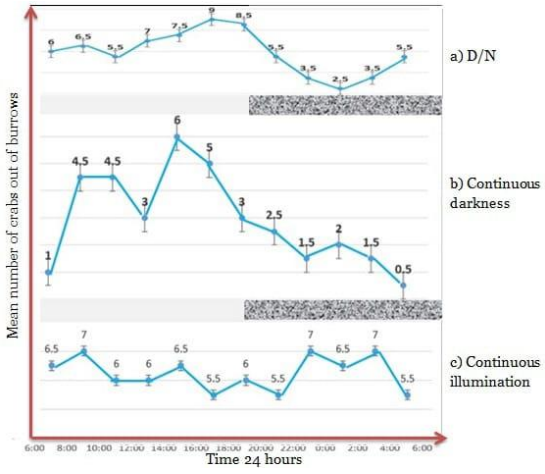

Figure 2. Emergence activity of the crab *Chiromantes boulengeri*, data of 5 consecutive days observations in a variety of lighting regimes summarized in 24 hr. estimates giving means and standard deviations.
a. Natural day light cycle, b. continuous darkness, c. continuous bright light

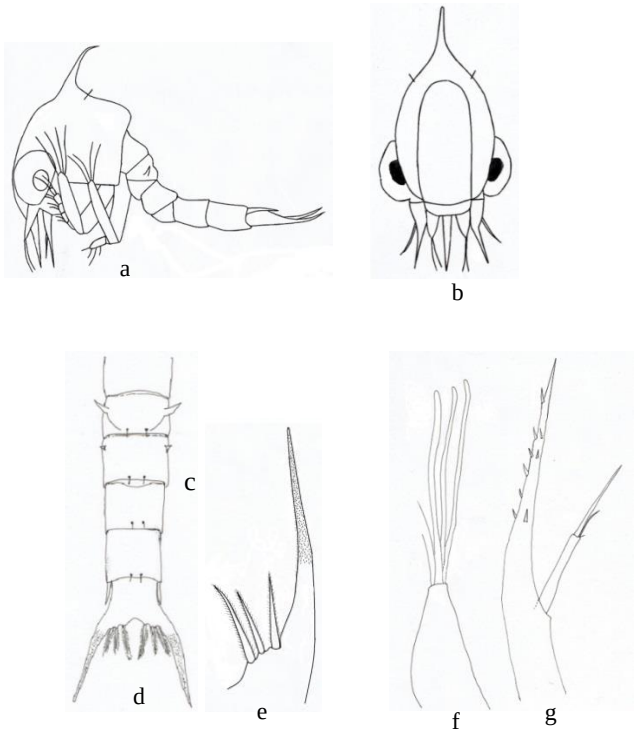

Figure 3. *Chiromantes boulengeri*, first zoea (a) lateral view of zoea; (b) vertical view of carapace; (c) abdomen and telson; (d) abdomen; (e) telson; (f) antennule; (g) antenna.

3.1b The timing of hatching and the release of larvae

The results of the experiments of hatching eggs of the gravid females shows that in all cases hatching and the release of the larvae into the water of rearing aquariums occurs during the night-time.

3.2 First larva of *Chiromantes boulengeri*

3.2.1 Description

Dimensions: total length 0.99 - 1.19 mm, (10 specimens),
Carapace length 0.40- 0.45 mm; Length of dorsal spine 0.13-0.18 mm; Length of rostral spine 0.16-0.22 mm;Tip of rostral spine to tip of dorsal spine 0.67-0.74 mm. Carapace (Figures. 3a,b) with dorsal spine well developed, curved backward and unarmed. Rostral spine about same length of dorsal spine. Two-minute setules at the base of dorsal spine. Postro-lateral margin of carapace smooth. Eyes sessile. Abdomen **(**Figure 3c) with five somites: somite one unarmed, somite 2 with two prominent dorso-lateral projections forwardly directed, somites two-five each with a pair of dorsal setules near posterior margin, somite 3 with three small dorso-lateral projections, somites 3 and 4 with slightly produced postero-lateral margins, somite 5 with a pair of long postero-lateral spines. Telson (Figures 3c,d) furca with two prongs, each covered with minute spinules; three inner spines at base of each prong middle shortest, innermost longest.

Antennule: (Figure 3e) conical with one stout and two slender aesthetics plus two fine setules.

Antenna (Figure 3f) Endopod absent, Exopod about 1/3 length of spine form process; surface unarmed, ending with one long and one short seta plus 1 short hair. spiniform process with coarse spines on surface.

Maxillae (Figure 3a) coxal endite with six setae. Basial endite with five processes (spines and setae). Endopod 2-segmented; proximal segment with one seta, distal one with one sub terminal and 4 terminal setae (in 2 groups of 2 each).

Maxilla (Figure 3b) proximal and distal lobes of coxal endite with five and two setae respectively. Proximal and distal lobes of basial endites with five and four setae, respectively. Endopod clearly divided, proximal and distal lobes, with two setae (one terminal and one subterminal) and three setae, respectively.

Scaphograthite with four plumose marginal and setae and a posterior plumose process. 1st Maxilliped (Figure 3c) basis with ten median setae arranged 2, 2, 3, 3. Endopod 5 segmented with setal formula 2, 2, 1, 2, 5; inner margin of 3rd segment with a minute hair.

Exopod partially segmented; distal segment with 4 natatory setae. 2nd maxilliped (Figure 3d) basis with 4 median setae. Endopod 3 segmented with setal formula 0, 1, 6. Exopod partially segmented; distal segment with 4 natatory setae.

Remarks

According to [16], it is clear that this larva is a grapsoid zoea because of the endopod maxillule with five setae, carapace without lateral spines, Antennal exopod< 1/3 spinous process. Basal segment of endopod of maxillule with one seta, endopod of Maxilla with four or five setae, proximal segment of endopod of 1st maxilliped with one or two median setae, endopod of maxilla with 5 or less setae, scaphosuathte with four setae.

The results of this experimental study show a positive relationship between the number of crabs outside their burrows and the time of day, as the number of emerged crabs increased during the expected daytime and decreased during the expected nighttime. During complete continuous darkness, the number of crabs were significantly higher during the expected period of daytime than the numbers at the expected period of nighttime. This pattern indicates an endogenous agent synchronized the circadian rhythm of the crab's emergence activity. However, the results of continuous light experiment proved that light is the exogenous factor induced the emergence activity of these crabs.

On the other hand, the results showed no evidence of rhythmic semi-diurnal tidal components to influence the crab's emergence behavior.

The circadian and tidal rhythms are important biological components synchronizing the behavior of intertidal animals, which are induced by exogenous agents and may persist in non-natural conditions by endogenous agents [17, 18, 19].

Some species show only a rhythm of circadian periodicity such as *C. boulengeri* of the present study whose peak of activity synchronized with daytime, while other species have, in addition to the 24 h rhythmic components, a lunar day rhythmic component, with both working simultaneously [20]. Unlike *C. boulengeri*, the crab *Sesarma reticulatum* has an emergence behavior synchronized with the tidal cycle. This difference is attributed to the difference in marine locations i.e., between the places of strong tidal components and those of weak tidal components [21, 22]. In

this regard, the estuarine environment of the Shatt Al-Arab, which is inhabited by the crab *C. boulengeri* is exposed to relatively weak tidal amplitude of 1.5 m, at a time when large quantities of fresh water were received especially of the two great rivers of Iraq, Tigers and Euphrates [23]. Apparently, this is the reason for the lack of the effects of tidal components, in contrast to the strength of the tidal components on the coastal environment of the open seas.

However, the existence of 24h or circadian rhythmic behavior controlled by endogenous agents, as in the case of *C. boulengeri* and as reported by other authors [24, 25, 26, 6] is an ecological adaptation of burrowing crustaceans which regulate their emergence from burrows to forage on the bottom during the appropriate period.

This adaptation is also beneficial for burrowing species such as *C. boulengeri*, as they have an endogenous regulator for their rhythmic behavior which enables them to interact with their habitat and its daily environmental fluctuations in a predictable way. On the other hand, it seems that the reduced activity of the emergence out of their borrow during the night is a strategic behavior of the crab *C. boulengeri* to avoid sub-tidal predators during the high tide period which invade the intertidal area and the land predators during the low tide period. These interactions between predation and rhythmicity have been documented in other macro-invertebrates [5, 27].

Also, from similar studies of some other related species of crabs in other regions, a broader knowledge can be concluded about the importance of the rhythmic behavior on the life strategy of *C. boulengeri* in the Shatt Al-Arab Estuary habitat. For example, [11, 12] and others agreed that the programmed rhythmic regulation of various crab's activities, such as the search for food and avoiding predators, are very useful in reducing the energy expenses necessary to sustain life.

On the other hand, other research reports that the crabs in the estuarine habitat, as in the case of *C. boulengeri* regulate the timing of the release of their planktonic larvae to the night, to avoid predation in the most vulnerable stages of life of the animal population [13, 14]. It is worth considering that these studies are related to the release of larvae during the nighttime, but at the same time it is confirmed that this is usually corresponding to the spring tidal cycle that occurs with the state of the new moon or the full moon. However, many other species of crabs exhibit other rhythmic synchrony.

Recent studies shed light on the existence of a close relationship between the climate changes and the biomechanics, behavioral activity and predator-prey interactions in the marine macro-benthos [28] and from this view point, we suggest that the considerable decline of the crab's density and their reduced abundance are attributed to the impact of climate changes on the crab's behavior. Therefore, more studies are recommended for understanding other biological activities of *C. boulengeri*, especially the mechanism and rhythmic behavior of larval release in the planktonic habitat and their retention to the intertidal habitat [29].

C. boulengeri is a brachyuran crab belonging to the Superfamily Grapsoidea, Family Sesarmidae. The species previously was included in the genus *Sesarma* Calman, and described as a new species from Basrah, Iraq (C. boulengeri) in 1920 and recently was removed from the genus *Sesarma* to *Chiromantes* as *C. boulengeri* from Iran [30]. It was reported that from about 230 species in the Family Sesarmidae, larval descriptions of only 54 species were published [31] including three species of the genus *Chiromantes*; *C. eulimene, C. haematocheir* and *C. dehanni*. However, there are no larval stage descriptions of *C. boulengeri*. Therefore, the new description of zoea I in the present article can be a very useful tool to the identification of zoea stages of this species in the planktonic habitats of Shatt Al-Arab River.

4. Conclusion

This laboratory experimental study concludes that the crab *C. boulengeri* has an endogenous rhythm of emergence and activity out of burrows with approximate circadian periodicity, with no significant effect of tidal rhythmic component. The study also showed that the first larval stage of *C. boulengeri*, which were hatched in laboratory for the first time and its morphometric characteristics were diagnosed, were released into water by ovigerous females during night periods, in order to avoid predation.

Conflict of interest

The authors declare no conflict of interest.

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