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Reproductive Cycle and Fecundity in Natural Population of Edible Freshwater Crab, *Oziothelphusa senex senex* (Fabricius, 1798) (Decapoda: Brachyura)

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Abstract

Rapid growth rate, high meat content, excellent palatability and resistance to white spot virus favored the culture of crab species in aquaculture industry. This study examined the natural reproductive cycle of edible fresh water crab *Oziothelphusa senex senex* by monthly measurement of ovarian index and histological examination of the gonads of the animals and determination of berried and young-one-bearing females collected monthly. The number of berried and young-one-bearing females was significantly higher in September-October. However, a small number of ovigerous and young-one bearing females were observed throughout the year. We also observed a breeding peak in September-October using ovarian index as marker. Within study catchments, mean number of eggs and young ones was 130 and 120 during September and 132 and 118 in October. The number of eggs spawned in the smallest (17 g body weight) crab is 80 and the largest (44 g body weight) crab is 140. We also observed a positive correlation between the numbers of eggs spawned and body weight. Surprisingly, no correlation was found between the breeding cycle and the environmental factors such as temperature, photoperiod and rain fall. The lesser dependence on climatological factors for the completion of reproduction in the crab indicates greater potential of this species for crab fishery.

Keywords: *Oziothelphusa senex senex*; Gonad index; Histological examination; Reproductive cycle; Fecundity

Introduction

Oziothelphusa senex senex is a freshwater edible crab normally inhabiting in rice fields and irrigation canals throughout South India. Although originally restricted to freshwater, they can also survive in 100% seawater. The outbreak of white spot disease in South India and susceptibility of prawns to virus infection resulted in search for alternate species for aquaculture. In view of resistance to white spot virus, together with a rapid growth rate, high meat content and excellent palatability, the crab species was preferred for culture in recent years. Research on *O. senex* has concentrated on aspects pertaining to changes in physiology during thermal and salinity adaptation [1,2]. Aspects of endocrine control of glucose, nitrogen metabolism were also studied [3,4]. Besides, the crab was used as a tool to monitor environmental contamination [5-7]. Few aspects that have been left unexamined for this crab are its potential for aquaculture, development of artificial feed, and potential fecundity of the crab. The above information is very essential to attempt culture of this species.

Historically, studies involving reproductive biology of crustaceans have relied on description of ovarian development such as colour and weight of ovary [8,9]. Histological examination of ovaries was considered by several workers to determine the breeding cycles in several crustaceans [10]. Recording of egg-bearing females was used as index to determine the reproductive cycle of several organisms [11-13]. Recently, determination of vitellogenin levels in circulation and in ovary was used as a sensitive tool to determine the reproductive stage in several crustaceans [14,15] including the crab *Oziothelphusa* [16]. Some aspects of the reproductive biology of *O. senex* (for example size at sexual maturity, colour and size of the ovary) have previously been examined [17]. Induction of ovarian maturation in this crab by leucine-enkephalin and hydroxyprogesterone was also reported [16,18,19]. Reddy et al. [20] also reported the role of prostaglandins in inducing ovarian growth in this crab. Very little is known about the natural recruitment of crabs entering in to reproduction during different months. The aim of this study was to describe the reproductive biology of the crab, *Oziothelphusa senex senex*. Changes in gonad index and gonad histology were also monitored on a circannual basis.

Material and Methods

Adult *Oziothelphusa senex senex* were collected from rice fields and irrigation canals around Tirupati. Animals were collected every month during the years of 2011, 2012 and 2013. Only mature female crabs were selected for sampling. Number of egg bearing and young one bearing females were noted. Number and weight of eggs and young ones were noted from these crabs. Without allowing the remaining animals to acclimatize to laboratory conditions, their somatic weights were noted. The ovaries from 30 crabs per month were isolated, blotted on a filter paper and weighed to the nearest mg in an electronic balance. The ovarian index was calculated using the equation:

$$\text{Ovarian index (OI)} = W_1/W_2 \times 100$$

where W_1 is wet weight of the ovary and W_2 is total wet weight of the crab.

Ovaries were assigned, on the basis of their macroscopic appearance, to one of the following five stages: 1) Immature; 2)

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developing (vitellogenic stage I); 3) mature (vitellogenic stage II); 4) ripe (vitellogenic stage III); and 5) spent. Ovaries from all females, were placed in Bouin's fixative (picric acid:formaldehyde:acetic acid, 75:25:5). After 24 h, they were washed in water and dehydrated with ascending alcohol series. Dehydrated ovaries were embedded in paraffin wax after clearing in xylene, sectioned transversely at 6 µm and stained with haematoxylin and counter stained with eosin. The maximum and minimum diameters of up to 50 randomly sectioned oocytes of each sectioned ovary were measured through the nucleus and the mean diameter was calculated. The proportions of oocytes at different stages of development in different months were also determined.

Statistical analysis

Statistical analysis was performed using SPSS 16.0 version. All statistical tests are two-tailed and probability levels <0.05 was considered significant. Values are presented as mean ± standard deviation.

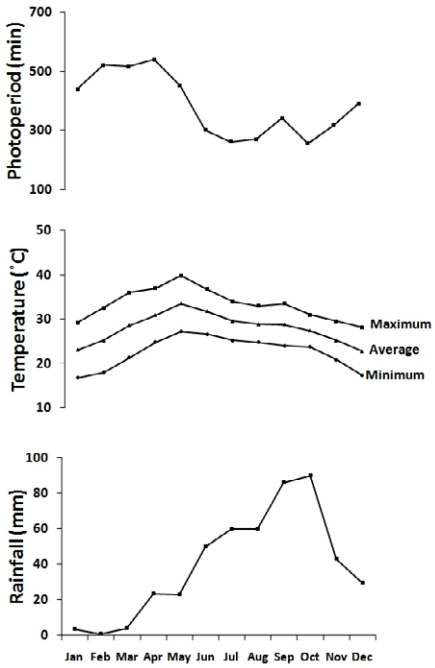

Figure 1: Recordings of monthly mean maxima and minima of temperature, photoperiod and rain fall in Tirupati (2011-2013).

Results

Environmental variables of animal collection area

Day lengths for the region declined progressively from maximum of 527 minutes in April to 251 minutes in October (Figure 1A). The temperature in the animal collection site varied between 25-40°C. In summer months (April-June) the temperature was high (>35°C) when compared to other months of the year (<32.5°C) in three years studied (Figure 1B). The rain fall for the study area also showed a seasonal pattern typical of monsoon pattern (i.e., high monsoon and low non-monsoon rainfall) (Figure 1C).

Breeding periodicity

The numerical incidence of egg-bearing and young-one-bearing crabs exhibited one breeding peak (Table 1). Maximum number of ovigerous and young-one-bearing animals was found (22% and 20% respectively) in September. Berried and young-one bearing females were also present in other months of the year but less in number. The mean ovarian index of crabs ranges from 0.18 to 1.97 with a maximum value falling in the month of September (Figure 2). Since a small population of animals in vitellogenic stage was found year-round indicating the crabs can reproduce under any natural environmental conditions.

Histological and macroscopic gonad descriptions

The macroscopic appearance of female gonads at various developmental stages (Table 2) and their histological characteristics (Table 3) are summarized. The ovaries of crabs collected in September exhibited a typical breeding color in this month of the year. In January, February and March the ovaries are of creamy hue. In April to June the color is pale brown to light orange and in July to October, the color is bright orange. In the remaining months the ovaries appear white in color.

A close association was observed between the macroscopic appearance of ovaries at the various developmental stages and their histological characteristics. Ovaries that were in early developmental stages had greatest proportion of perinuclear oocytes with a size range of between 30-90 µm and a mean oocyte diameter was 53 µm; however similar size oocytes were also present in all other stages with different proportions (Figure 3A). Developing (early yolk vesicle) oocytes (90-

Month	% of Egg bearing crabs (n=300)	% of Young one bearing crabs (n=300)	Number of eggs/ crab	Weight of egg (mg) (n=124)	Egg diameter (mm) (n=124)	Number of young ones/crab	Weight of young ones (mg) (n=106)
Jan	--	1	--	--	--	95	11 ± 0.12
Feb	2	2	106	8.5 ± 0.15	1.95 ± 0.05	98	11 ± 0.23
Mar	5	4	110	10 ± 0.12	1.95 ± 0.05	102	11 ± 0.21
Apr	6	9	107	10 ± 0.14	1.97 ± 0.04	97	12 ± 0.24
May	9	7	105	9 ± 0.16	1.97 ± 0.06	96	12 ± 0.18
June	4	7	110	9 ± 0.20	2.01 ± 0.04	108	12 ± 0.19
July	5	4	106	9.5 ± 0.16	1.98 ± 0.06	100	11 ± 0.21
Aug	5	4	99	10 ± 0.21	2.12 ± 0.06	108	12 ± 0.22
Sept	22	20	130	10 ± 0.22	2.21 ± 0.06	120	13 ± 0.21
Oct	18	19	132	11 ± 0.19	2.16 ± 0.05	118	13 ± 0.18
Nov	10	8	122	10 ± 0.18	2.11 ± 0.05	120	12 ± 0.19
Dec	1	4	120	10 ± 0.22	2.02 ± 0.06	116	12 ± 0.14

Table 1: Monthly analysis of brood size and morphometric observations relation to egg bearing and young-one bearing female crabs.

Ovarian stage	Macroscopic description
I – Immature/recovering (previtellogenic stage)	Ovaries are thin strand-like and translucent to opaque white; recovering spent ovaries in few crabs.
II – Developing (vitellogenic stage I)	Ovaries are thickened and dark yellow; oocytes are arranged compactly and are not clearly visible within the ovary.
III – Mature (vitellogenic stage II)	Ovaries further thickened and orange in colour; oocytes are clearly visible in the ovary.
IV – Ripe (vitellogenic stage III)	Ovaries swollen and large; dark brown or bright orange; oocytes are clearly visible in the ovary.
V – Spent ovary	Ovaries are slightly thick and white in colour; few unspent dark orange or dark brown oocytes are also present in some crabs.

Table 2: The macroscopic description of the different stages of ovarian development in the crab *O. senex sene*.

Ovarian stage	Histological description	Oocyte diameter*(µm)
I – Immature/recovering (previtellogenic stage)	Thick ovarian wall with centrally located germanium surrounded by number of oocytes; cells are spherical or oval in shape and arranged at the periphery of oocytes; ovary consists of primary and secondary oocytes; follicle cells are surrounded by bigger oocytes	30-90 (53 ± 9.2)
II – Developing (vitellogenic stage I)	Oocytes consist of a band of yolk globules located peripherally with a prominent nucleus and nucleolus; oocytes are enveloped by follicle cells.	90-120 (110 ± 8.8)
III – Mature (vitellogenic stage II)	Peripherally located yolk globules migrate towards the centre of oocytes.	120-170 (162 ± 11.1)
IV – Ripe (vitellogenic stage III)	Large accumulation of yolk globules occupying the entire oocyte; disappearance of nucleus and nucleolus; fine inter dispersed protein globules in between the large lipid yolk globules; appearance of disintegrated follicle cells around oocytes.	170-210 (198 ± 8.9)
V – Spent ovary	Ovary shows several post-ovulatory follicles along with unextruted ova. Atretic oocytes also present.	50-100 (71 ± 12)

Values in the parentheses are mean ± S.D. *n=12 crabs (360 oocytes)

Table 3: Histological description and diameters of the oocytes of the different stages of ovarian development in the crab *O. senex senex*.

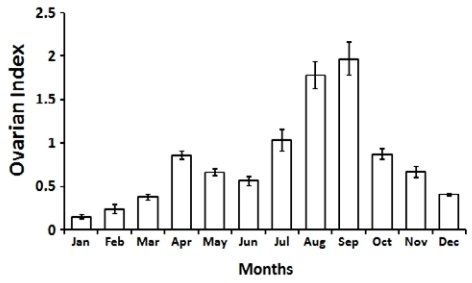

Figure 2: Monthly analysis of ovarian index of *O. senex senex*. Values are mean (± S.D) of 30 crabs.

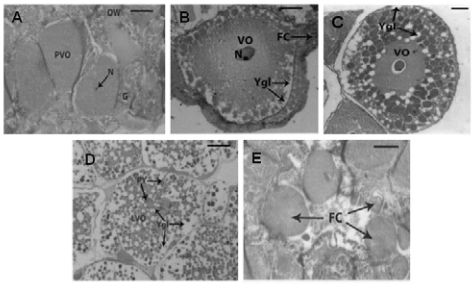

Figure 3: Microscopic appearance of the different gonadal developmental satges of female *O. senex senex*. A: Immature; B: Vitellogenesis Stage I (developing); C: Vitellogenesis Stage II (mature); D: Vitellogenesis Stage III (ripe); E: Spent. N: Nucleus; FC: Follicle Cell; PVO: Previtellogenic Oocyte; VO: Vitellogenic Oocyte; LVO: Late Vitellogenic Oocyte; G: Germanium; OW: Ovarian Wall; YGL: Yolk Globule; PY: Protein Yolk.

120 µm) represented the greatest proportion of oocytes in stage II gonads, with a mean diameter of 110 µm (Figure 3B). The oocytes were filled with protein yolk located peripherally with a thick ovarian wall and a prominent nucleus. Mature (late vitellogenic) oocytes had a size range of 120-170 µm with a mean diameter of 162 µm (Figure 3C). At this stage, the peripherally located yolk globules tend to migrate towards centre, gradually replacing the protein yolk. Ripe oocytes had a size range of 170-210 µm with a mean of 198 µm. The cytoplasm of oocytes contains large accumulation of yolk globules occupying the whole oocyte (Figure 3D). Spent ovaries contained several degenerative oocytes, few late vitellogenic oocytes and many active follicle cells preparing the germanium for the next cycle of oogenesis (Table 3 and Figure 3E). The majority of spent ovaries were in individuals that were berried with eggs ranging in diameter from 50 to 100 µm (S.D ± 12 µm).

Temporal descriptions of gonad development

The mean oocyte diameter of different gonad stages of female *Oziothelphusa senex senex* was shown in Figure 4. The monthly proportions of different size oocytes in ovary of *Oziothelphusa senex senex* were presented in Figure 5. In the month of January, February and March the oocytes were in early development stages. The absence of vitellogenic oocytes is a characteristic feature in these months. The proportion of females with stage I ovaries was greatest in January with 90%. In the months of April and May the oocyte diameter increases slightly and the yolk globules started accumulating at the periphery of the oocytes. Very few late vitellogenic oocytes were seen in these months. Larger oocytes were observed in the months of June and July. The oocytes contain large accumulation of yolk globules occupying the whole of oocyte. Ripe gonads (stage IV) were dominant in September, with 64% of females exhibiting this stage. The ovarian index in this month is at peak (Figure 2). In the months of October to December most of the ovaries were in spent condition. Several degenerate oocytes and few late vitellogenic oocytes were observed in these months. Appearance of vacuole in the ooplasm is a characteristic feature of degenerating or resorbing oocytes in the crabs in these months. This is in agreement with the decreased ovarian index during these months (Figure 2). Since a small population of animals in vitellogenic stage (I

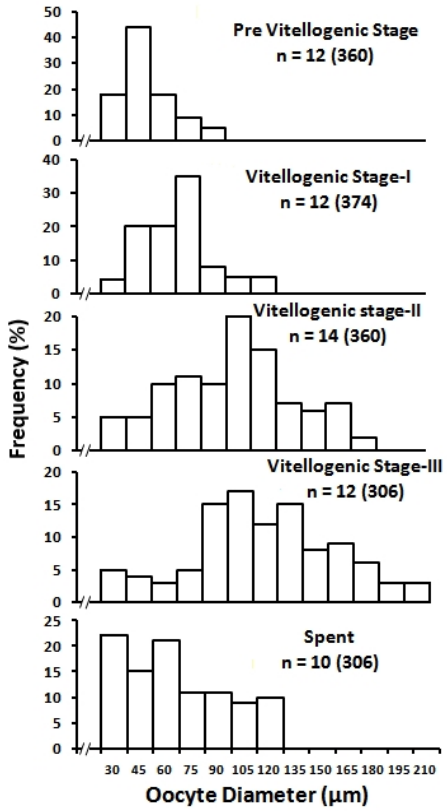

Figure 4: Mean oocyte diameters of the different gonad stages of female *O. senex senex*. n=Number of crabs (number of oocytes measured is in parentheses).

to III) was found year-round indicating the crabs can reproduce under any natural environmental conditions throughout the year.

Furthermore, monthly proportions of different ovarian stages mirrored the monthly proportions of egg-bearing and young-one-bearing females. The percentage of mature females that were berried (egg bearing) increased from 5% in August to 22%, 18%, 10% in September, October and November respectively (Table 2).

Relationship between body weight and fecundity

The effective fecundity of female *Oziothelphusa senex senex* was represented by number of eggs in brood and ranged from 80 to 146 (Figure 6). The number of eggs spawned in the smallest crab (17 g body weight) is 80 and the largest crab (44 g body weight) is 146 (Figure 6). There was a positive correlation ($F_{1,53}$=3.791; p<0.001) between effective fecundity and body weight of *O. senex*. The relationship between the effective fecundity and body weight of *O. senex* was best described by the equation: EF=56.43+1.80 BW (r=0.926), where EF, effective fecundity; BW, body weight.

Discussion

Seasonal breeding cycle

The reproductive cycle, usually, includes a series of morphological, physiological, histological and metabolic events like gametogenesis, spawning, growth and development of egg [21]. These events are very useful to determine the scope and potentiality of the species for commercial exploitation. In decapods crustaceans, different reproductive end points were used to determine the reproductive stage of the organism. Recently, we have demonstrated that hepatopancreas but not the ovary is the site of vitellogenesis in female *Oziothelphusa senex senex* [22]. Further, we have reported that retinoid X receptor, ecdysteroid receptor and ecdysteroid responsive gene (E75) mRNA levels vary significantly during different vitellogenic stages in the crab, *Oziothelphusa senex senex* [23]. In the present study, ovarian maturation has been assessed using ovarian index, oocyte diameter,

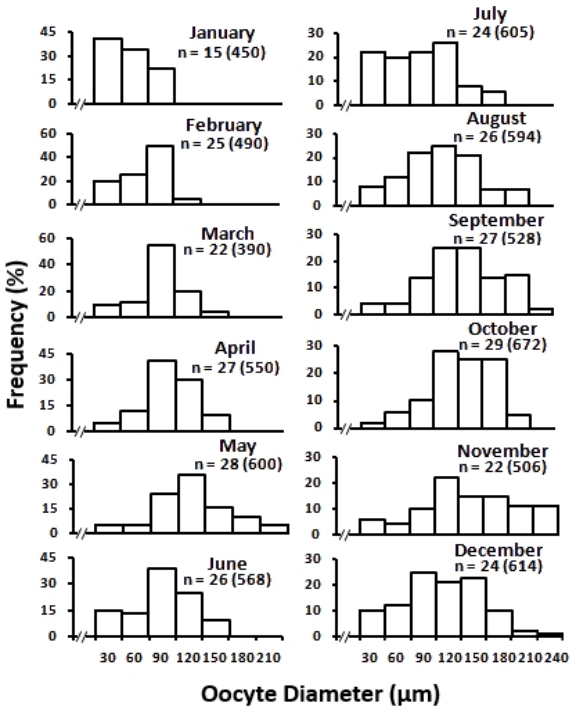

Figure 5: Monthly distributions of the diameters of oocytes in ovaries of *O. senex senex*. n=Number of crabs (number of oocytes is in the parentheses).

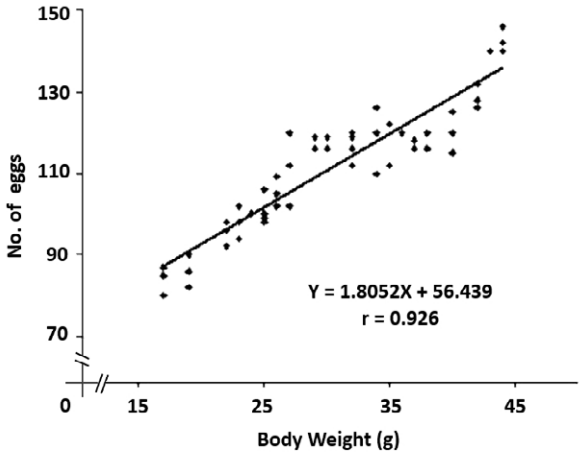

Figure 6: Regression analysis showing correlation between the body weight and the number of eggs freshly spawned.

and histological alterations, and reproductive cycle has been judged based on incidences of egg-bearing and young-one-bearing animals in nature. The data indicate a significant breeding period as indicated by more number of females that were berried with eggs and young-ones falling in the colder part of the year (August-November). The above estimate of reproductive season was also supported by the trends in gonad stages in different months of the year. These trends in gonad development based on macro- and micro-scopic examination of gonads were mirrored in the trends of the ovarian indices.

The numerical incidence of berried females observed in the present study (maximum 22% in September) is relatively low when compared to marine crustaceans. The marine crustaceans show consistently high percentage of females in berry in a catch during the breeding period. Boolootien et al. [24] reported 100% berried *Hemigrapsus nudus*, *Petrolisthes cinctipes*, 90% berried *Emerita analoga* and 80% berried *Pachygrapsus crassipes* in a catch during breeding season. Beatty et al. [8] also recorded higher spawning rates (95.6%) for mature female crayfish *Cherax cainii* at Lake Navarino. The possible reason for the high incidence of berried females in a catch may be the marine habitat with the uniform and ideal environment will provide favorable breeding place for the organism for the complete breeding expression of the females of a population during breeding period.

In most crustaceans, the eggs are attached to the pleopods and the abdomen is held deflexed after the formation of the berry. In *O. senex* the eggs are not attached to the pleopods but are protected within the tightly inflexed abdomen making a brood. In *O. senex* egg diameter is relatively large (>1.95 mm) when compare to egg diameter of spiny lobster *Palinurus homarus* (0.54 mm) [25,26], mangrove crab *Sesarma rectum* (0.611 mm) [27] hermit crab *Eupagurus bernhardus* (0.75 mm) [28], and spider crab *Mithraculus forceps* (0.56 mm) [29]. Pillai [13] reported that in marine species, the number of eggs varies from 3,000 to 10,000,000 per female and the size of each egg varies from 0.2 to 1.8 mm. The number of eggs in fresh water crustaceans varied from 10 to 800 per female and the size of egg is between 0.5 to 2.8 mm. Such differences in the number of eggs laid and in the size of eggs between the marine and fresh water forms may be explained by the nature of egg development and the survival rate of young ones. The marine and estuarine crustaceans that return to sea for breeding bear a large number of small yolky eggs [30]. The fresh water crab *O. senex senex* remain in fresh water for spawning bear less number of large yolky eggs. The difference in size and number of eggs laid by the marine and fresh water crustaceans is explained in terms of the nature of egg development as well as the survival rate of young ones [31]. In *O. senex senex*, the entire larval duration will be completed within the egg and the young ones (crab-lets) are retained in the brood of the female. In the crab, though the number of eggs spawned are less but the survival rate is relatively large (>96%). The number of eggs present in the berry of *O. senex* increases as the body weight increases. Cobo and Okamori [29] observed strong correlation between fecundity and body size in spider crab *Mithraculus forceps*. Arshad et al. [32] showed a positive correlation between fecundity and different parameters like carapace length, carapace width and body weight in blue swimming crab *Protunus pelagicus*.

Influence of environmental variables on reproduction

A positive correlation between temperature and gonadal gravimetry is evident during summer, especially from January-May. During these months the average temperature rises from 23.36°C to 33.48°C and this rise is parallel by a gradual rise in ovarian index during these months. High temperature was found to hasten the growth and maturation of ovaries in different crustaceans. Temperature induced quick maturation was also observed in *Cambarus shufeldti* [33]. Habashy and Hassan [34] showed that optimum level of temperature i.e. 29°C is required for increased reproductive success in *Macrobrachium rosenbergii*. In *O. senex senex,* temperature as a gonad stimulating environmental factor is applicable only during the warmer part of the year, whereas this temperature correlation does not holds good with regard to the winter period peaking of ovarian gravimetry.

During winter, photoperiod shows a correlation with the ovarian index and maturation. Similar photoperiod associated gonad maturation was observed in *Cambarus virilis* [35], *Procambarus simulans* [36] and in *Procambarus clarkii* [37,38]. In *Litopenaeus vannamei* feeding activity, growth and survival of smaller animals were affected by photoperoid [39]. Further, studies of Tidwell et al. [40] indicated that continual light conditions have positive impact on survival of *Macrobrachium rosenbergii* juveniles during the nursery phase. In *Homarus gammarus* hatching of eggs also was found to be stimulated by long photoperiod [41]. Co-variation analysis carried out with regard to the number of eggs in the brood purse of berried females against the various physical factors (temperature: r=0.0657; photoperiod: r=0.1495; rainfall: r=0.5759) yielded only a small and insignificant correlation coefficients. It is precisely this freedom from physical factors like temperature and photoperiod that renders the breeding biology of *Oziothelphusa senex senex* pliable to manipulation. This freedom can be exploited in the development of aquaculture technology for this edible crab.

This study represents the first comprehensive study of reproductive biology of a wild population of *Oziothelphusa senex senex* and the first histologically based study on the reproductive biology of a freshwater edible crab. Lack of dependence on the climatological factors for the completion of reproductive cycle can be exploited in the development of aquaculture technology for this edible crab.

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