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Sex specific quantitative estimation of total haemocytes in mud crab, *Scylla serrata* (Forskâl, 1775): An evidenced based report

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Abstract
Laboratory experiment was conducted to study sex specific total haemocytes count (THC) of mud crab, *Scylla serrata* (Forskâl, 1775) during the month of August, 2019 from the estuary of river Subarnarekha, West Bengal, India. The quantitative analysis of haemocytes has shown that THC of adult female ($2.94\times10^7\pm$ 0.15 cells/ml) is higher than that of adult male ($1.89\times10^7\pm$ 0.11cells/ml). The higher THC count in female seems to be associated with the reproductive functions in female.

Keywords: mud crab, *Scylla serrata*, total haemocyte count, river Subarnarekha

Introduction
A large number of colorless stellate amoebocytes or corpuscles also referred to as leucocytes are found in plasma, which are collectively called as haemocytes (Gustafson and Stoskop, 2005; Kambale *et al*., 2010) [1, 2]. Haemocytes constitute the cellular component of the haemolymph, but they are also resident in other sites such as the connective and vascular tissues (Cheng, 1981; Lokar, 2010) [3, 4]. Haemocytes are known to transports nutrients, respiratory gases, enzymes, metabolic wastes and also toxicants throughout the body. In invertebrates, particularly mollusks and crustaceans, haemocytes play a major role in cellular immune functions, such as phagocytosis (Ratcliffe *et al*., 1985; Smith, 1991; Söderhäll and Cerenius, 1992; Hanington *et al*., 2010) [5, 6, 7, 8], encapsulation (Ratcliffe *et al*., 1982; Lokar *et al*., 1982; Götz, 1986) [9, 10, 11]. Crustacean haemocytes are colourless nucleated cells freely floating in the plasma of the haemolymph and have been reported to play a significant role in certain physiological functions, such as moulting and cuticular tanning (Strutman and DoUiver, 1968; Vacca and Fingerman, 1983) [12, 13], synthesis of chitin and haemocyanin and carbohydrate metabolism (Johnston *et al*., 1973) [14]. Being a water dwelling crustacean, the mud crab *Scylla serrata* (Forskâl, 1775) is regarded as non-indigenous species within coastal waters and known for their succulent meat and delicate flavour and texture. Mud crabs are highly sought after in seafood restaurants, both locally and internationally (Fazhan *et al*., 2017) [15], thus gaining the importance of the researchers. Haemocytes of the mud crab *Scylla serrata* (Forskâl, 1775) were characterized based on morphological features using light and electron microscopy, and cytochemistry and the cells were identified as hyaline, semi-granular and granular haemocytes (Kumar *et al*., 2013) [16]. As the authors are aware that the reports on haemocyte count of mud crab is scanty (Kumar *et al*., 2013) [16], thus considering the role of haemocyte in body system, the authors set the present experiment to count the haemocyte in mud crab (in both the sexes) from the estuary of river Subarnarekha, West Bengal, India as a report for further research.

Materials and Method
Test material
Live specimens of mud crabs, *Scylla serrata* (Class: Crustacea; Subclass: Malacostraca; Order: Decapoda; Family: Portunidae) were collected during the month of August, 2019 from the estuary of river Subarnarekha, West Bengal, India (Lat: 21degree 33 min 18 seconds N and Long: 87 degree 23 min 31 seconds E) by the efficient collector. *Scylla serrata* weighing between 20 and 25 grams were dried by using blotting paper and then segregated for experimentation.

Total haemocyte count

Haemolymph of *Scylla serrata* was collected by cutting walking legs of healthy male and female crab with fine sterile scissor and quickly drawn into W.B.C pipette as per the method of Tauber and Yeager (1934) [17]. The pipette was filled with haemolymph up to 0.5 mark and diluted with diluting fluid (Tuerk's fluid) and shaken for 2-3 minutes. The first three drops of the mixture were discarded before filling the W.B.C counting chamber (Jones, 1962) [18]. Free total haemocytes (THC) were counted by using a haemocytometer with improved double Neubauer ruling as per the method of Kolmer *et al*. (1969) [19]. The formula used for counting THC is as follows:

$$THC = \frac{X}{4} \times 10 \times Y$$

Where, X=Total number of haemocytes counted in 4 chambers of the cones.
4=Number of the chamber.
10=Depth of the Chamber.
Y= Dilution.

Result and Discussions

The mud crab, *Scylla serrata* is a widely cultured species of high economic significance. This species gaining the interest in aquaculture due to its demand/price, high flesh content and rapid growth rates in captivity. Since the species is tolerant to white spot syndrome virus which causes heavy mortality in penaeid shrimp, basic understanding of haemocytes will be of great value in understanding the innate immune mechanism (Somboonna *et al*., 2010) [20]. The identification and classification of crustacean haemocytes is essential to elucidate their specific immune functions and to allow comparisons among different crustacean species (Gargioni and Barracco, 1998) [21]. Literature depicts that the haemocyte count in different crustaceans varies both inter and intra-specifically (Table -1). The first report on the characterization of haemocytes of the mud crab was made by Kumar *et al* (2013) [16]. They reported that THC in mud crab was 7.31 x 10^6 to 7.18 x 10^7 with a mean of 2.86 x 10^7 cells/ ml, but the sex specific THC has yet been listed anywhere. Considering the role of THC for assessing the physiological state of an animal, the present paper reports that the THC in female and male *Scylla serrata* is found to be $2.94\times10^7\pm 0.15\times10^7$ and $1.89\times10^7\pm 0.11\times10^7$ respectively in the the estuary of river Subarnarekha, West Bengal, India (Table-2). Previous study on Hemipteran insect, *Chrysocoris purpureus* shows that THC in the adult female (6660 ± 440 cells/cu.mm) is higher than adult male (4220 ± 165 cells/cu.mm) (Pugazhvendan and Soundarjan, 2012) [22]. In the present study the same scenario is observed for mud crab, a known crustacean (see table-2). The exact reason of this type of sex specific variation is not known, but the higher concentration of THC in female seems to be associated with their reproductive functions (Pugazhvendan and Soundarjan, 2012) [22]. Although the present result is an evident based report, but there may be other environmental and related factors which may modulate THC of *Scylla serrata*, warrants its further evaluation.

Table 1: Comparison of THC of various crustaceans

Species	THC/ ml	Reference
Carcinus granulatus Smith, 1857 [currently as: *Carcinus maenas* (L., 1758)]	31.0 X 10^6	Tauber and Yeager (1935) [23]
Carcinus maenas (Linnaeus, 1758)	5.4 X 10^8	Chisholm and Smith (1992)
Macrobrachium rosenbergii (De Man, 1879)	1.9 X 10^6	Gargioni and Barracco (1998) [21]
Penaeus setiferus (Linnaeus, 1767) [currently as: *Litopenaeus setiferus* (L., 1767)	8.9 X 10^6	Tauber and Yeager (1935) [23]
Penaeus monodon Eabricius, 1798	50.9 X 10^6	Van de Braak *et al*. (1996) [25]
Sicyonia ingentis (Burkenroad, 1938)	13.7 X 10^3	Martin and Graves (1985) [26]
Earfantepenaeus paulensis (Pérez Farfante, 1967)	44.5 X 10^6	Gargioni and Barracco (1998) [21]
Scylla serrata (Eorskâl, 1775)	2.86 X 10^7	Kumar *et al*. (2013) [16]

Table 2: THC for Male and female *S. serrate*

	Female				Male			
No. of Observation	**Number of haemocyte counted in 4 chamber**	**THC using the method of kolmer *et al*. (1969) [19].**	**Mean (cells/µl)**	**Cells/ml**	**Number of haemocyte counted in 4 chamber**	**THC using the method of kolmer *et al*. (1969) [19].**	**Mean (cells/µl)**	**Cells/ml**
1.	59	2950	2940.00 ±145.26	$2.94\times10^7\pm$ 0.15	39	1950	1892.00±114.52	$1.89\times10^7\pm$ 0.11
2.	67	3350			32	1660		
3.	58	2900			46	2300		
4.	49	2450			34	1700		
5.	61	3050			37	1850		

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