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SEASONAL VARIATIONS IN THE VOLUME OF THE HAEMOLYMPH AND BODY WEIGHT OF THE HORSESHOE CRAB, *TACHYPLEUS GIGAS* (MULLER)

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The changes in volume of haemolymph of the horseshoe crab, *Tachypleus gigas* (Muller) were more pronounced in females of size above 160 mm with maximum values in size group 241-260 mm. In males, the variations in the volume of haemolymph was not significant in sizes between 121-140 mm. An inverse relationship was observed between the volume of haemolymph and salinity of the environment. Maximum volume of haemolymph (57.3 ml) in females was observed when the salinity of the environment was low in October whereas, minimum (30 ml) at higher salinities during summer and post flood periods. The body weight of the crab was also found to be affected by the fluctuations in salinity. During flood period (Oct. - Nov.) average body weight of the carb increased by 9.6 % (size group V), 6.4 % (size group VI) and 9.8% (size group VII) in case of female, where as males showed only 3.09 % higher body weight. During post flood period (Dec. - Jan.), the average body weight decreased by 5.45; 7.2 and 18.2 % in size group V, VI and VII respectively in females and 6.1 % in males. The variations in body weight with salinity were more pronounced in females than in males, during different seasons.

Key words: Seasonal variations, Haemolymph, Body weight, *Tachypleus gigas*.

Introduction

In recent times, the biomedical properties of the horseshoe crab, a living fossil, has attained unanticipated importance due to marketing of Limulus Amoebacyte Lysate as an endotoxin tester in food, drug and pharmaceutical industries [1]. A discrete population of the horseshoe crab, *Tachypleus gigas* (Muller) is abundantly found along north-east coast of India with maximum population density at Balramgari (Lat 21° 17' N; Long 87° 00' E), Orissa.

The horseshoe crab is a hardy animal and can thrive well in extensive dilution or saturation of seawater by maintaining osmotic steady state. Salinity, significantly influences the volume of the haemolymph and body weight of the marine arthropods during different months [2-4]. In the present investigation, an attempt has been made to study the effect of the seasonal variations in salinity on the volume of haemolymph and body weight of the horseshoe crab.

Materials and Methods

The present investigation is based on the specimens collected during their migration towards the intertidal zone for spawning, at Balramgari in Orissa, India. Monthly observation, extending from March' 86–April' 87 were carried out by collecting a total of 306 specimens (140 females and 166 males) from a marked 200 m transect during highest spring or neap tides. After determining the sex of each individual [5] specimens were blotted lightly with filter paper after washing off debris and sand, and then weighed to nearest 1 mg on a single pan electronic balance. The carapace length in each individual was measured to the nearest 1 mm by Vernier Calipers and then the sample was grouped in eight different size groups of 20 mm each (I: 100-120; II: 121-140; III: 141-160; IV: 161-180; V: 181-200; VI: 201-220; VII: 221-240 and VIII: 241-260 mm). Haemolymph of each of the crab was collected by a sterile disposable, pyrogen free 18-gauge 3.8 cm hypodermic needle. The neele was inserted into the heart to allow the haemolymph to flow into a 250 ml sterile polythene bottle. All the specimens were bled until the flow showed an intermittent drip. The weight of the crab was again recorded carefully and the difference between the two weights was considered as the approx volume of haemolymph removed. Simultaneously, water samples for salinity measurement were collected and analysed following the method described by Grasshoff [8].

Results and Discussion

Range and mean values of volume of haemolymph in different size groups of female and male, are presented in Fig.1. The difference in the volume of haemolymph in females of size group I and II (from sizes 100-140 mm) was not significant (P>0.05), whereas, from size group IV (161-180) to size group VIII (241-260 mm), a proportionate increase in the volume of haemolymph with increasing six was observed. The value of haemolymph was observed maximum in female of size group VIII (241-260 mm). The females of size group III (141-160 mm) were totally absent in the samples. Similarly, male specimens of size group I (100-120 mm) and size group IV to VIII (from sizes 161- 260 mm) were also not observed.

The volume of the haemolymph increased with the size in males but difference was not significant (p>0.05) as compared to females (Fig. 1).

Seasonal variations in the volume of haemolymph in relation to salinity are shown in Fig. 2. An inverse relationship in the volume of haemolymph with salinity was observed. Maximum value of haemolymph in females, was recorded in Oct. '86 when the salinity values were minimum. Similarly, minimum values were found in July '86 in both the sexes when maximum salinity value was recorded (Fig. 2). The differences in the haemolymph volume with salinity were more pronounced in females as compared to males. During summer period (Feb.-Sept.) when the maximum salinity was recorded, the values of haemolymph in females were relatively low (average volume 40.6 ml) whereas, in flood period (Oct. - Nov.) with low salinity, the values of haemolymph were maximum (average 54.9 ml). However, in post flood period (Dec. - Jan.), fluctuations in the values of haemolymph in females were relatively of low magnitude (average 47.4 ml) (Fig. 2). In males, during summer period, the average value of haemolymph was 16.2 ml followed by 28.5 ml during flood period, and 19.6 ml during post flood period.

The body weight of the horseshoe crab, displayed distinct seasonal fluctuations. Minimum body weight coinciding with highest salinity values was recorded in both the sexes in the month of July '86. Maximum body weight in females was found in the month of Oct. '86 and the fluctuations were more pronounced as compared to males (Fig. 3).

As the females specimens of size group V, VI, VII and male specimens of size group III, occurred regularly and collected in all the months, the specimens from these size groups were considered for studying seasonal fluctuations in body weight in the present study. During summer period, the average body weight of females of size groups V (181-200 mm), VI (201-220 mm) and VII (221-240 mm) were 397.8 g, 454.0 g, and 640.0 g respectively, whereas, the average body weight of male of size group III (141-160 mm) was 184.9 g (Fig. 4). During flood period, the average body weight of female was 440.0 g (size group v), 485.0g (size group VI), 710.0g (size group VII) and of males 190.8 g. The percentage increase in body weights in females of size groups V, VI and VII were 9.6, 6.4 % and 9.8 % respectively., whereas, in male, it was only 3.09. During post flood period, the average body weight in females decreased to 416.0 g (size group V) and 450.0 g (size groups VI) and 580.5 g (size group VII) whereas, in males it was 179.1 g. The percentage of decrease in average body weight of females was 5.45% (size group V), 7.2% (size group VI), 18.2% (size group VII) and 6.1 % in males. Maximum fluctuations in the body weight, during flood period was recorded in females of size group VII.

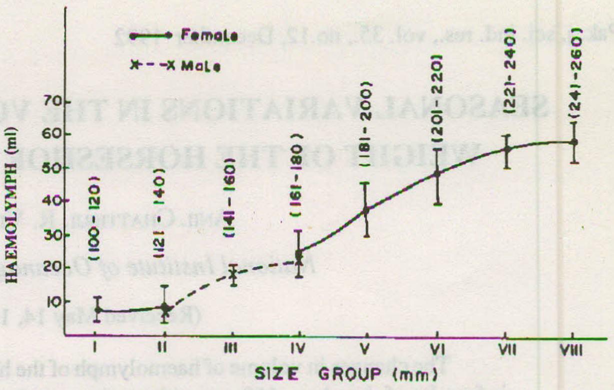

Fig. 1. Variations in the volume of the haemolymph of *T. gigas* with different size groups.

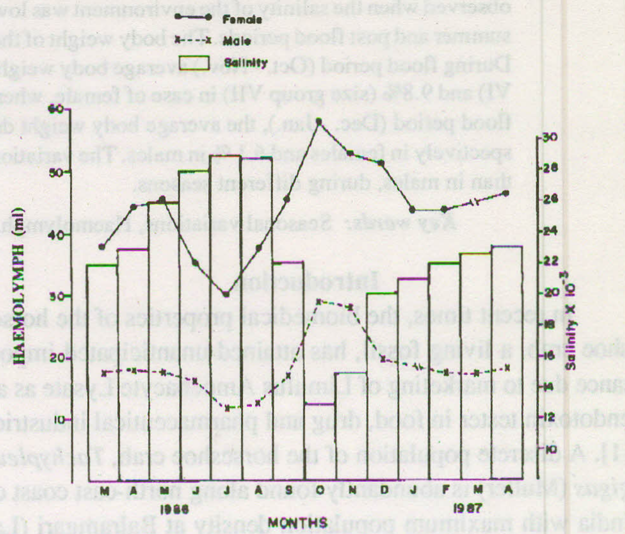

Fig. 2. Seasonal variations in the volume of haemolymph of *T. gigas* with salinity.

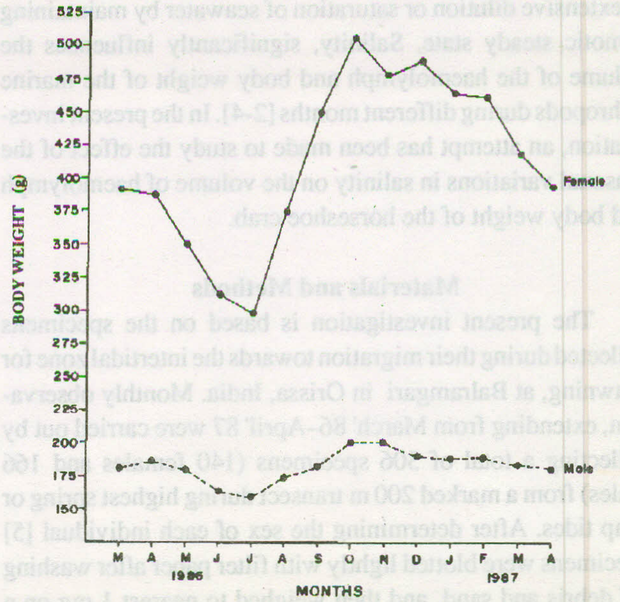

Fig. 3. Seasonal variations in the body weight of *T. gigas*.

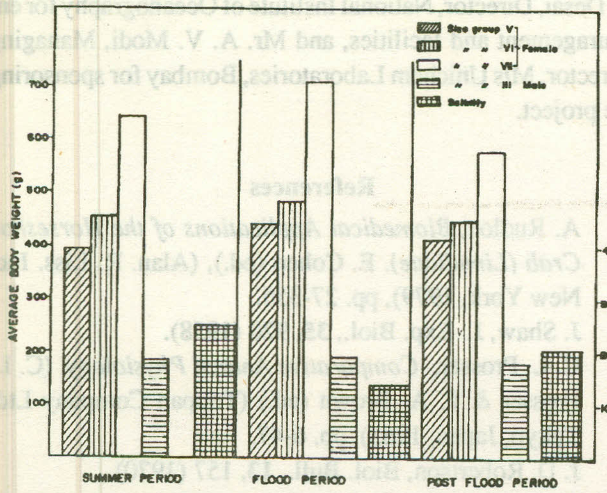

Fig. 4. A comparison in the fluctuations of average body weight of *T. gigas* during different seasons and with salinity.

Marine arthropods including horseshoe crabs exhibit all possible patterns of osmoregulation [7] and extra cellular fluids are mostly in osmotic equilibrium with the surrounding seawater [8]. The osmoregulation deals basically with the effect of salinity on the osmotic and ionic properties of the haemolymph [9]. The responses by haemolymph basically related to hyporegulation at low salinity when the volume of haemolymph increases considerably during flood period and hyper-regulation or conformity at higher salinities when the volume of the haemolymph decreases during summer and post flood period.

The horseshoe crab is mainly confined to the polyhaline region of the estuaries but immature specimens have also been found to migrate into mesohaline waters [10]. In fact, all stages of the horseshoe crab are able to tolerate a wide fluctuation in the salinity of the environment. *Limulus* has been found to occur in waters of salinity 6-8 x 10^{-3} showing a lower salinity tolerance when the animal faced a gradual acclimatization [4]. The horseshoe crab shows a perfect mechanism of osmoregulation which enables the animal to ensure a proper intracellular water balance in particular environment. The animal successfully, maintains more or less hypo-osmotic state in dilute media whereas, in more concentrated media, the haemolymph osmolarity strictly follows the osmolarity of the external environment.

Mature specimens of *Tachypleus gigas* migrate towards the shore and lay their eggs in nests on sandy beaches, round the year. The metamorphosis of the larvae takes place in oceanic waters where the salinity does not vary greatly. But in the intertidal zone, the animal is subjected to great osmotic stresses due to high and rapid fluctuations in salinity besides dessication during the low tide. Adult specimens of the *Limulus* have also been reported migrating, shoreward during spring and summer for spawning purposes [11] and thereby face salinity variations ranging from 6-32 x 10^{-3}.

In the present study it was observed that during rainy season when the salinity decreased considerably, due to land run off, the volume of haemolymph increased by 26.0 % in females. This is basically due to hypo-osmotic condition when water from the environment passes inside the body of the carb to maintain equilibrium between internal and external osmotic state. Decrease in salinity of the environment result in diffusive movement of water into the animal body across permeable surface. The permeability of water is largely through highly chitinized gills of the horseshoe crab.

The most important factor to minimize the diffusive movement of water is to reduce the concentration and osmotic gradients between the haemolymph and the external conditions. Similarly, during summer and post flood periods when the salinity of the environment is relatively high and the hyper-osmotic condition prevails, water passes outside the body of the horseshoe crab resulting in decrease in the values of haemolymph-the maximum of 19.8 % in females. During flood period, the haemolymph volume increased by 15.7 % as against 12.9 % during post flood period in males. However, in normal seawater, *Limulus* has been found to be isosmotic and showed no significant difference in volume of haemolymph of the horseshoe crab and the salinity of the surrounding water are supposed to be in isosmotic condition. The animal tissues act adequately in regulating the haemolymph concentrations over this wide range of salinity changes [8]. It has also been observed that the ionic composition of haemolymph vary from the medium regardless of the osmostic relationships. At high salinities, the haemolymph of the crab has been found hypo- ionic and hyper-osmotic to the medium [12-14]. The haemolymph collected from 6 samples of *Limulus* over a period of 3 summers showed hypo-osmotic state [15], whereas, McManus [16] reported an iso-osotic state of haemolymph on *L. polyphemus* when kept in tanks of running seawater of 25 x 10^{-3} salinity.

At high salinities, the body weight of the horseshoe crab has been reported to decrease considerably, as against an increase at lower salinities [4]. In shore crab, *Carcinus maenas*, the body weight of the animal increased when the animal moved into diluted seawater of 50 % to the normal seawater [3]. In a similar way, during % investigation, body weight of *T. gigas* increased by approx 9.8 % in female (221-240 mm size) and by 3.09 % in male (141-160 mm size). During post flood period, the body weight decreased by 18.2 % in females and 6.1 % in, males. These fluctuations in body weight are more pronounced in females rather than in males of *T. gigas*. In an another study [4], it has been reported that the body size has no relation with osmotic steady state even though it is related to salinity.

Several laboratory studies have been carried out to assess the extent of changes of the body weight in different salinities [2,4,12,13]. The body weight of the crab increased when transferred from 100 % seawater to 15 % seawater [4]. The increase in weight has been reported to be low (about 2-4 %) upto 33 x 10^{-3}, salinity; greater (about 5-8) %)at 20 x 10^{-3}, Salinity, greater (about 5-8%) at 20x10^{-3} salinity and very high (15%) at 13-22 x 10^{-3} salinity. Similarly in concentrated seawater of 150x10^{-3} and 200x10^{-3} the loss in body weight has been calculated to be 3 % and 6 %, respectively [4]. The above studies support the persent findings where the *T. gigas* looses and gains the body weight at higher and lower salinities, respectively. While working on the osmotic and ionic regulation of *Limulus*, Robertson [4] reported that at 66 x 10^{-3} seawater only few *Limulus* were osmoregulating and maintaining higher osmotic and chloride concentration in the haemolymph. The specimens weighing upto 32-36 g and having 7 cm carapace width, have been found to tolerate lowest dilution upto 5 x 10^{-3} seawater [4].

The lysate which is prepared from the haemolymph of the horseshoe crab, formed an opaque gel in the presence of minute amount of endotoxins [17]. The pyrogen testing by lysate, at present, is a promising method for the detection of endotoxins even upto 100 g/ml. Though salinity plays an important role in fluctuating the volume of haemolymph in different seasons, it is still not ascertained whether the variations in the salinity have any signifiacnt effect on the quality of lysate or not. Jorgenson and Smith [18] reported that in June/July through Oct., maximum lysate preparations could be possible. The variability in lysate in the haemolymph is related to ecobiological changes rather than any other factor. The possibility of causing the variability is supported by the fact that the activity of lysate increases when the horseshoe crab gets acclimated to dilute seawater [4]. In *Uca pugilator* the activity of certain enzymes have been reported higher when the animal acclimated in low salinity medium [19]. Sullivan *et al.* [20] reported that in dilute seawater, the concentration of copper based hemocynin increased considerably. At present, the problem of lysate variability with season is of great concern as a causative factors for these variability have still not been elucidated. This aspect deserves investigations in detail. Future studies on this particular aspect may elucidate methods whereby potency/quality of the lysate can be assessed by the measurement of lysate composition on the haemolymph of the horeseshore crab in Indian waters during different seasons.

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