

Is Sex Reversal Common Phenomenon In Horseshoe Crabs Too?

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ABSTRACT

The capacity of organisms to change their sex has evolved independently in several species. Sex change has been widely studied, but research approaches have differed and conclusions have often been specific. Although sex allocation theory provides a unifying framework for the study of sex change, this unity has not always been appreciated, Environmental sex reversal (ESR), which results in a mismatch between genotypic and phenotypic sex, is well documented in numerous species and may be induced by chemical exposure. In the present study, an attempt was made to hypothesize the phenomenon of sex reversal in both Asian species (*Tachypleus gigas* and *Carcinoscorpius rotundicauda*) of the horseshoe crab in accordance with the size of the animals. Our findings suggest that both Asian species of horseshoe crabs ultimately show a positive sign of sex reversal phenomenon, however, this hypothesis needs further confirmation by studying the hormonal changes at all moulting stages of the horseshoe crab.

Keywords: sex reversal, horseshoe crab, environmental.

INTRODUCTION

Two species of the horseshoe crab namely; *Tachypleus gigas* (Müller) and *Carcinoscorpius rotundicauda* (Latreille) are abundantly found throughout the year along the north-east coast of India. Extensive studies have been conducted on various aspects of Atlantic and Asian species of the horseshoe crabs [Chatterji, 1999; John et al., 2018; Fairuz-Fozi et al., 2018; Shuster et al., 2003; Pati et al., 2020a,b; Pati et al., 2021a,b; Mohamad et al., 2021]. However, the phenomenon of sex reversal in horseshoe crab has not been reported so far. In fact, sex reversal is a common phenomenon

in several marine organisms especially in crustaceans [Mutalipassi et al., 2018]. Generally, while studying the population dynamics of a species in an aquatic ecosystem, data of both males and females are jointly collected. However, during the course of routine collection and interpretation of data, the phenomenon of sex reversal is often being ignored and not examined critically. There could be many reasons for ignoring such an important phenomenon in any aquatic crustaceans. Proper grouping and analysis of data of males and female's individuals of a given population helps in understanding the sex reversal phenomenon to some extent [Bauer, 2000; Mutalipassi et al.,

2018]. Such valid dataset (s) would also be able to provide several scientific insights including the possibility of sex reversal—an interesting phenomenon—common among many crustaceans [Cooper, 1965; Percy and Forss, 1969; Wenner and Haley, 1981]. The phenomenon of sex reversal has earlier been reported in several invertebrates including molluscs [Guo et al., 1998; Kasyanov, 2001; J. S Lee et al., 2012; Jung Sick Lee et al., 2013] and decapod crustaceans [Bauer, 2000; Mutalipassi et al., 2018]. Previous studies conducted in the last century have demonstrated that specimens experiencing a male stage at smaller sizes and switched to females at their larger sizes. It has also been found that sex ratio in invertebrate changes in accordance with size of the animals as reported in *Mercenaria mercenaria* [Eversole, 2001] and *Crassostrea gigas* [Gosling, 2004]. In *C. gigas* differences in sex ratio was observed in accordance with shell size as the male ratio was higher in the one-year-old class whereas the female ratio in the two-year-old class [Guo et al., 1998]. Similarly, population dynamic investigations in shrimp have also clearly shown the sex reversal phenomenon after one year of hatching [Mutalipassi et al., 2018; Valerio Zupo and Messina, 2007]. Zhang et al. (2018) have observed functional males up to 2 years and all females in the third year in the population of black porgy, *Acanthopagrus schlegelii* (Bleeker) which confirmed sex reversal in this marine organism [Zhang et al., 2018]. Baeza (2007) observed that sex allocation in a given population

of decapod crustaceans is size-dependent where smaller groups of animals were allocating to the male individual than larger ones [Baeza, 2007]. It has further been confirmed that the sex-reversed females were restricted only in larger sized groups based on a population dynamic investigation of shrimp [Valerio Zupo, 1994].

During the last few decades' field studies undertaken along the northeast coast of India showed an interesting phenomenon partially supporting the phenomenon of sex reversal in horseshoe crab. In the present study, an attempt was made to hypothesize the phenomenon of sex reversal in both Asian species of the horseshoe crab in accordance with the size of the animals. The present study is comprising of data collected for almost two decades from 1996 to 2016 at the Balramgari (Balasore) and Hukitola coastal villages of Odisha in India where the occurrence of two species of the horseshoe crab was earlier reported [Nelson et al., 2016]. We statically analysed the comprehensive data collected during the study period for both Asian species of horseshoe crabs to ultimately show a positive sign of sex reversal phenomenon in these species.

MATERIALS AND METHODS

The data being considered for this publications were collected between the years 1996 and 2016 at Balramgari coast of Balasore (Lat. 21°43'99"

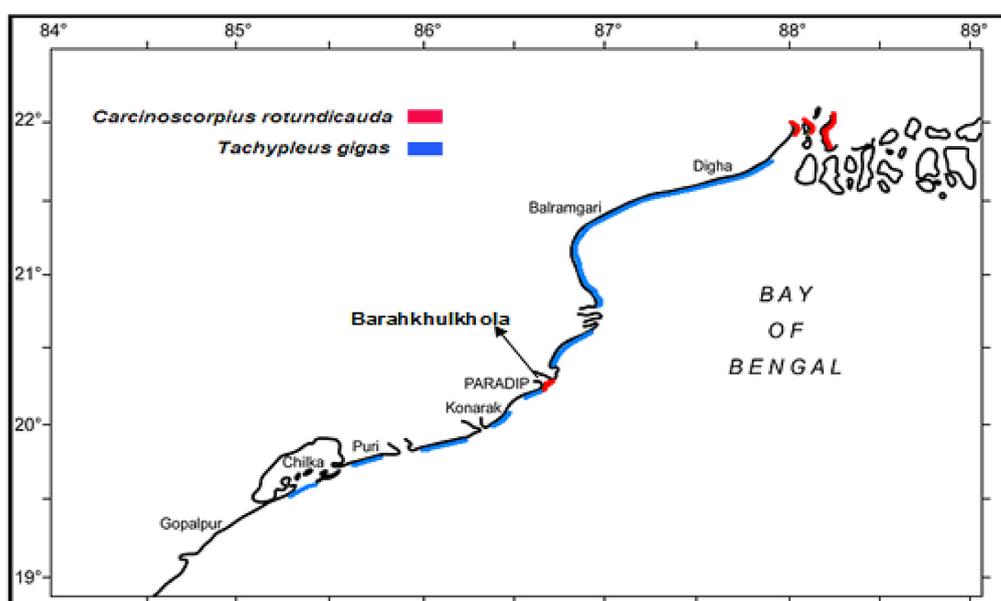


Fig. 1. Study area along the north-east coast of Odisha, India

N; Long. 87°01'49" E) and at Hukitola village of Odisha (Lat 20°24'12.5" N; Long 86°47'22.7" E) along the northeast coast of India (Figure. 1).

Being located in the lower reaches of the River Mahanadi Delta, the beach and mangrove areas in the vicinity of Hukitola are supporting the nesting activity of both *T. gigas* and *C. rotundicauda* [Chatterji, 1999]. The fieldworks were conducted in different phases during March 1996 - February 1997 (24 months), February 2004 - December 2006 (35 months), March-May 2007 (3 months), and April-July 2016 (4 months) through various scientific projects (Technical report, 1988, 1996, 1998, 2001, 2005, 2007). Monthly samplings were coincided with either full or new moon phases and with the attainment of the highest high tides in this area. For this, a shore seine having an area of 100 m² was operated with the help of six fishermen and live specimens of horseshoe crabs were collected from the natural environment during the attainment of the highest high tides [Chatterji, 1999]. All specimens of male and female of breeding couples of *T. gigas* and *C. rotundicauda* were counted and their carapace length (in mm) was measured separately with a caliper from the uppermost part of the carapace to the tip of the telson. A total of 2973 males (size range: 80-140 mm) and 2952 females (size range: 141-260 mm) of *T. gigas* whereas 2609 males (size range: 60-120 mm) and 2605 females (size range: 121-220 mm) of *C. rotundicauda* were randomly selected during the present study. All specimens were released back into the sea immediately after recording all parameters for their survival.

The number of specimens of horseshoe crab collected during one fishing operation was considered as catch per unit effort (CPUE). A null hypothesis was applied to confirm the effect of tide height and abundance of the horseshoe crab using at first one-way ANOVA considering CPUE is the dependent variable. Secondly, we used univariate *F* analysis to test the effects of the dependent variable (using SAS 9.2, North Carolina, USA) [Pati et al., 2020; Sarkar et al., 2021]. Further statistical estimates on crab's sex ratio and percent number of male and females were represented through scatter plots and histograms using Originpro 8.5.

RESULTS

The dense time-series data, obtained for 66 months represent the occurrence of 5925 *T. gigas* and 4,784 *C. rotundicauda* in total. While the carapace length of *T. gigas* was ranged between 80 and 140 mm (male) and 140 and 260 mm (female), it varied between 60 and 120 mm (male) and 110 and 220 mm (female) for *C. rotundicauda*. Figure 2 and Figure 3, depicted the sex ratio and percentage of male and female within the species *T. gigas*. Similarly, Figure 4 and Figure 5, described the same for species *C. rotundicauda*.

DISCUSSION

However, during routine population studies, the phenomenon of sex reversal mostly go

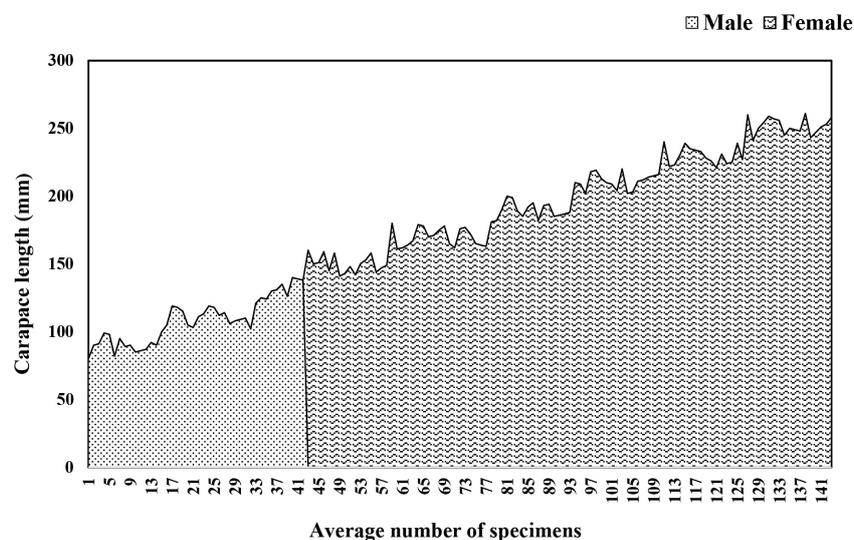


Fig. 2. Sex ratio of *T. gigas*

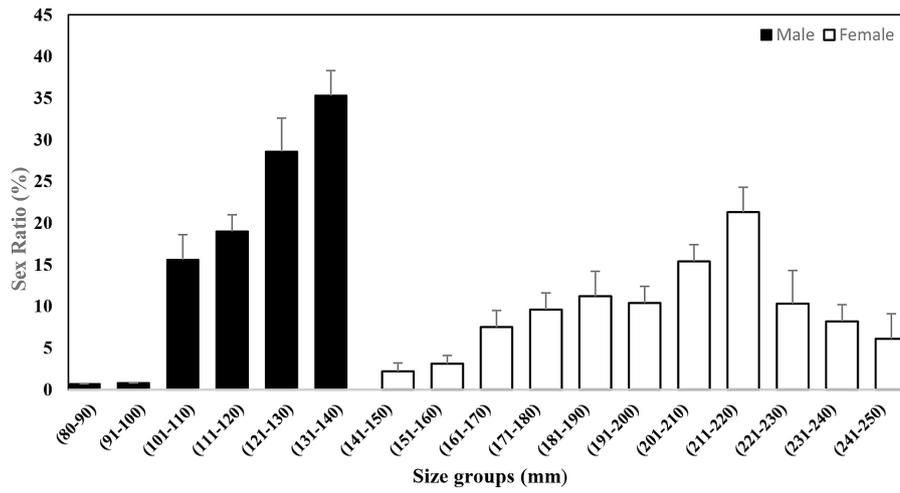


Fig. 3. Percentage of males and females of *T. gigas*

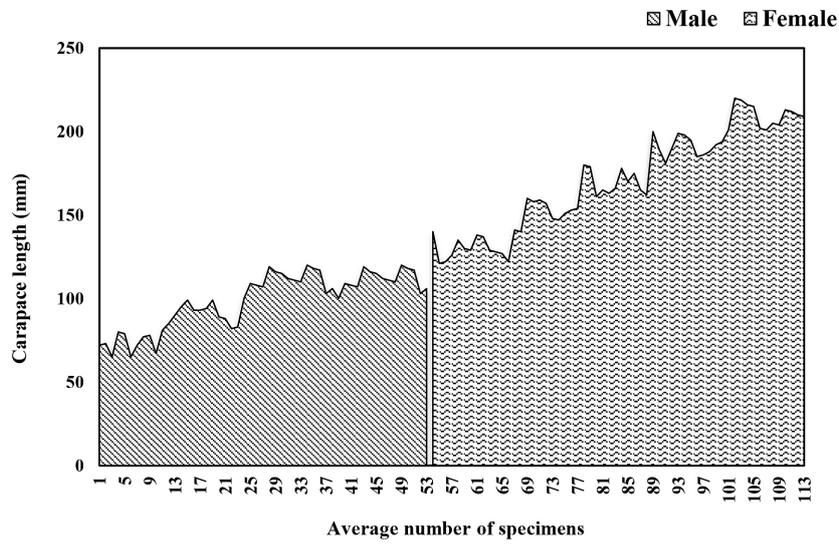


Fig. 4. Sex ratio of *C. rotundicauda*

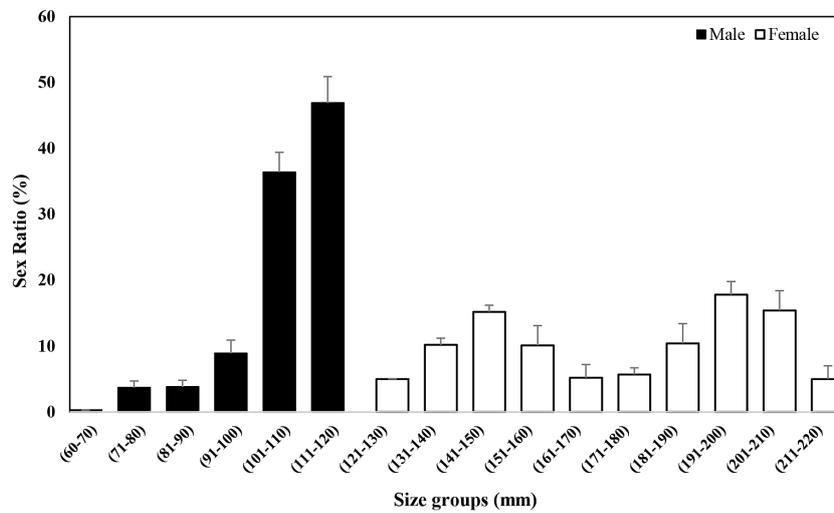


Fig. 5. Percentages of males and females of *C. rotundicauda*

unnoticed. In a study conducted on the population of mole crabs, analysis of some field data supported a protandric hermaphroditism hypothesis where even with a several month influx of young crabs onto beaches, sex ratio varied with size in a characteristic manner when data were combined for the entire year [Barnes and Wenner, 1968].

This phenomenon to some extent depends if the animal changes their sex with their size either due to sudden environmental disturbances or even changes in normal physiological processes. Particularly for this study, data analysis needs to be done systematically.

Biogenic activities of the horseshoe crab mostly take place in the open deep ocean however, only for spawning purpose, mature pairs in amplexes, migrate towards suitable breeding beaches round the year [Chatterji, 1999; Sekiguchi et al., 1988]. The mature pairs of the horseshoe crab lay their gametes on sandy or muddy beaches that are protected from the rough tidal waters of the sea. The fertilized eggs generally require 40 to 45 days of incubation in either sandy or muddy nests. Once the larvae hatch out, they swim around in the shallow intertidal areas near the beaches for some time and then migrate back to their nursery grounds for further development [Chatterji, 1995]. There could be many reasons for not studying the morphometric variations in males and females sexes in horseshoe crab at their early stages of larval development. Although the young larvae of the horseshoe crab show the formation of all appendages immediately after hatching identification of sex following morphometric differentiation cannot be possible. Secondly, the developing larvae undergo to several moulting stages at deeper zone of the sea as such the time of actual allocation of morphometric characters showing a particular gender is not possible due their slow growth rate.

Several studies conducted on decapod crustaceans showed sexual differentiation successfully those have always been controlled by the presence or absence of androgenic gland (AG) [A Sagi et al., 1997]. Development of male sex at larger sizes of crustaceans was prevented due to reduction in the hemolymph levels of the hormone resulting in sex reversal due to an auto-differentiation of the ovary in many crustaceans [Baeza, 2006; Amir Sagi and Aflalo, 2005]. At smaller sizes, the germinative zone has essentially been reported for determining the testis tissue because of the presence of the androgenic hormone

[Charniaux-Cotton, 1967]. In several other studies conducted under simulated conditions, it has been shown that many marine crustaceans are simply gonochoristic, primarily based on the absence of ovotestis development. Researchers have cultured postlarvae of *H. inermis* in petri dishes and regularly monitored exuviae to study the sex and the size of each individual [Zupo et al., 2008]. Several developing larvae losing their appendices masculinae have shown the transformation of males into females suggesting a mechanism of sex reversal in decapod crustaceans [Zupo et al., 2008]. However, such studies on developing larvae of horseshoe crab under controlled conditions were not possible due to their very slow growth rates and occupying different ecological niche during their developments. Hence phenomenon of sex reversal in these species were considered on analysis of field data collected on morphometric variations of both species for several years of study.

Earlier studies have clearly shown the sex reversal phenomenon in the natural population of crustaceans [Wenner and Haley, 1981]. In those studies, analysis of grouping of data for males and females crustaceans supported appropriately the sex reversal assumption [Cooper, 1965; Percy and Forss, 1969]. Analysis of field data collected for the year on the population of mole crabs also supported a protandric hermaphroditism hypothesis in which several month influxes of young crabs onto beaches showed significantly varied sex ratio with size in a specific manner [Barnes and Wenner, 1968]. Reverberi (1950) for the first time reported a peculiar mechanism of sex reversal where males of *H. inermis* showed sex reversal phenomenon once they completely lost their testis and showed the development of ovary from embryonic undifferentiated cells [Reverberi, 1950]. Similarly, Mutalipassi et al., 2018 also confirmed the sex reversal phenomenon in shrimp after one year of hatching [Mutalipassi et al., 2018]. Further to these studies, Zupo (1994) also reported the presence of sex-reversed females based on a population dynamic investigation where small size females developed simultaneously with the male spring group (Valerio Zupo, 1994). In our study, direct observation on morphological changes and allocation of the particular reproductive system of the growing larvae of horseshoe crab under simulated conditions could not be performed due to their very slow growth. It is also difficult to rear horseshoe crabs in

the laboratory under simulated conditions and hence there is no record available that showed when the horseshoe crabs attain sexual maturity. However, researchers do believe that generally, the horseshoe crab takes about 10-12 years' time to attain sexual maturity. Considering the growth estimates of the juveniles it is believed that they undergo 12 moults before becoming fully grown males whereas 13 moults in the case of females [Sekiguchi et al., 1988]. Charnov (1982) explained very well the sex allocation theory which has been confirmed by many researchers [Charnov, 1982]. It has been observed that the rapid sex change in shrimps could be related to two narrow periods of reproduction in each year. It has been suggested that rapid sex change is beneficial to shrimps to change their sex quickly rather than prolonging through intermediate stages. However, the Indian horseshoe crab breeds throughout the year as such the necessity of sex change might not be as rapid as reported in growing shrimps. In our study, out of 4782 specimens, 2609 males ranging in size between 60 and 120 mm were collected. Surprising none of the individual showed any morphometric characters of female. Similarly among 2175 individual ranging in size between 121 and 220 mm, not a single male individual was collected between these size ranges.

Morphologically male and female horseshoe crabs are different and identification of their morphometric characters are based on observing the claspers along with dactylopodite, marginal opisthosomal spines and gonophores. In males, the terminal front pedipalps modified resembling like boxing gloves whereas in females it remained unchanged [Shuster et al., 2003]. Sex determination can also be done by observing the genital operculum. Male shows genital papillae underside the genital operculum whereas female's gonopore appear to be softer bumps with slits [Shuster et al., 2003]. Researchers also reported that in shrimp males are identified by appendix masculina containing mature testis whereas in females these appendices are not present and show mature ovary as they grow [Zupo et al., 2008]. These observations lead to the conclusion that the species is capable of sex reversal from male to female with their sizes [Motalipassi et al., 2018; Valerio Zupo and Messina, 2007]. However, Zupo et al. (2008) further confirmed the sex reversal by studying the morphological changes and histological investigations in laboratory cultured shrimps supporting

the changes the external sex characteristics with growing larvae [Zupo et al., 2008].

Sex reversal phenomenon has also been observed in many other invertebrates. Several researchers have confirmed sex reversal in bivalve species [Guo et al., 1998; Kasyanov, 2001; Lee et al., 2012; Lee et al., 2013]. In all their studies sex ratio have been observed to be in accordance with size of animals such as *Ostrea virginica* [Hillman, 1965], *Mercenaria mercenaria* [Eversole, 2001] and *Crassostrea gigas* [Gosling, 2004]. It has also been observed that in *C. gigas* female ratio increased with the age as the result of sex reversal from male to female [Guo et al., 1998]. The male ratio reported being high in the one-year-old class, whereas the female ratio in the two-year-old class [Lee et al., 2013; Park et al., 2012].

During the last 2 decades, morphometric studies were conducted as a part of resource assessment surveys of both species of horseshoe crabs occurring along the north-east coast of India. In our observations, the identification of male individuals had a strong positive correlation with the size of the horseshoe crab. We have also not encountered any degenerated male parts in larger size individuals which also suggest that after certain moulting processes, male individuals transformed into a female at a larger size. However, this hypothesis needs further confirmation by studying the hormonal changes at all moulting stages of the horseshoe crab. After careful analysis of the data collected over several years, the possibility of sex reversal in horseshoe crab cannot be ruled out. We also know that if the hypothesis of sex reversal is confirmed in the horseshoe crab, the sex allocation is totally size-dependent and occurring slowly related to the moulting of the developing juveniles. The critical stage at which the sex reversal phenomenon occurs should be investigated more closely.

CONCLUSION

The carapace length of *T. gigas* was in the range of 80-140 mm for male and 140-260 mm for female, the same is varied between 60-120 mm for male and 110-220 mm for female in case of *C. rotundicauda*, in the samples collected over a period of 66 months and 5925 numbers of *T. gigas* and 4,784 *C. rotundicauda*. The statistical analysis of the sex ratio in both the species, the phenomena of sex ratio can be presumed and

reported for first time, though the hypothesis need circumstantial evidences and may initiate a totally new domain of research in the field of conservation study of horseshoe crab.

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