Ecobiology of horseshoe crab in Brebes, Northern Coast of Java: Preliminary study

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Abstract. Horseshoe crabs are known as living fossils and three species are found in Indonesia where all of them are being protected. The research aimed to assess ecology, species composition, and biological aspects in Brebes estuary. The research was conducted in August and October 2017 in Brebes Estuary. Samples were caught using local fishermen and experimental gears. Parameters of water quality were observed in situ and substrate was analyzed in the laboratory. The horseshoe crabs were measured morphologically, their stomach and eggs were taken to be analyzed in the laboratory. Total specimens of horseshoe crabs were 33 individuals (Tachypleus gigas=8; Carsinoscorpius rotundicauda=25). T. gigas has 16.3-22.6 cm of prosomal width and 216.1-717.0 gram of weight. C. rotundicauda has 11.2-14.5 cm of prosomal width and 96.0-275.5 gram of weight. Stations with a muddy sand substrate (silt >50%) have the highest number of captured horseshoe crabs. Food habits of T. gigas are macrophytes and detritus while C. rotundicauda are macrophytes, molluscs, annelids, and coral. Niche breadth of C. rotundicauda were wider than T. gigas. High niche overlap value between T. gigas and C. rotundicauda indicates high competition in utilizing food sources. The fecundity of C. rotundicauda was higher than T. gigas but the egg diameter of C. rotundicauda smaller than T. gigas.

Keywords: ecology; egg; food habit; horseshoe crab; nice breadth; nice overlap

1. Introduction
Horseshoe crabs are called ancient creatures where they have changed from 200 million years and the lineage of evolution is at least ~ 510 million years [1, 2]. Rudkin et al. [3] also reported that these creatures had an age longer than 445 million years. The horseshoe crab currently consists of only four species. Limulus polyphemus (Linnaeus, 1758) occurs in the coastal waters of the eastern continental shelf of North America and in the Gulf of Mexico and three other species (Tachypleus gigas (Müller, 1785), Carcinoscorpius rotundicauda (Latreille, 1802) and Tachypleus tridentatus (Leach, 1819)) are found in the coastal waters of India, Southeast Asia, China, and Japan [4]. Indonesian coastal waters are reported to have three species of Asian horseshoe crabs, namely T. gigas, C. rotundicauda and T. tridentatus, which are distributed in several regions [5-7].

The horseshoe crab's role in ecology is food source for other species, a voracious eater, and can disrupt extensive benthic and coastal habitats [4]. The blood of L. polyphemus and T. tridentatus has a substance known as Limulus Amebocyte Lysate (LAL) [8]. The other two species T. gigas and C.
**C. rotundicauda** can also produce LAL. LAL is used by the US Food and Drug Administration (FDA) as a test for the presence of endotoxins in biology, pharmaceutical drugs, and medical devices and is used worldwide [9]. *Tachypleus Amebocyte Lysate* (TAL) is an alternative to LAL [10].

Despite its important role in ecology, the horseshoe crab poses several threats to its survival. Significant changes in the natural habitat of horseshoe crabs for nesting are caused by continuous anthropogenic activities [1, 10]. Development in coastal areas is one of the contributors to the loss of suitable habitat for horseshoe crab nesting, especially in the Eastern Asian region [11-14]. In the Asian region, the population of horseshoe crabs has some pressures in terms of medical use and human consumption [15].

The IUCN Red List of Threatened Species releases the status that *Limulus polyphemus* is vulnerable [16], *T. tridentatus* is endangered [17]. *T. gigas* is data deficient [18] and *C. rotundicauda* is data deficient [19]. Three species of horseshoe crabs (*T. gigas, C. rotundicauda* and *T. tridentatus*) in Indonesia have a protected status through Regulation of the Minister of Environment and Forestry of the Republic of Indonesia No. P.20/MENLHK/SETJEN/KUM.1/6/2018 concerning protected flora and fauna species [20].

Several studies related to the ecology and habitat of *T. gigas* and *C. rotundicauda* have been conducted [13, 21-23]. Studies on the food habit of *T. gigas* have been conducted in India [24], Malaysia [25, 26], and Indonesia [27]. Meanwhile, the food habit study of *C. rotundicauda* has been conducted in India [28], Hong Kong [29], Malaysia [12], and Indonesia [27]. Studies on the eggs of *T. gigas* and *C. rotundicauda* have been conducted in Malaysia and Sunderban Estuarine Region [28, 30, 31].

The horseshoe crab in Indonesia has been recorded in several areas such as Sumatera [32-34], Java [6, 7, 35-37], Madura [7], Kalimantan [37-39] and Sulawesi [37]. Several studies on horseshoe crab have been conducted in Indonesia related to composition [35], morphometric [6, 7, 36, 38, 40, 41], genetic [6, 39], population [34, 41], food habit and reproductive aspect (egg) [27].

In Indonesia, studies on the ecology and biology (food habit, reproductive aspect) of *T. gigas* and *C. rotundicauda* are still scant. Ecological and biological studies of *T. gigas* and *C. rotundicauda* are needed in Indonesia to fill in the lack of data on horseshoe crab in Indonesia. This research aimed to assess ecology, species composition, and biological aspects of horseshoe crab in Brebes estuary.

### 2. Materials and methods

#### 2.1. Study site

This study was carried out on August and October 2017 in Brebes, Northern Coast of Java. The research stations cover the entire delta Pemali estuary area which is located in seven locations determined according to the typology of the waters. The seven stations are Mangrove creek (station 1), Polang Canal (station 2), Mouth of Pemali (station 3), Mouth of West Pemuda Canal (station 4), East Pemuda Canal (station 5), River embankment (station 6), and Kaliwlingi (station 7) (figure 1).

#### 2.2. Data collection

Sampling was carried out using local fisherman’s fishing gear (single-layer nets/snapper nets/three-inch gillnet monofilament nets, trammel nets, and trash nets), and also experimental gear (centipede nets, and fyke nets). The horseshoe crab was recorded for gender, measurements of prosoma (length and width), and weight. The horseshoe crabs were dissected and their gonads and stomachs were preserved in 5% formaldehyde for further analysis. Gonads and stomach content observation were conducted in Research Institute for Fish Resources Enhancement. In the laboratory, ovaries were weighted to the nearest 0.001 g, then some eggs of each ovary were counted as subsample and its diameter was measured under a microscope equipped with an ocular micrometer.
2.3. Data analysis

Species diversity is a comparative analysis of the horseshoe crab species composition caught at each station that represents habitat typology. The comparison is displayed in histogram form so we can find out which species are mostly caught in the estuary of the Pemali Delta.

Index of preponderance is used to determine the feeding habits of horseshoe crab [42]:

\[
I_i = \frac{V_i \times O_i}{\sum V_i \times O_i} \times 100
\]  

Where:
- \( I_i \) = index of preponderance
- \( V_i \) = the volume index of food item \( i \) (as indicated by their percentages)
- \( O_i \) = the occurrence index of food item \( i \) (as indicated by their percentages)

Nice breadth describes the amount of food resources that can be utilized by a group of organisms. Nice breadth is calculated using the Levins Index [43, 44]:

\[
B = \left( \sum p_{ij}^2 \right)^{-1}
\]  

Where:
- \( B \) = Levins nice breadth
- \( p_{ij} \) = the proportion of the individuals of species \( i \) which is associated with resource state \( j \)

Nice breadth is standardized using Hulbert's formula [44, 58]:

\[
B_a = \frac{(B - 1)}{(n - 1)}
\]
Where:
\[ B_a = \text{standardized index of niche breadth} \]
\[ B = \text{Levins nice breadth} \]
\[ n = \text{total number of item (resources)} \]

The niche breadth \((B_a)\) values vary from 0 to 1. The niche breadth \((B_a)\) values 0 mean species consume a single item and 1 mean species exploits available items in equal proportion. Values of \(B_a\) are considered low when below 0.4, moderate when between 0.4-0.6, and high when the value is higher than 0.6 [45].

Niche overlap between the most common species was analyzed using Pianka's index [59]:
\[
O_{jk} = \frac{\sum (P_{ij}P_{ik})}{\sqrt{\left( \sum P_{ij}^2 \sum P_{ik}^2 \right)}} \tag{4}
\]

Where:
\[ O_{jk} = \text{Pianka’s index of niche overlap between species } j \text{ and } k \]
\[ P_{ij} = \text{the proportion of the i resource in the diet of species } j \]
\[ P_{ik} = \text{the proportion of the i resource in the diet of species } k \]

The value of niche overlap \((O_{jk})\) is classified as low (<0.33), medium (0.33-0.67) and high (> 0.67) [46]. The assumption here is that all species can access different food sources equally [47, 48], because it did not collect data on the availability of resources in the study area [47].

The Gonadosomatic Index (GSI) was calculated using Effendi’s formula [49], representing the percentage of the gonad weight with total horseshoe crab weight. Fecundity was determined using a combination of the gravimetric method [49, 50] against the ovaries of mature female organisms [51] determined by the formula:
\[
F = \frac{G}{Q} \times X \tag{5}
\]

Where:
\[ F = \text{fecundity (numbers of egg in ovary)} \]
\[ G = \text{Weight of ovary (g)} \]
\[ Q = \text{Weight of sub-sample ovary (g)} \]
\[ X = \text{Numbers of egg subsample} \]

3. Results and discussion

3.1. Composition, distribution of horseshoe crab and water environmental conditions

3.1.1. Composition and distribution of horseshoe crab. Two species were found in Pemali Delta Estuary during the study, there are \(T. gigas\) and \(C. rotundicauda\). These two species are the dominant species of three species commonly found in Indonesian waters. \(T. gigas\) has characteristics: brownish, has a size of up to 40 cm, has a convex body shape with a horseshoe-like carapace, a triangular telson-shaped cross section, and has special characteristics in the form of a protoreceptor in the caudal spin [52], while \(C. rotundicauda\) is the smallest species, with the largest sizes ranging from 25-30 cm, the body shape is like a steel shell, brownish, and the cross-section of the telson is round [53].

A total of 33 individuals (Eight \(T. gigas\); 25 \(C. rotundicauda\)) of horseshoe crab were caught during the study. The composition of horseshoe crab species caught at each research station is presented in figures 2 and 3. Based on figures, we discovered that horseshoe crab caught in five out of seven stations, Mangrove Creek, Polang Canal, Mouth of West Pemuda Canal, East Pemuda Canal, and Upstream Estuary. In this study, horseshoe crab were not found in the Muara Pemali and the river embankments, but based on information from local fishermen, horseshoe crabs were generally caught there.
Figure 2. Composition of horseshoe crab caught at the research stations.

Figure 3. The amount of horseshoe crab caught in the Pemali Delta Estuary.

*C. rotundicauda* and *T. gigas* can be found at Polang Canal and East Pemuda Canal, while at the other three stations (Mangrove Creek, Mouth of West Pemuda Canal, and Kaliwlingi) only found *C. rotundicauda*. *C. rotundicauda* is a species that dominates the catch compared to *T. gigas* and almost found in all research stations with a percentage ranged from 67.67 - 100%. The Northern Coast of Java generally has shallow waters with soft, sandy bottoms or broad mudflats or mangrove-mudflat ecosystems and these characteristics are suitable as habitat for *C. rotundicauda*. Horseshoe crab usually spends most of its life near or at the bottom of brackish swamp waters [5, 7, 54, 55, 56]. *T. gigas* is found in much lesser numbers. This condition is presumably because this species prefers a slightly different or more specific habitat than *C. rotundicauda*. All sampling locations were thought to be less suitable or ideal as a habitat for genus *Tachypleus*, so that this species was found in a smaller percentage. These findings similar to previous studies where the genus *Tachypleus*, *C. rotundicauda* is almost always found in greater numbers than *T. tridentatus* and *T. gigas* [57].

The distribution of carapace length frequencies of the two horseshoe crabs caught in the Pemali Delta Estuary is showed in figure 4. The horseshoe crab species that dominates Pemali River estuary were *C. rotundicauda* with carapace lengths ranged from 11.4-13.3 cm which is divided into three interval classes (10-11, 12-13, and 14-15 cm). The highest number of horseshoe crabs was found in the 12-13 cm interval class which reached 17 individuals (77.27%) and were found mostly in St.2 and St. 5, while the remaining 22.27% were in the interval class between 10-12 cm that found in St.2, st. 4 and st. 5. The largest *C. rotundicauda* (with a carapace length 15.3 cm) was found in St. 7 located in the upstream estuary area.
Figure 4. The distribution of carapace length frequencies of the two horseshoe crab species.

*T. gigas* species found in the research locations had a carapace length ranged between 16.1-24.5 cm and were in class intervals of 16-17, 22-23, and 24-25 cm. Overall *T. gigas* had the largest size and wide class intervals. Mashar *et al.* [7] in their research along the north coast of Java, found this type of crab in the size range between 15-16 cm to 25-26 cm. The variation in the size of horseshoe crabs is most likely influenced by geographical conditions, population density, food availability, and environmental conditions [60, 61]. Malaysian mangrove horseshoe crab has the carapace length longer than India and Thailand [62].

Table 1. Water quality in research stations.

| Parameters     | Unit | St.1             | St.2             | St.3             | St.4             | St.5             | St.6             | St.7             |
|----------------|------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
| Depth          | m    | 1                | 0.7              | 1.8              | 0.6              | 1.2              | 1.7              | 1.5              |
| Transparancy   | cm   | 10-30 (50)       | 40-60 (50)       | 30-40 (35)       | 20-60 (40)       | 20-40 (30)       |                  |                  |
| Bottom Salinity| ppt  | 22 (19.5)        | 14-25 (15)       | 4-26 (15)        | -                | 15.6-31.0 (23.3) | 0.1-26 (13.05)  | 0.5              |
| Temperature    | ºC   | 30.08-31.55 (30.80) | 26.62-28.58 (27.60) | 29.09-29.72 (29.40) | 28.9-29.91 (29.40) | 27.15-27.35 (29.65) | 27.35-27.83 (27.83) | 18.3-27.3 (22.76) |
| Dissolved Oxygen| mg/l | 7.33 (6.25)  | 6.04-6.38 (5.32) | 3.60-6.41 (5.32) | 4.74-6.14 (5.53) | 5.66-7.23 (6.47) | 4.70-6.31 (5.38) | 6.16-6.56 (6.36) |

Note: - No data; ( ) average

3.1.2. Water environmental conditions. The condition of aquatic environment based on six parameters in the research locations shows that these waters still support aquatic fauna, including mangrove horseshoe crab resources related to living habitats and food sources, where the temperature ranged from 18.3-32.15ºC (average 28.21ºC), salinity ranged from 0.5-31‰ (average 15.55‰), dissolved oxygen ranged from 3.60-7.33 mg/L (average 5.98 mg/L), and water transparency ranged from of 10-380 cm
The depth of the waters ranged from 0.6 to 1.8 m and was strongly influenced by daily tidal dynamics, while the bottom substrate for most of the area was dominated by mud (St. 2, St. 5), and muddy sand (St. 1, St. 3 and St. 4), only St. 6 that the substrate dominated by sand (table 1 and 2).

The results showed that the *T. gigas* species were only found limited to the estuary areas which had the lowest salinity range up to 14‰. Another specific characteristic that is quite light is the typology of the substrate as its habitat. This species found in locations that have a muddy sand substrate with a silt percentage more than 50%. However, salinity is thought to be a limiting factor compared to substrate types for the distribution of this species, as explained in previous studies that the Asian Limulidae *T. tridentatus* and *T. gigas* live in shallow and calm seas, to river estuaries with muddy sand bottoms [63].

### Table 2. Percentage of substrat fractions and substrat organic matter.

| Parameters                  | Unit | Category | Stations |
|-----------------------------|------|----------|----------|
|                             | %    |          | St.1  | St.2  | St.3  | St.4  | St.5  | St.6  | St.7  |
| Substrat fractions          |      | Sand     | 33,75 | 17,15 | 31,17 | 43,26 | 24,28 | 84,92 | -     |
|                             |      | Silt     | 30,22 | 52,04 | 44,07 | 31,57 | 56,71 | 8,46  | -     |
|                             |      | Clay     | 36,03 | 30,81 | 24,76 | 25,17 | 19    | 6,62  | -     |
| Substrat organic matter      | %    |          | 2,76  | 2,89  | 2,84  | 2,48  | 2,74  | 0,92  | -     |
| Note: - No data             |      |          |        |       |       |       |       |       |

*C. rotundicauda* was found in all study sites. This condition is similar to the results from previous studies where *C. rotundicauda* have a wider range habitat preference, that were brackish, muddy waters in mangrove ecosystems, and shallow beaches with soft sandy bottoms or large mud flats [54, 56, 63]. In this study, *C. rotundicauda* species were found from the end of estuary to the upstream estuary (salinity 0.5‰), and a wider range of muddy substrate type, between 8.46% to more than 50%, even more, frequent found in sandy mud type habitat with a balanced composition of sand and mud.

#### 3.2. Biology of horseshoe crab

##### 3.2.1. Food habit

The food habits of horseshoe crabs were analyzed from the stomach contents of the horseshoe crabs which have food inside. The number of stomach content analyzed were 4 individuals of *T. gigas* and 21 individuals of *C. rotundicauda*. *T. gigas* index of preponderance consisted of macrophyte (95.45%) and detritus (4.55%), while *C. rotundicauda* consisted of macrophyte (68.99%), molluscs (29.07%), annelids (1.54%), and corals (0.41%) (figure 5). In this study, the food composition of *C. rotundicauda* was more varied compare to *T. gigas*. These results are similar to research conducted by Nuraisah et al. [27]. The index of preponderance is a useful tool to study the food preferences of organisms [42]. Nikolsky [51] distinguishes the food preferences of organisms into three categories, main foods with an Index of Preponderance of more than 25%, complementary foods between 5-25%, and supplementary foods less than 5%. The index of preponderance of *T. gigas* shows that its main food was macrophyte, and its supplementary food was detritus. *C. rotundicauda* has the main foods of macrophytes and molluscs, and supplementary foods of annelids and corals. Both of them have commonly used macrophytes as their main food. Recent studies among horseshoe crabs suggest a variety of foods, including particulate organic matter such as algae/macrophyte, may play a significant role in the diet of horseshoe crabs [64, 65, 66].

The index of preponderance of *T. gigas* in Brebes shows that its main food is macrophyte and supplementary food is detritus. Food habit of *T. gigas* in Balikpapan (Indonesia) consists of gastropods, bivalves, scaphopods, litter, and others with the main food being gastropods and bivalves [27]. The food composition of *T. gigas* in Cherok Paloh (Pahang, Malaysia) showed that echinoderms dominated food item during the open sea migration phase and the dominations were substituted by macrophyte as the *T. gigas* migrated inshore [25]. Hajisamae et al. [26] found that *T. gigas* in the east coast of Peninsular...
Malaysia have a high food preference on molluscs. Chatterji et al. [24] research showed that *T. gigas* was a selective feeder, and analysis of its stomach content in different months showed that molluscs and decayed organic matter were the main constituents of its diet. The major portion of the mollusca was contributed by bivalves. From July to October, the percentage of decayed organic matter, sand particles, and pieces of plant material was high in the stomach of *T. gigas*. Unidentified organic matter (mostly plant material) in decayed condition constituted about 28.0% of the total diet. It occurred regularly and throughout the year with a maximum contribution in October (50%).

**Figure 5.** Index of preponderance of *T. gigas* and *C. rotundicauda*.

The index of preponderance of *C. rotundicauda* in Brebes shows that its main foods are macrophyte and molluscs, supplementary foods are annelids and corals. The food habit of *C. rotundicauda* in Balikpapan (Indonesia) is consists of gastropods, scaphopods, bivalves, crustaceans, echinoderms, Polychaeta, litter, and others with the main food being gastropods and leaf litter [27]. In Sunderban Estuarine, *C. rotundicauda* feeds mainly on gastropods, bivalves, polychaetes, and decayed organic matter. Decayed organic matter consisting mainly of plant material [28]. Seasonal variations of the food composition of *C. rotundicauda* in Pahang (Malaysia) showed that mollusks formed the main item especially gastropods. Unidentified organic matters in the stomach content analysis of *C. rotundicauda* showed a high preference for plant materials [12]. Zhou & Morton [29] found that the food composition of *C. rotundicauda* juveniles from Pak Nai beach (Hongkong) consisted mainly of insect larvae, followed by polychaetes and amphipods.

The food compositions of horseshoe crabs were closely related by the abundance and availability of food in the environment [25]. The Malaysian peninsular land barrier effect has been shown in macrophyte [67, 68] and marine animals [69, 70], which depends on the water currents for their dispersal. In several studies, macrophytes were found to be the main food in the intestines of male horseshoe crabs [12, 24, 25, 28, 71, 72]. Sediment analysis encountered that the composition of macrophyte was higher in the beach area [25], so it is suspected that this affects the stomach contents of horseshoe crabs in coastal areas. Though horseshoe crabs are selective feeders, their adaptation to prefer a variety of macrobenthic communities (adaptive feeding) could have helped them in maintaining their population over time [73].

3.2.2. Niche breadth. Nice breadth describes the amount of food resources that can be utilized by a group of organisms and the selectivity of a type of organism between species and between individuals within the same species to food resources in certain habitats [44]. The niche breadth (*B_a*) between the *C. rotundicauda* and *T. gigas* two types of horseshoe crabs is different.
Table 3. Niche breadth of horseshoe crabs *T. gigas* and *C. rotundicauda* in Brebes.

| Species                  | Nice Breadth (Ba) |
|--------------------------|-------------------|
| *Tachypleus gigas*       | 0.10              |
| *Carcinoscorpius rotundicauda* | 0.78          |

*T. gigas* has a niche breadth (B_a) value of 0.10, lower than *C. rotundicauda* which has a value of 0.78 (table 3). Nice breadth of *T. gigas* in Brebes is low (B_a = 0.10), similar to the nice breadth of *T. gigas* in Balikpapan (B_a = 0.15) [27]. The low value of nice breadth indicates *T. gigas* is selective in nature’s food preferences. This can be seen from the number of food types on *T. gigas* less than *C. rotundicauda*. *T. gigas* has only two types of food (macrophyte and detritus). Low nice breadth characterizes that the species is selective towards the food resources available in the waters [44, 74]. The low value of nice breadth indicates that the species has a lower adaptability to the new environment [75].

The nice breadth of *C. rotundicauda* in Brebes is high (B_a = 0.78), higher than the nice breadth of *C. rotundicauda* in Balikpapan (B_a = 0.53). A high value of nice breadth indicates *C. rotundicauda* is a generalist species or not selective in choosing food resources in nature. *C. rotundicauda* has more food types than *T. gigas* in Brebes. *C. rotundicauda* has four types of food (macrophyte, molluscs, annelids and corals). A high value of nice breadth characterizes that the species is not selective towards the food resources available in the waters [44, 74]. The dietary pattern of an organism that is a generalist (high-value nice breadth) indicates that the species has high adaptability to new environments [75].

3.2.3. Niche overlap. The niche overlap value between *T. gigas* and *C. rotundicauda* is high (O_ijk = 0.97) (table 4). This niche overlap value illustrates the high competition opportunities between *T. gigas* and *C. rotundicauda* according to the classification by Moyle & Senanayake [46]. The niche overlap value between *T. gigas* and *C. rotundicauda* in Brebes is different from Balikpapan. In Balikpapan, the niche overlap value between *T. gigas* and *C. rotundicauda* has a small niche overlap value (O_ijk = 0.28) which indicates low competition in food utilization [27]. Niche overlap of food is an area of space-niches inhabited by two or more niche inhabitants [59]. If the value of the niche overlap approaches 1, it indicates that two species being compared have almost the same type of food [74]. Niche overlap occurs when two or more organisms use the same food resource so that it can describe the competition between organisms in an ecosystem. Sa *et al.* [76] has explained that niche overlaps can occur in similar organisms that utilize the same food resource. *T. gigas* has high competition against *C. rotundicauda* due to their high similarity in using macrophyte as the main food.

Table 4. The niche overlap value of *C. rotundicauda* and *T. gigas* in Brebes.

| Species                  | Nice Overlap (O_ijk) |
|--------------------------|----------------------|
| *Tachypleus gigas*       | *Carcinoscorpius rotundicauda* |
| *Tachypleus gigas*       | 0.97                 |

3.2.4. Egg. Males were dominant from both species, from 33 horseshoe crab, only four females horseshoe crab that caught in the field while the rest were males. Comparison of male and female from horseshoe crabs that found in the study site showed in table 5. Only three mature female horseshoe crab that can be observed in the laboratory, one *C. rotundicauda* (32.4 cm), and two *T. gigas* (44.8 and 47.2 cm).

The reproductive aspects of *T. gigas* and *C. rotundicauda* showed in table 6. Unfortunately, we have not been able to determine the spawning period of the two species due to the very limited number of samples. The GSI of *T. gigas* ranged from 0.79 to 14.33%, while the GSI of species *C. rotundicauda*...
was 22.1%. The research conducted by Chatterji [30] found that GSI from *T. gigas* ranged from 18.03 to 29.11%. with carapace length were 121-170 mm.

Table 5. Number of male and female from both species of horseshoe crab.

| Species name               | Female | Male |
|---------------------------|--------|------|
| *Tachypleus gigas*        | 3      | 5    |
| *Carniscorpius rotundicauda* | 1  | 24   |
| **Total**                 | 4      | 29   |

Table 6. The reproductive aspects of *T. gigas* and *C. rotundicauda* from Brebes waters.

| Species                          | TL (cm) | CL (cm) | GSI (%) | Fecundity (eggs) | Diameter (mm) |
|----------------------------------|---------|---------|---------|------------------|----------------|
| *Tachypleus gigas*               | 47.2    | 24.5    | 0.79    | 330              | 2.2-4.33       |
| *Carniscorpius rotundicauda*     | 44.8    | 22.3    | 14.33   | 4,109            | 2.0-4.03       |
| **Total**                        | 32.4    | 15.2    | 22.1    | 10,236           | 1.53-2.88      |

The fecundity of *T. gigas* ranged from 330 to 4,109 eggs, while *C. rotundicauda*’s fecundity was 10,236 eggs. It means the fecundity of *C. rotundicauda* is higher than *T. gigas*. The size of oocytes from both species showed different sizes, where the oocytes of *C. rotundicauda* is smaller than *T. gigas*. This result was similar to a study that has been conducted by Chatterji [30], that stated fecundity of *T. gigas* (maximum fecundity was 6565 eggs) is lower than *C. rotundicauda* (maximum fecundity was 10,982 eggs) and eggs diameter of *T. gigas* (ranged between 1.54 to 2.09 mm) is smaller than *C. rotundicauda* (1.59 to 2.35 mm). In a study conducted by Khan [28] states that *C. rotundicauda* has fecundity between 3,540 to 13,490 eggs. The fecundity of both *C. rotundicauda* and *T. gigas* increasing with body size and weight [28, 30, 31].

The mature females from both species found in estuarine area, with shallow water (Polang Canal and Mouth of west pemuda canal). Due to limited samples, we cannot conclude the spawning period and its location of the two species of horseshoe crab in Brebes waters. However, Ehlinger & Tankersley, 2009 [77] stated that the timing of spawning of horseshoe crabs varies among areas with different tidal regimes. According to Brockman& Smith [78], all four species of horseshoe crabs migrate to shallow water for spawning.

4. Conclusion

A total of 33 individuals (Eight *T. gigas*; 25 *C. rotundicauda*) of horseshoe crab were caught during the study. The condition of aquatic environment based on six parameters in the research locations shows that these waters still support aquatic fauna. Stations with a muddy sand substrate with a silt percentage of more than 50% have the highest number of captured horseshoe crabs. Food habits of *T. gigas* are macrophytes and detritus while *C. rotundicauda* are macrophytes, molluscs, annelids, and coral. Niche breadth of *C. rotundicauda*’s was wider than *T. gigas*. Niche overlap between *T. gigas* and *C. rotundicauda* was high that indicates high competition between them in utilizing food sources. The fecundity of *C. rotundicauda* was higher than *T. gigas* but the egg diameter of *C. rotundicauda* smaller than *T. gigas*.

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