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Influence of environmental variability on the body condition of the mangrove horseshoe crab *Carcinoscorpius rotundicauda* from Banyuasin Estuarine, South Sumatra, Indonesia

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Abstract
The body conditions indices were useful to determine an individual's well-being, and favorable food availability indicating a good environmental condition. The conditions of the mangrove horseshoe crab (*Carcinoscorpius rotundicauda*) might be related to several environmental parameters. The study's aim was to analyze the key environmental parameters affecting the body condition of *C. rotundicauda* found in Banyuasin Estuary Waters. The sampling was conducted in July 2019 in Banyuasin Estuary Waters. The data of weight and prosomal width for *C. rotundicauda* were used to estimate the body conditions indices (relative condition factor). While the environmental parameters data were recorded for each sampling site. The backward stepwise regression was used to determine the key environmental parameters affecting the body condition indices. The best-fitted model (adjusted $R^2 = 91.9\%$; $F = 60.102$; $p < 0.05$) indicated several environmental parameters (salinity, pH, seafloor temperature, clay and silt contents) significantly affecting the body condition indices. Changes in the key environmental parameters could generate changes in the availability of favorable food for *C. rotundicauda*. The result also can be used as baseline data for determining the marine conservation areas as well as horseshoe crab management plan.

Key words: Banyuasin Estuarine, Body condition, *Carcinoscorpius rotundicauda,* Environmental variability.

Introduction

In ecological studies, a body condition is used to determine an individual's nutritional or physiological status as well as evaluate the stored energy quantity, and indicate an individual's well-being (Bolger and Connolly 1989; Stevenson and Woods 2006; Pablo et al. 2015). The body condition indices become a useful tool to estimate the fish body with favorable food availability indicating a good environmental condition, a habitat quality as well as estimate fish and population abundances (Bennet 1970; Blackwell et al. 2000; Arismendi et al. 2011; Pablo et al. 2015).

Several factors (endogenous or exogenous) were related to the fish condition. The major endogenous factor affecting the fish conditions were sex, reproductive state, and age while the exogenous factors were parasitism as well as environmental conditions or food availability (Murphy et al. 1990; Pablo et al. 2015). The several environmental parameters (salinity, DO, temperature, and the percentage of pollutants) were influencing the larval development rate of horseshoe crabs (Botton et al. 2010).

There are three horseshoe crabs in Asia which one of them is known as the mangrove horseshoe crab (*Carcinoscorpius rotundicauda*), and their distribution includes India, Philippines, Japan, Korea, China, Thailand, Malaysia, Singapore, and Indonesia (Cartwright-Taylor et al. 2009; Cartwright-Taylor et al. 2011; Chen et al. 2015). Since 1996, the IUCN red list reported that the category and criteria of *C. rotundicauda* were data deficient (World Conservation Monitoring Centre 1996). In Indonesia, the Asian horseshoe crabs are the protected genetic resources (Minister of Forestry Decree No. 12/Kpts-II/1987 and Government Regulation No. 7/1999). In Banyuasin Coastal Waters, the horseshoe crab was found as a discard catch for the trammel net fishing (Fauziyah et al. 2018a). The first investigation of the horseshoe crabs from these waters and their morphometric variability have been reported (Fauziyah et al. 2019b; Fauziyah et al. 2019c) however the body condition and their environmental variability have not been analyzed.

These species have a high risk of species extinction due to the threat combination of the high exploitation levels and the high degradation level of habitat. Salinity, pH, temperature, DO and substrate type were the environmental factors that influenced their spawning activity (Nelson et al. 2016; Jawahir et al. 2017).

The study's aim was to analyze the key environmental parameters affecting the body condition of *C. rotundicauda* found in Banyuasin Estuary Waters. The study results were expected to provide important information for the conservation management of horseshoe crabs concerning the main environmental parameters influencing the horseshoe crabs conditions of well-being.

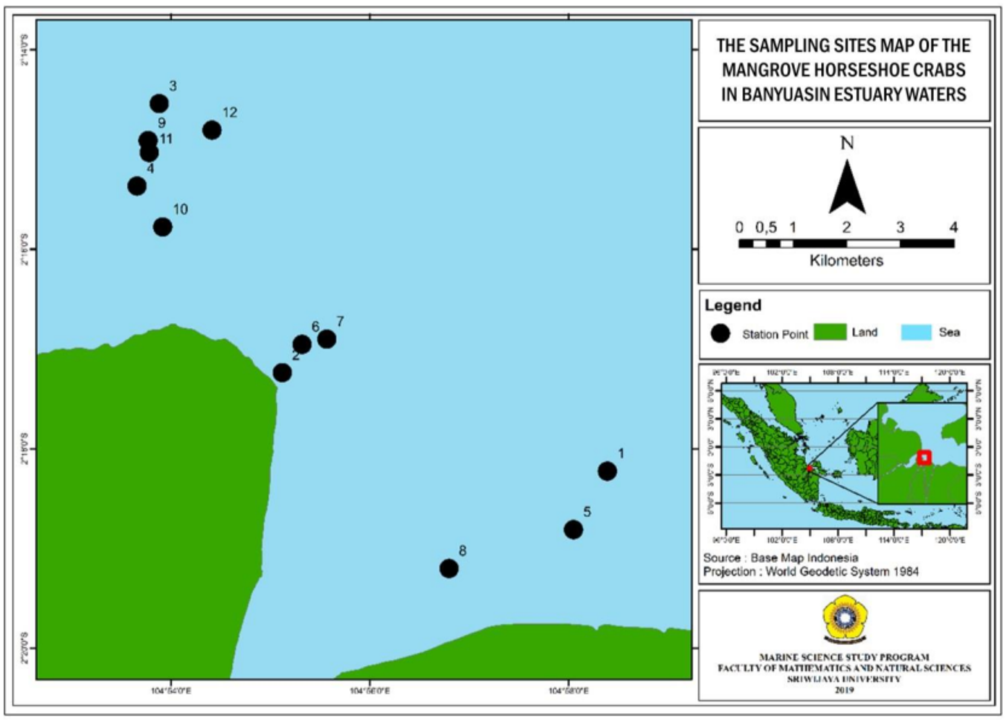

Figure 1. The sampling sites for capturing *C. rotundicauda* in Banyuasin Estuary Waters. The sampling was conducted together with local fishermen using a trammel net.

Material and methods

Sampling

The sampling location (Figure 1) was part of investigation survey of the horseshoe crabs from Banyuasin estuaries which were conducted in July 2019 (Fauziyah et al. 2019b) and the sampling sites (stations) were determined purposively based on the fishermen's experience when capturing the horseshoe crabs as a discarded catch. Identification and morphometric measurement of the horseshoe crabs refer to the previous study (Cartwright-Taylor et al. 2009; Tanacredi et al. 2009; Yang and Ko 2015; Fauziyah et al. 2019b; Fauziyah et al. 2019c).

In addition, salinity, temperature, and DO samples were taken using a water sampler and then measured using a refractometer, digital thermometer, and DO meter respectively. Whereas the sediment samples were taken using an Ekman grab and then would be analyzed on Laboratory.

Data Analysis

The wet sieving technique was used to determine the size grains of the sediment samples (Haris et al. 2015). Substrates texture was analyzed using Shepard's triangular diagram for the samples containing silt, clay, and sand (Costa et al. 2013; Ningsih and Supriyadi 2013; Fauziyah et al. 2018b).

The body condition indices of *C. rotundicauda* were estimated using the relative condition factor (Le Cren 1951; Blackwell et al. 2000; Froese 2006; Pablo et al. 2015):

$$K_n = \frac{W}{W_r}$$

$$W_r = aL^b$$

where K_n is the relative condition factor, W is the actual weight in grams, W_r is the predicted weight from the weight-prosomal width relationship and L is the prosomal width in millimeters. This equation was especially for the species with allometric growth (Fauziyah et al. 2020). To avoid any influence of sex factors that might affect the horseshoe crab condition, The K_n values would be calculated separately between females and males. This equation used for the species with an allometric growth

In addition, the stepwise multiple regression (Fauziyah et al. 2019a) was used to determine the key environmental parameters affecting the body condition indices. A backward selection approach was used to determine the fitting regression models. For this analysis, the dependent variable was the relative condition factor (Y) while depth (X_1), salinity (X_2), pH (X_3), DO (X_4), sea bottom temperature (X_5), sand percentage (X_6), clay percentage (X_7), and silt percentage (X_8) were independent variables. The SPSS software was used for all statistical analyses with a significant level of 0.05.

Results

Body condition indices

During the observation period, 12 sampling sites were determined, and a total of *50 C. rotundicauda* were collected (Table 1). The body weight-prosomal width relationships and the body condition indices (K_n) for both sexes were shown in Figure 2 and Figure 3. Figure 2 showed a negative allometric growth for males ($b < 3$, $p < 0.05$) and an isometric growth for females ($b = 3$, $p < 0.05$). The body weight (BW) of males ranged from 70 gram to 294 gram while the BW of females ranged from 100 gram to 880 gram.

Furthermore, Figure 3 showed variation in the K_n value for both sexes by the prosomal width. The K_n values for males range from 0.83 - 1.21 with their mean values of 0.97 while females range from 0.83 - 1.27 with their mean value of 1.07. The K_n values for both males and females were significantly different ($p < 0.05$) based on t-Test. The most K_n values for males (60%) were less than 1 but on the contrary, the most K_n values for females (67%) were greater than 1. These values indicated that the female's body condition was more well-being than males.

Table 1 presented a variation in the K_n values (dependent variable) and the environmental parameters (independent variables). The K_n values for both males and females fluctuated from 0.83 to 1.27 with a mean value of 1.04. The highest K_n value was found at station 6 (K_n=1.27) with the value of the environmental parameter for depth (X_1), salinity (X_2), pH (X_3), DO (X_4), sea bottom temperature (X_5), sand percentage (X_6), clay percentage (X_7), and silt percentage (X_8) were 6.25 m, 20.6‰, 7.62, 6.73 mg/l, 31.1°C, 20.86%, 2.85%, and 76.29% respectively. Conversely, the lowest K_n value was found at station 3 (K_n=0.83) with the value of the environmental parameter for depth, salinity, pH, DO, sea bottom temperature, sand percentage, clay percentage, and silt percentage were 2.6 m, 20.6‰, 7.89, 5.93 mg/l, 28.5°C, 12.09%, 11.52%, and 76.39% respectively.

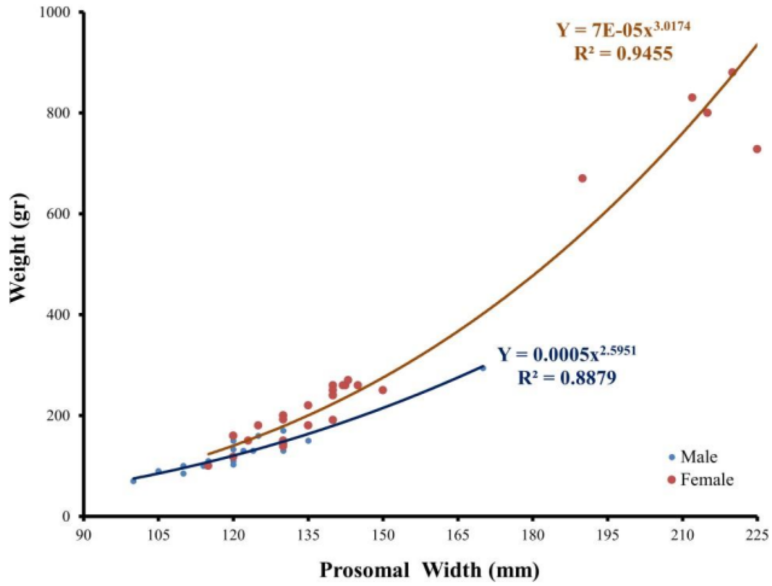

Figure 2. The prosoma width-weight relationship of *C. rotundicauda* from Banyuasin Estuary Waters. There was a different growth pattern for both sexes where males indicated negative allometric and females indicated isometric.

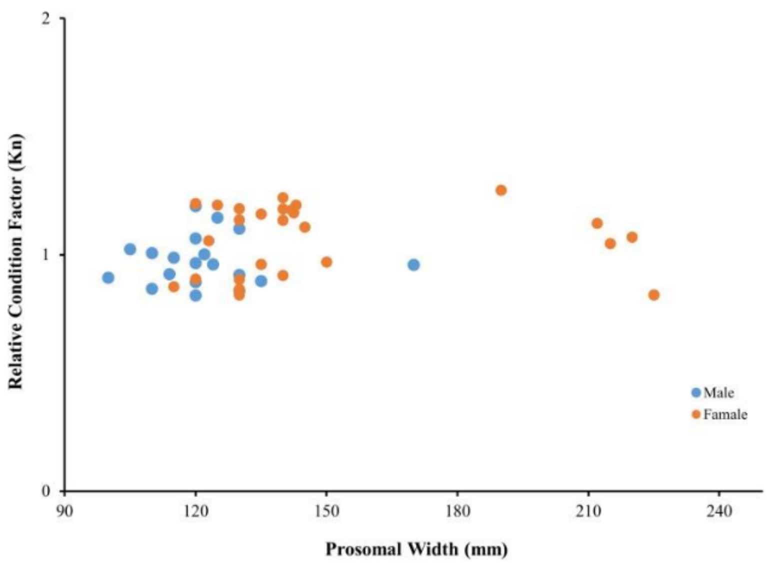

Figure 3. The relative condition factor (Kn) of *C. rotundicauda* from Banyuasin Estuary Waters. There was a significant difference between Kn values for males and females at a significant level of 0.05.

Table 1. The relative condition factor of *C. rotundicauda* from Banyuasin Estuarine and environmental parameters at each station. These data would be analyzed using a backward stepwise regression.

Station	Y	X1	X2	X3	X4	X5	X6	X7	X8
1	1.02 M	1.93	20.67	7.7	5.9	28.2	9.91	5.45	84.64
2	1.21 M	1.6	19.67	7.6	6.13	28	29.01	6.77	64.22
3	0.83 M	2.6	20.6	7.89	5.93	28.5	12.09	11.52	76.39
4	1.01 M	0.55	20.33	7.99	6.23	30.2	24.89	6.14	68.97
5	1.00 M	1.45	20.8	7.9	6.06	28.3	29.18	8.94	61.88
6	0.96 M	6.25	20.6	7.62	6.73	31.1	20.86	2.85	76.29
8	1.16 M	1.4	18.96	7.75	7.5	29	30.67	6.59	62.74
9	0.88 M	1.85	17.6	7.96	6.6	30.4	19.95	6.26	73.79
10	0.85 M	1.5	19.3	7.93	6.63	31.3	9.49	12.68	77.83
10	1.01 M	1.5	19.3	7.93	6.63	31.3	9.49	12.68	77.83
11	0.92 M	1.5	20	8.09	6.76	29.6	38.2	12.5	49.3
11	0.90 M	1.5	20	8.09	6.76	29.6	38.2	12.5	49.3
11	0.91 M	1.5	20	8.09	6.76	29.6	38.2	12.5	49.3
11	0.99 M	1.5	20	8.09	6.76	29.6	38.2	12.5	49.3
11	1.11 M	1.5	20	8.09	6.76	29.6	38.2	12.5	49.3
11	0.97 M	1.5	20	8.09	6.76	29.6	38.2	12.5	49.3
11	0.89 M	1.5	20	8.09	6.76	29.6	38.2	12.5	49.3
11	0.96 M	1.5	20	8.09	6.76	29.6	38.2	12.5	49.3
11	0.86 M	1.5	20	8.09	6.76	29.6	38.2	12.5	49.3
12	1.07 M	1.6	20.67	8.1	6.67	31.2	20.57	9.08	70.35
1	1.19 F	1.93	20.67	7.7	5.9	28.2	9.91	5.45	84.64
3	1.22 F	2.6	20.6	7.89	5.93	28.5	12.09	11.52	76.39
3	1.18 F	2.6	20.6	7.89	5.93	28.5	12.09	11.52	76.39
6	1.07 F	6.25	20.6	7.62	6.73	31.1	20.86	2.85	76.29
6	1.27 F	6.25	20.6	7.62	6.73	31.1	20.86	2.85	76.29
7	1.13 F	4.7	19.67	7.61	6.9	28.9	19	6.75	74.25
7	1.05 F	4.7	19.67	7.61	6.9	28.9	19	6.75	74.25
9	1.19 F	1.85	17.6	7.96	6.6	30.4	19.95	6.26	73.79
9	1.15 F	1.85	17.6	7.96	6.6	30.4	19.95	6.26	73.79
9	1.24 F	1.85	17.6	7.96	6.6	30.4	19.95	6.26	73.79
10	1.22 F	1.5	19.3	7.93	6.63	31.3	9.49	12.68	77.83
10	1.19 F	1.5	19.3	7.93	6.63	31.3	9.49	12.68	77.83
10	1.17 F	1.5	19.3	7.93	6.63	31.3	9.49	12.68	77.83
10	0.86 F	1.5	19.3	7.93	6.63	31.3	9.49	12.68	77.83
10	1.21 F	1.5	19.3	7.93	6.63	31.3	9.49	12.68	77.83
11	0.96 F	1.5	20	8.09	6.76	29.6	38.2	12.5	49.3
11	0.90 F	1.5	20	8.09	6.76	29.6	38.2	12.5	49.3
11	1.19 F	1.5	20	8.09	6.76	29.6	38.2	12.5	49.3
11	0.96 F	1.5	20	8.09	6.76	29.6	38.2	12.5	49.3
11	1.12 F	1.5	20	8.09	6.76	29.6	38.2	12.5	49.3
11	1.06 F	1.5	20	8.09	6.76	29.6	38.2	12.5	49.3
11	1.15 F	1.5	20	8.09	6.76	29.6	38.2	12.5	49.3

Station	Y	X1	X2	X3	X4	X5	X6	X7	X8
11	0.97^{F}	1.5	20	8.09	6.76	29.6	38.2	12.5	49.3
11	1.21^{F}	1.5	20	8.09	6.76	29.6	38.2	12.5	49.3
11	0.83^{F}	1.5	20	8.09	6.76	29.6	38.2	12.5	49.3
11	0.85^{F}	1.5	20	8.09	6.76	29.6	38.2	12.5	49.3
11	0.91^{F}	1.5	20	8.09	6.76	29.6	38.2	12.5	49.3
12	1.15^{F}	1.6	20.67	8.1	6.67	31.2	20.57	9.08	70.35
12	0.90^{F}	1.6	20.67	8.1	6.67	31.2	20.57	9.08	70.35
12	0.83^{F}	1.6	20.67	8.1	6.67	31.2	20.57	9.08	70.35

Note: the dependent variable was the relative condition factor (Y) while depth (X_1), salinity (X_2), pH (X_3), DO (X_4), sea bottom temperature (X_5), sand percentage (X_6), clay percentage (X_7), and silt percentage (X_8) were independent variables. Superscript on Y indicated males (M) and females (F)

Key environmental parameters

In general, the environmental conditions varied at all sampling sites. The depth waters ranged from 0.55 to 6.25 m, the salinity ranged from 17.60‰ to 20.8‰, pH ranged from 7.6 to 8.1, DO ranged from 5.9 to 7.5 mg/l, the sea bottom temperature ranged from 28°C to 31.2°C. While the sand percentage ranged from 9.49% to 38.2%, the clay percentage ranged from 2.85% to 12.68%, as well as the silt percentage ranged from 49.3% to 84.64%. Based on Shepard's triangular analysis, there were two types of substrates, namely Clay and Sandy Clay.

The results of filtering the environmental parameters using backward stepwise regression were presented in Table 2. Based on the method, some environment parameters (Depth, % sand, and DO) were removed from the multiple regression model. Thus, the best-fitted model was expressed as follows:

$$Y = 3.816 - 0.128X_2 - 0.474X_3 - 0.06\,X_5 + 0.022X_7 + 0.035X_8$$

Table 2. The resume results of backward stepwise regression that used to determine the key environmental parameters affecting the body condition of *C. rotundicauda* from Banyuasin Estuarine. Some independent variables (Depth, % sand, and DO) were removed to obtain the best-fitted model.

Model	Adjusted R^2	F-test		β_i	t-test	
		F	Sig.		t	Sig.
Regression	0.919	60.102	0.000*			
a (constant)				3.816	7.868	0.000*
X2 (salinity)				-0.128	-5.637	0.000*
X3 (pH)				-0.474	-3.058	0.006*
X5 (bottom temperature)				-0.06	-2.1	0.048*
X7 (% Clay)				0.022	3.781	0.001*
X8 (% Silt)				0.035	8.999	0.000*

* Significance level at 5%

The coefficient of determination (adjusted R^2) was 0.919 (91.9%) that indicated 91.1% of the body condition for *C. rotundicauda* (K_n) could be explained by the key environmental parameters (salinity, pH, bottom temperature, clay percentage, and silt percentage), while 8.1% was explained by other factors. The F-Test result ($F = 10.103$; $p < 0.05$) indicated that in simultaneously, the key environmental parameters affected the body condition of *C. rotundicauda*. The t-Test results indicated that partially, each of the key environmental parameters significantly influenced the body condition of *C. rotundicauda* ($p < 0.05$). Thus, the key environmental parameters that influenced the body condition of *C. rotundicauda* were salinity, pH, bottom temperature, clay percentage, and silt percentage.

Discussion

The body condition of *C. rotundicauda* varied in line with variations in environmental parameters at each sampling site. In this context, it's assumed that heavier horseshoe crabs of a given prosomal width were in better condition. This index (condition factor) has been believed to be a good indicator of well-being “fitness” of the fish population (Bolger and Connolly 1989). The body condition indices were also useful in comparing single-specific populations that living in similar/different conditions, establishing the timing and duration of gonad maturation, as well as indicating changes in the gross nutritional balance during the food supply activities (Stucky and Klaassen 1971; Chang and Navas 1984; De Silva 1985; Bolger and Connolly 1989).

The highest body condition indices for *C. rotundicauda* was found at station 6 where represented the habitat closest to the mangrove area. Conversely, *C. rotundicauda* with the lowest body condition indices was found at station 3 that represented an environment condition near the offshore. The environmental parameters were also different for both locations either related to depth, salinity, temperature, pH, DO or the substrate types. This result showed that environmental parameters might be able to influence the variation in the body condition indices due to differences in food availability. But not all environmental parameters have a significant effect because the horseshoe crabs have a different tolerance limit for every environmental parameter. The environmental parameters were not the single factor that influenced this index. The maturity stages and sex were also significantly affecting the horseshoe crab's weight and its Length-weight relationships (Graham et al. 2009).

In the contexts of the environmental biology and conservation (Stevenson and Woods 2006), the condition indices were useful for the key drivers of environmental degradation (pollution, climatic change, and habitat loss), life history patterns (migration, juvenile survival, and reproduction) as well as ecological interactions of threatened/endangered species (diet and density, social dominance, and parasite contents).

The best-fitted model that selected using the backward stepwise regression explained 91.9% of the variance (adjusted R^2) for the body condition of *C. rotundicauda* (K_n) could be explained by the key environmental parameters (salinity, pH, bottom temperature, clay percentage, and silt percentage). The excellent model performance has been produced as indicated by the value of adjusted R^2 greater than 85% (Makungo and Odiyo 2017). The sand percentage, depth and DO were removed from the model due to their correlation was not significant with the body condition indices.

Highly value of the condition factor was related to the high percentage of silt and clay (positive correlation), as well as linked with low the seafloor temperature, salinity, and pH (negative correlation). Fluctuation in sea temperature might be influencing the physiological stress or changes in prey availability (Lloret et al. 2013). The temperature indicated planktonic productivity, but on the other hand, the warmer temperatures could reduce upwelling and water mixing (Calvo et al. 2011), avoiding horseshoe crabs to obtain sufficient food resources. The sea temperature affected the development rate of horseshoe crabs (Ehlinger and Tankersley 2004) and became one of the key factors affecting their movement patterns (Wada et al. 2016). For their embryonic development, the optimal temperature was between 25–30°C (Laughlin 1983).

All species of Asian horseshoe crabs were mostly found at lower salinity (Chiu and Morton 2004; Vestbo et al. 2018). Although *C. rotundicauda* could be adapting from the low salinity to the high salinity gradually (Liao et al. 2011), the high abundance of *C. rotundicauda* probably linked to low salinity (Jawahir et al. 2017). And only in conditions of the salinity and temperature extreme influence the survival rate of horseshoe crabs (Ehlinger and Tankersley 2004). For the embryonic development of the horseshoe crab *Limulus polyphemus*, their larvae survived between 10 to 70 ppt and the optimal salinity conditions were between 30-40 ppt (Ehlinger and Tankersley 2004). In laboratory experiments, the optimal salinity conditions for *T. gigas* were between 25-35 ppt (Zaleha et al. 2011). Salinity was one of the key factors probably could control the molting of horseshoe crabs (Chatterji et al. 2014).

Most aquatic orgasms were sensitive to changes in pH values, the ideal pH value for horseshoe crabs was between 6.5-8.5 (Gunarto 2004; Mahfud et al. 2017). In the present study, the pH variation (7.61-8.1) still under ideal conditions for the horseshoe crabs. Statistically, pH was negatively correlated to the body condition which indicated that decreasing the pH value (closer to 7) had an impact on improving the body condition of horseshoe crabs. When the crabs live in conditions of pH 7, the stress level was low thus the pH 7 was the optimum condition for crabs (Hastuti et al. 2016).

According to these study results, the higher contents of clay and silt in the seafloor would be affecting the higher value of the body condition indices. This result indicated that *C. rotundicauda* prefer to live in close to the mangrove area (muddy and brackish areas) similar with the common natural habitat of *C. rotundicauda* in others location (Robert et al. 2014; Jawahir et al. 2017). Mudflats also were important maturation grounds for juvenile and sub-adult *C. rotundicauda* (Chen et al. 2015). During its life cycle, Juveniles *T. tridentatus* forage on mudflats, grow and mature to be adults in deeper subtidal areas, then spawn on open, sandy beaches close to the high-tide zone (Sekiguchi et al. 1988; Chen et al. 2004; Almendral and Schoppe 2005; Chen et al. 2015).

Besides, change in the key environmental parameters could generate the supply of unfavorable food for *C. rotundicauda*. The key environmental parameters also can be used as baseline data for determining the marine conservation areas as well as the horseshoe crab management plan. For maximizing conservation results, understanding of predator and their impacts was required (Nordberg et al. 2019).

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