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Analysis of Substrate, Gastric Content and Nutrient Content of *Varuna litterata*

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Abstract: *Varuna litterata* is a secondary freshwater crab found in tidal areas. This crab is caught, its eggs are taken, and it is considered a delicacy. Continuous fishing without any cultivation efforts can cause a decline in the population, leading to extinction. This study aims to determine the characteristics of the habitat substrate, stomach, and nutritional content of *V. litterata*. The method used in this study is descriptive exploratory. The study was conducted in the Serayu River for four months (October 2023 - January 2024). The observation location was carried out at three stations. The study showed that *V. litterata* prefers waters with clay sand substrates (75% sand, 25% dust, and 0% clay). This habitat is very suitable for cultivation development, as shown by the population of captured crabs. The results of observations of stomach contents show that this crab is an opportunistic omnivore. This crab has a high nutritional content with an average water content (60.72%), ash (4.57%), fat (0.52%), protein (25.66%), and carbohydrates (8.52%). Further research is essential to understand the reproductive system of *V. litterata*, its life cycle, and suitable habitats for spawning.

Keywords: Nutrient content; Serayu River; Stomach contents; Substrate; *Varuna litterata*

Introduction

Crustaceans belonging to the infraorder Brachyura show extraordinary diversity and inhabit aquatic environments worldwide, with a wide geographic distribution (Farhadi & Harlioglu, 2018; Kawai & Cumberlidge, 2016). There are more than 7,200 species that have been identified in 104 families (Ma et al., 2019; Lau et al., 2021; Cui et al., 2021). The Brachyura infraorder includes three major ecological categories: marine crabs, secondary freshwater crabs, and primary freshwater crabs. Sea crabs remain in the sea throughout their life cycle. In contrast, secondary freshwater crabs, also known as "partially freshwater crabs", begin their initial development in the sea before moving to freshwater habitats. In contrast, primary freshwater crabs inhabit freshwater ecosystems exclusively throughout their lifetime (Vogt, 2016).

Members of the Varunidae family are a type of secondary freshwater crab that is often found in the intertidal zone (Hobbs et al., 2017; Lapolo et al., 2018; Hsu & Shih, 2020; Shih et al., 2019). Currently, there are around 160 species belonging to the Varunidae family, distributed in 38 genera in various parts of the world (Cuesta & González-Gordillo, 2020). One of the genera included in the Varunidae family is Varuna, which consists of two species, namely *Varuna litterata* (Fabricius, 1798; Devi et al., 2013) and *Varuna yui* (Hwang & Takeda, 1986; Lin et al., 2018). In certain nations like the Philippines, *V. litterata* is captured for commercial purposes and utilized as a dietary option in countryside regions (Taguiam et al., 2022). Crab eggs stuck to the female's stomach are considered a delicacy. Thailand is also actively fishing for this crab, which can usually be found in markets during the spawning season. Consumption of *V. litterata* crabs in Thailand reaches around 18,000 tons per year, but only around

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12,000 tons can be produced domestically (Jumawan et al., 2022). Therefore, around 6,000 tons of *V. litterata* must be imported annually from Myanmar and Cambodia (Suppapan et al., 2017). Unsustainable fishing can threaten crab populations, as happened in the Philippines (Subang et al., 2020). Cultivation efforts are needed to maintain the *V. litterata* population. The International Union for Conservation of Nature (IUCN) has also highlighted that *V. litterata* is among the species facing endangerment (Palomares MLD, 2022).

Although much research has been conducted on the global brachyuran crab fauna, relatively little research has been conducted on *V. litratata*, especially concerning Indonesia. Susilo et al. (2020) carried out research on *V. litterata* regarding new records of *V. litterata* from Meru Betiri National Park, Habitat of *V. litterata* in Bengawan Solo (Fariedah et al., 2023), but research on habitat ecology, analysis of stomach content, and nutritional content has not been carried out.

Knowledge of the habitat, feeding habits and nutritional content of a crab species is very important to understanding its nutritional needs and interactions with other animal groups (Rady et al., 2018; Ben et al., 2019; Paul et al., 2018). This information is also useful for successful cultivation. This information can help us understand the role of crabs in the food chain and their potential impact on the ecosystem (Marchessaux et al., 2023). Additionally, it can provide insight into the nutritional value of crabs as a food source, which is important for human health and fisheries sustainability (Nanda et al., 2021; Yogesh Kumar et al., 2019). Crustaceans dwell in various ecological niches and populate diverse environments across different parts of the world, which is evident in their wide array of dietary preferences (Prado et al., 2022). The majority of species continue to adhere to this dietary pattern, with uncommon instances of extreme specialization. Nonetheless, many crab species maintain their capacity to consume a diverse range of foods, displaying a preference for specific types, like grapsids favoring herbivory and portunids predominantly opting for carnivory (Wang et al., 2020; Udoh & Jimmy, 2015).

Several reports have been made on the diet and feeding behaviour of several species, including *Scylla serrata* (Paul et al., 2018), *Portunus segnis* (Ben et al., 2019), *Callinectes sapidus* (Rady et al., 2018), *Opilio Chionocetes* (Burukovsky et al., 2021), as well as analysis of the nutritional content of crabs from *Calinetes sapidus* (Zotti et al., 2016), *Scylla paramosain* (Yusof et al., 2020), *Eriocheir sinensis* (Czerniejewski et al., 2023). However, information about the habitat preferences, diet, type of food preferred and nutritional content of *V. litterata* is still limited. Therefore, a study was conducted to understand the habitat ecology, feeding habits and nutritional content of *V. litterata* in the lower reaches of the Serayu River.

Method

Location and Time of Research

This research was conducted in the downstream part of the Serayu River in Central Java, where a dam blocks the flow of water downstream. Monitoring is carried out at three points: 1) Upstream of the dam where the water is fresh. 2) Downstream of the dam where the water remains fresh. 3) Below the dam, the water transitions to brackish (Figure 1). The geographical coordinates of the research location are 07°01'52" - 07°31'54" South Latitude and 108°50'16" - 110°04'20" East Longitude. This research lasted for four months, from October 2023 to January 2024. Samples were collected using a gill net measuring 4 meters long and 1.5 meters high and a mesh size of 2 millimetres.

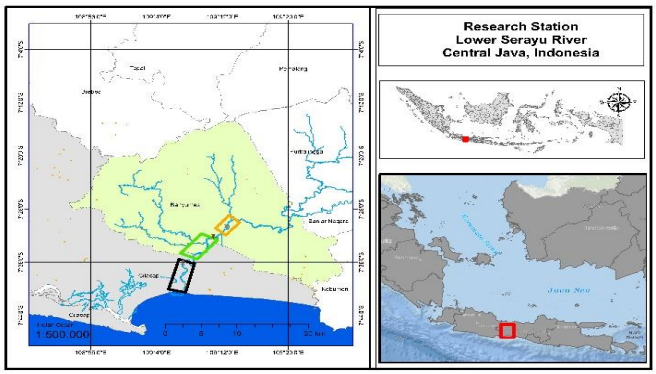

Figure 1. Research location. Station 1 (yellow box), station 2 (green box), station 3 (black box)

Habitat Substrate

Substrate observations were carried out by Sørensen et al. (2021), Zhu et al. (2022), Zhang et al. (2022), as the main approach. The specimens underwent a treatment involving dehydration at a heat of 220^{0}C for approximately four hours, followed by subsequent cooling and pulverization. The filtering process uses a Sieve Shaker using a Sieve Net measuring 0.063 mm. Samples that pass through the filter are categorized as silt and clay, while those that do not pass through are called sand. To determine the percentage of each sediment fraction, calculations are carried out using the following formulas:

- Percentage fraction of sand = (Total weight (g) / 25) x 100%.
- Weight of silt fraction = (a - b) + (b - c) + (c - d) + (d - e).
- Percentage of silt fraction = (Total weight of silt fraction (g) / 25) x 100%.
- Clay percentage = 100% - sand fraction percentage - mud fraction percentage.

Stomach Contents

Observation of the stomach contents of *V. litterata* was carried out as done by Rady et al. (2018): a.) Crustacean remains, such as body parts of shrimp, crabs and stomatopods. b) Fish remains, including fins, scales, bones and spine. c) Remains of molluscs, such as gastropods and bivalves. d) Other materials include algae filaments, nematodes, polychaetes, ophiuroides, and unknown objects. e) Dust and other small particles, such as sand and mud.

The occurrence rate of stomach contents was determined by dividing the count of stomachs containing a specific food category by the total observed stomach count. The method for calculating the percentage of each gastric content type's presence followed the procedure outlined in Devi et al. (2013). Percentage of appearance of prey i:

$$\sum_{j=01}^{n} aij/A) * 100\% \quad (1)$$

Where: aij: The number of *V. litterata* whose stomachs contain prey category j; A: The number of *V. litterata* observed along with their food in the stomach; and N: The total points for all crabs and all prey in all hulls examined.

Nutritional Content

The nutritional analysis of *V. litterata* followed the methodology outlined by Marques et al. (2010). Each sample (10 g in a Petri dish) underwent dehydration in an incubator (ULE500) at 105^0C until reaching a consistent weight. Ash content was determined by subjecting samples (10 g) to a 16-hour burn at 500^0C. The Kjeldahl method, with a conversion factor of 6.25, was applied to assess crude protein content in each sample (1 g), while total lipids were extracted via the Soxhlet hot extraction method using ethyl ether (AOAC, 2000). Energy content was computed as follows: protein, 4.27 kcal/g wet weight; lipids, 9.02 kcal/g wet weight; carbohydrates, 4.11 kcal/g wet weight, with 1 kcal being equivalent to 4.184 kJ (FAO, 1989).

Data Analysis

The acquired data underwent ANOVA analysis to identify variances, followed by a Tukey test to ascertain distinctions among data collected from different observation stations (Silaban et al., 2020).

Result and Discussion

Habitat Substrate

Crab samples were collected from three observation stations at a linear distance of ± 27,000 m from the mouth of the Serayu River. The research location has different ecosystem characteristics, which impact the substrate characteristics at each observation station (Table 1).

Table 1. Observation Station Substrate Characteristics

Station	Parameter	Results
01	Sand	3
	Dust	82
	Look	15
	Texture class	Dusty clay
02	Sand	75
	Dust	25
	Look	0
	Texture class	Clayy sand
02	Sand	7
	Dust	73
	Look	20
	Texture class	Dusty clay

Stomach Contents of V. litterata

The results of observations on the stomach contents of *V. litterata* showed that this type of crab consumes the remains of crustaceans, fish, plants, sand and mud, and various things (Figures 2 and 3). The study also showed that *V. litterata* had different stomach contents each month (Table 2). The environment where the crab resides affects its capability to capture prey.

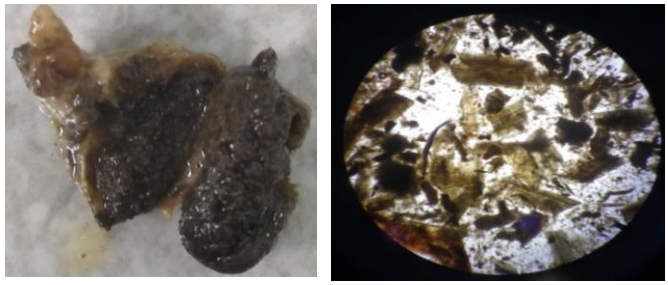

Figure 2. Appearance of the stomach contents of *V. litterata*

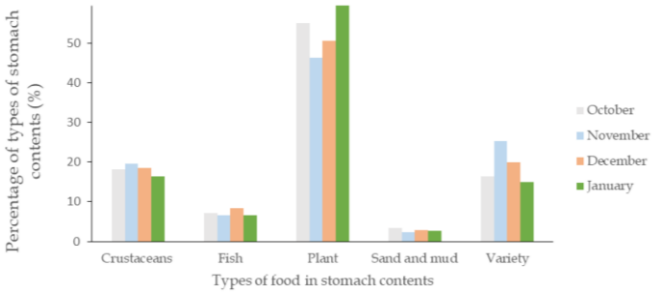

Figure 3. Types of food in the stomach contents of *V. litterata*

Table 2. Average Stomach Contents of *V. litterata*

Month	Average stomach contents STD (%)	Min (%)	Max (%)
October	79.90 ± 8.81^{c}	60.00	95.00
November	69.47 ± 14.03^{a}	40.00	98.00
December	71.37 ± 13.15^{ab}	35.00	90.00
January	76.13 ± 10.97^{bc}	50.00	95.00

Nutrient Content of V. litterata

Crabs are considered an important fisheries product among other invertebrates (Kunsook & Dumrongrojwatthana, 2017; Hungria et al., 2017). In this study, the proximate or nutritional composition (protein, fat, carbohydrate, ash, and water content) from three stations was analyzed. The chemical content in the *V. litterata* crab studied was quite high compared to other crabs (Table 3).

Table 3. Biochemical Content of *V. litterata*

Analysis Parameters	Station		Average (%)
	02(%)	03(%)	
Water	60.61	60.54	61.01	60.72
Ash	4.68	4.39	4.65	4.57
Fat	0.47	0.54	0.56	0.52
Proteins	25.67	25.65	25.67	25.66
Carbohydrate	8.58	8.88	8.11	8.52

Discussion

V. litterata substrate

Territorial behaviour involves all forms of interaction between animals and the area where they live, including claims of ownership, adjustment, and adaptation to their environment. It also includes relationships between the residents of the area and other individuals in or around it. A number of these factors are considered important in the study of territorial behaviour, as has been observed by Saher et al. (2018) and Arechavala-Lopez et al. (2019). In aquatic environments, especially decapods, territorial behaviour tends to be strongly influenced by the type of substrate around them. Particle characteristics and substrate hardness can influence how they dig burrows and the territorial behaviour strategies of *Portunus trituberculatus* crabs and mud crabs (*Scylla olivacea*) (Zhu et al., 2022; Waiho et al., 2015). Areas with inappropriate substrates can reinforce territorial behaviour in animals, which can lead to physical injury, death, and reduced survival (Darnell et al., 2020). Apart from that, the availability of substrate types in an area also greatly influences the life of crabs (Dapeng et al., 2019).

Research by Zhu et al. (2022) on the crab *Portunus trituberculatus* showed that sand substrate was effective in increasing survival due to its natural burying behaviour. Putri et al. (2022) on *Scylla serrata* and *Scylla olivacea* also showed that the type of substrate, such as clay, can influence burrowing behaviour and their abundance and growth.

Stomach Contents of V. litterata

In this research, it was noted that *V. litterata* displays opportunistic omnivorous behavior, primarily consuming vegetation in its stomach, alongside various crustaceans, fish, sand, and mud (Figure 3). Crabs are opportunistic, omnivorous animals that prefer animal foods and tend to have predatory characteristics (Krieger et al., 2016; Rady et al., 2018). This eating pattern still occurs in most crab species. Nevertheless, the majority of crab species retain the ability to ingest a diverse range of nourishment. Among them, certain species exhibit preferences; for instance, grapsids lean towards herbivorous diets, while portunidae predominantly favor carnivorous fare (Lee & Lee, 2022; Rasheed, 2018). A Safaie (2016) study also shows that crabs are opportunistic omnivorous animals that prefer animal prey but rarely eat more mobile prey, such as fish and shrimp. Ben Abdallah et al. (2019) also argue that crabs show primitive behaviour as opportunistic omnivores with predatory tendencies and a preference for animal food.

Another investigation carried out by Tadi-Beni et al. (2023) examined *Portunus pelagicus* from the Persian Gulf in Iran and Sadeghi et al. (2018) on crabs collected from the northwestern Persian Gulf showed that the food preferences of these crabs vary. However, the majority tend to consume crustaceans, fish and molluscs. Various studies also note that various types of crabs, such as *Scylla serrata*, *Callinectes sapidus*, and *Lithodes scintilla*, tend to consume a mixture of foods that include molluscs, crustaceans, fish, and polychaetes (Paul et al., 2018; Rady et al., 2018; Andrade et al., 2022).

The various types of stomach contents found in the stomach of *V. litterata* indicate that this crab is an omnivorous creature that consumes various plant and animal components. The majority of the detritus seen in the stomachs examined came from plants. Increasing information shows that the food chain in the lower reaches of the Serayu River mainly depends on detritus and organic matter. This shows that the plant remains found in the crab's stomach contents were most likely caused by leaf litter and river vegetation. Some types of grapsid crabs prefer leaf litter with a certain C/N ratio (Rani et al., 2023), while others consume a variety of food types (Rady et al., 2018). This research shows that *V. litterata* inhabits areas rich in organic carbon. Large amounts of sand particles in the gut seem to match the texture of the sediment at the study site because sand is the main component of the sediment at the three study sites with the most dominant *V. litterata* population. Thus, it can be concluded that many sand particles were found in the intestinal contents, probably accidentally ingested while seeking food from the Substrate.

Mollusks significantly contribute to the stomach contents of the majority of omnivorous crabs, such as *Plagusia depressa* (Santana & Carneiro, 2019), *Portunus segnis*, *Portunus sanguinolentus* (Rasheed, 2018) and *Scylla serrata* (Khan, 2018). However, in this study, no mollusc remains were found in the stomach of *V. litterata*. This could be caused by the strength of the

crab's shell, which may not be sufficient to crush mollusc shells, or perhaps the crab does not like food that consists of sedentary organisms such as molluscs. This is based on research conducted by Devi et al. (2013) in remote areas of Cochin, Kerala, India. Previous research conducted by Jiménez et al. (2023) also reported the absence of molluscs in the stomach of the crab *Callinectes sapidus*. In its native area, *Callinectes sapidus* is a generalist and omnivorous crab and can eat meat as a scavenger, shrimp and crab, and various plants. The crab's feeding pattern generally lasts throughout the year, except during shell moulting and mating periods when feeding stops or is minimal. Several studies, such as Rady et al. (2018) and Gommer et al. (2018), also reported crabs with empty stomachs during certain seasons, such as pregnant females, parasitized crabs, and newly moulted crabs. Additionally, food consumption patterns may vary depending on the reproductive cycle, with some crab species consuming more food after spawning while others consume less when not spawning.

The results of research conducted by Devi et al. (2013) showed variations in stomach contents in various size groups. The results of this study showed that the maximum number of empty stomachs among the specimens analyzed was in the carapace width size class 2.5 – 3.0. A crab with a size of 1.5-2.0 and a carapace width of 2.0-2.5 looks like its stomach is almost filled with food. Razali et al. (2020) reported that the feeding intensity of adult horseshoe crabs is relatively lower. Nguyen et al. (2022) stated that small crabs eat more intensively than large crabs because the efficiency of moulting in small crabs is greater, and energy requirements are greater. Differences in diet composition in different size groups may be related to changes in cheliped strength and foraging behaviour. The contents of the crab's stomach cannot be ascertained whether the prey was still alive or not when it was eaten. Dapeng et al. (2019) have explained the prey capture mechanism in portunid crabs (*Portunus trituberculatus*).

In this investigation, the quantity of food ingested by males and females showed no noteworthy significant, as Devi et al. (2013) reported. Crabs occupy various habitats in different geographic regions, resulting in variations in the type of food consumed. Crabs' foraging strategies vary, with the type of food consumed depending on the location where the crab lives. The nutritional makeup of crab sustenance may differ across various geographical regions encompassing both marine and estuarine environments and their respective habitats. Prey availability, ontogeny, and moulting stage can also influence crab diets. Crab size distribution can also influence diet, as crabs of different sizes inhabit different habitats and have different prey availability. Seasonal changes in prey availability can also result in changes in diet. Variability in crab diets is influenced by their habitat, as stated by Campbell et al. (2021).

Analysis of the crab's stomach contents shows that its habitat influences its diet and preferences. *V. litterata* consumes various foods, including crustaceans, plants, sand, etc. However, the most dominant food is plant remains, followed by miscellaneous and crustaceans. The presence of sand and debris in their hulls is related to the sandy substrate in which they live and reproduce. In addition, the presence of plant residue in his digestive tract could be attributed to foliage found along the Serayu River. These observations suggest that *V. litterata* exhibits opportunistic omnivorous behavior, showing an inclination towards consuming both animal and plant matter, particularly favoring plant-derived food..

Nutrient Content of V. litterata

The results of the analysis of the nutritional content of *V. litterata*/100g are still higher than research conducted by Avenue (2024) with nutritional content of water: 68.1%, ash: 6.2%, fat: 3.8%, protein: 13.8% and carbohydrates: 8.1%. The results of research on the nutritional content of *V. litterata* are higher than other brachyuran crabs, such as the results of research conducted by Paul et al. (2021) showing that mud crabs have a biochemical content of 15% - 25% protein, 1% fat and 2% - 3% minerals. Compared with other types of Brachyuran crab (*Portunus pelagicus*) carried out by Shibana et al. (2018) showed that the collected crabs' carbohydrate, protein and lipid content was 3.35g/100g, 23.8g/100g and 5.54g/100g. Kanwal et al. (2016) also reported the biochemical content of commercially important Brachyuran crabs (*Portunus pelagicus*, *Portunus sanguinolentus*, *Scylla serrata* and *Scylla olivaceae*) showing the percentage of protein in the crab *Portunus pelagicus* (24.45%) in *Scylla serrata* (22.49 %), *Scylla olivaceae* (22.15%) and in *Portunus sanguinolentus* (15.34%). Carbohydrate percentage values in *Scylla olivaceae* (2.82%), *Portunus pelagicus* (2.32%) and *Scylla serrata* (2.28%). Water content in *Portunus pelagicus* (23.2%), *Scylla serrata* (18.4%) and *Portunus sanguinolentus* (17.9%). The fat content of the crabs studied ranged from 8 - 9%.

The high protein content in *V. litterata* can play a role in supporting life. The high protein content functions as catalysis and transportation protects the immune system, helps form movement, controls impulses, and supports nerve growth (Kanwal & Saher, 2016). The protein content in crabs varies depending on environmental conditions such as pollution levels and other factors such as collection time, season, and food availability (Yu et al., 2022; Rekha et al., 2014). The high protein content of *V. litterata* makes this crab very popular in the Chaoshan region of China. This crab is processed into pickles, and research conducted by Wang

et al. (2021) demonstrates that the crude extract from this crab exhibits potent bacteriostatic properties against both gram-negative and gram-positive bacteria.

Conclusion

Observations in the Serayu River's lower reaches show that *V. litterata* prefers habitats with clayey sand substrates (75% sand, 25% dust, and 0% clay). The largest population of *V. litterata* indicates this found at station 02. *V. litterata* is an opportunistic omnivore, with plants being the most dominant stomach contents, followed by a variety of crustaceans, fish, and mud. This crab has a high nutritional content compared to other brachyuran crabs, with an average content of water (60.72%), ash (4.57%), fat (0.52%), protein (25.66%), carbohydrates (8.52%). Further research is essential to understand the reproductive system of *V. litterata*, its life cycle, and suitable habitats for spawning.

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Author Contributions
Each author played a role in completing this research activity. The main author provided the basic idea and research materials. The second and third authors designed the research methods. All authors were involved in data collection, table preparation and data analysis, the review process, and writing the article.

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Conflicts of Interest
The author declares that there is no conflict of interest in this research.

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