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**Bioscience Research**

**Print ISSN: 1811-9506 Online ISSN: 2218-3973**

***Journal by Innovative Scientific Information & Services Network***

**RESEARCH ARTICLE** BIOSCIENCE RESEARCH, 2020 17(4): 2976-2987. **OPEN ACCESS**

# Some Aspects of Biology of Terrestrial Crab, *Pudaengon wanonniwat* in Waeng Sub-district Sawangdandin District, Sakon Nakhon Province, Thailand

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The study of terrestrial crab, *Pudaengon wanonniwat* in the forests at Waeng Sub-district, Sawangdandin District, Sakon Nakhon Province, Thailand was determined in September 2017-June 2019. The habitat result found that they lived in the forests adjacent to rice-fields that were always watery in the rainy season. Stomach content analysis can be classified into six groups, i.e., insect, plant, snail, earthworm, phytoplankton and unidentified. The analysis of frequency percentage and food composition ratio was mainly insect group. Crab mating season indicated in November to December. Then spawning was beginning in January to May and hatched during mid of May. Their average carapace width (CW) and carapace length (CL) of brood stock were 5.01±0.80 cm, and 3.92±0.48 cm. For reproduction characteristics, crab fecundity was 68.18±23.18 with 200 crabs survive after hatching. Their average body weight (W), CW, and CL were 0.29±0.02 g, 0.87±0.16 cm, and 0.76±0.19 cm, respectively. After one year of culturing, their growth performance in terms of average of W, CW, and CL was 21.33±3.36 g, 3.91±0.51 cm and 3.14±0.16 cm, respectively. Growth performance analysis can be concluded from the relationships between W and CW, and W and CL equations. Their equations were $W = 1.462CW^{2.538}$ ($R^2 = 0.857$), and $W = 1.445CL^{3.028}$ ($R^2 = 0.926$).

**Keywords: *Pudaengon wanonniwat*, stomach content, mating season, spawning, growth performance**

## INTRODUCTION

*Pudaengon* sp. is a freshwater crab in the Potamidae and is classified into the terrestrial crabs which considered an endemic freshwater crab (Supajantra, 2002). Ng and Naiyanetr (1995) reported that four species of *Pudaengon* were found in Thailand. The first species, *P. mukdahan* was found in Na Si Nuan village, Mueang District, Mukdahan Province, while the second species, *P. sakonnakorn* was found in Chiang Khruea village, Mueang District, Sakon Nakhon Province. The third, *P. thatphanom* was found in Fung Daeng village, That Phanom District, Nakhon Phanom Province, and the fourth species, *P. wanonniwat* was found in Wanon Niwat District, Sakon Nakhon Province. Another three species were found in the Lao People's Democratic Republic, i.e., *P. khammouan*, *P. hinpoon* and *P. arnamicai*. Other than, *P. sakonnakorn* was found in Si Songkhram District, Nakhon Phanom Province, Akat Amnuai District, Kham Ta Kla District, Sakon Nakhon Province. *P. thatphanom* was found in Mueang, Nakhon Phanom Province. *P. wanonniwat was* found in Muang District, Sakon Nakhon Province (Supajantra, 2002). *Pudaengon* spp. was an endemic species that could be found in several areas, and their products were served for local consumptions. In some case, the excess productions were sold at several local markets, such as That Phanom District, Tha Uthen District, Mueang District, Nakhon Phanom Province, Mueang District, Sakon Nakhon Province, and Mueang District, Mukdahan Province markets. They have been popularly eaten as food in the villagers because of big size, high meat yield, and better taste than rice-field crabs. As a result, they can sell at a high price, which ranges 100-250 Baht/kg from local market survey compared to rice-field crabs 40-50 Baht/kg (Doolgindachabaporn et al., 2002). It was also revealed that Lao people had brought these crabs from their country to sell at several markets in Thailand, such as That Phanom District and Tha Uthen District, Nakhon Phanom Province. Because of the high demand, these crabs are at risk of being extinction. In addition, due to the problem of destruction, encroaching on forested areas as habitats, pollution that affects living, has led to the changes in ecological conditions caused the reduction in crab numbers in wild environment. Besides, they have direct and indirect benefits to the ecosystem, such as adding oxygen to the soil and increasing the soil's nutrients (Supajantra, 2002). Therefore, the biological study of these species is necessary.

Biological study of *Pudaengon* has indicated that it was similar to *Thaipotamon* species, both in the general shape of the carapace and physiognomy and in the structure of the guidelines. *Pudaengon* however, differs from *Thaipotamon* in having the carapace more rounded; the anterolateral regions mildly to distinctly rugose (vs. smooth); anterolateral margin distinctly cristate; a proportionately shorter exopod of the third maxilliped, with the tip not reaching, subequal or slightly over-reaching the inner distal edge of the merus; and the exopod of the third maxilliped completely lacks a flagellum (vs. presence of a short but distinct flagellum). However, to the best of our knowledge, there is no information regarding the reproduction characteristics of *Pudaengon* species. Therefore, the study on habitats, stomach content, mating season and spawning season, molting as well as relationship between body weight (W), carapace width (CW), and carapace length (CL) of *Pudaengon wanonniwat* was intended to study as new knowledge. This research can be used as guidelines for managing terrestrial crab resources and leading to culture, breeding well as conservation practices later.

## MATERIALS AND METHODS

The biological studies of *P. wanonniwat* were conducted in the field and the laboratory by studying and collecting the crab's samples from September 2017 to June 2019. The study areas and gathering relevant information were in the forested area of Na Ngua village, Non Sao Khwan village and Khok Si Khai village, Waeng Sub-district, Sawangdandin District, Sakon Nakhon Province, which was the inhabited of *P. wanonniwat*. This research was investigated at Fishery Program, Faculty of Agriculture and Technology, Nakhon Phanom University, and Department of Fisheries, Faculty of Agriculture, Khon Kaen University, Thailand.

### Habitat

The study of habitat was in September 2017 to October 2018. Habitat parameters, including area and living conditions, as well as the depth of holes, were collected. Tape measure or ruler was used to measure the slope of crab holes, while a full-circle protractor was used to measure the slope in degrees, totaling 100 holes.

### Stomach content

The stomach content was conducted in the laboratory from June 2018 to May 2019 by collecting 20 crabs per station in 3 seasons, resulting in 180 crabs. Sample collections were scheduled in the rainy season (June to September 2018), winter season (between October 2018 to February 2019), and summer season (from March to May 2019). The crab were stunned by the cold water after being caught and then preserved in 75% ethanol. A surgical scissors was used to take crab's stomach for stomach content analysis. Afterward, the stomach was cut into fine pieces and placed in the Petri dish, and the content of stomach was taken via a needle for checking food types under stereomicroscope. The next step was calculation to find the percentage of food content of each type in the stomach and frequency percentage of each foods as to analyze the types and proportions present in the stomach by frequency of occurrence method by formula (1) with numerical composition by formula (2), which was modified from the method of Stehlik (1993) cited by Sharifian and Kamrani (2017).

The percentage frequency of occurrence (FO%) (formular 1) and its percentage points (PP) of each food (formular 2) were calculated following Stehlik (1993) method with some modification as mentioned in Sharifian and Kamrani (2017).

$$FO\,\% = (N^{o} / N^{i}) \times 100 \qquad (1)$$

Where $N^o$ = Number of stomachs with particular food group, and $N^i$ = Total number of stomachs with food

$$PP = (P^o / P^i) \times 100 \quad (2)$$

Where $P^o$ = Point of particular food group, and $P^i$ = Total points of all food groups

**Mating season and spawning**

The mating season was conducted in the field and the laboratory from September 2017 to December 2018. Thirty pair adult crab samples of both sexes were collected from studied areas and placed inside the circular plastic tray (30 cm in diameter and 29 cm in height) at a density of 1 crab per tray. They were then fed with instant shrimp food pellets (40% protein, 4% fat and ash 4%) one time per day at 06.00 p.m. After that, 15 pairs of the adult crabs were transferred into a plastic tray (35x52x32 $cm^3$) and cultured from June to September 2018, and another 15 pairs were stocked from October to December 2018 for behavior and mating studying.

Egg numbers of each crab (total 30 berried females) were counted for fecundity and development of fertilized egg determinations. After that, 15 berried females were moved to plastic tray (35x52x32 $cm^3$), a density of 1 crab per tray, and fed with instant shrimp food pellets one time a day at 06.00 p.m. for egg development every seven days.

**Growth performance**

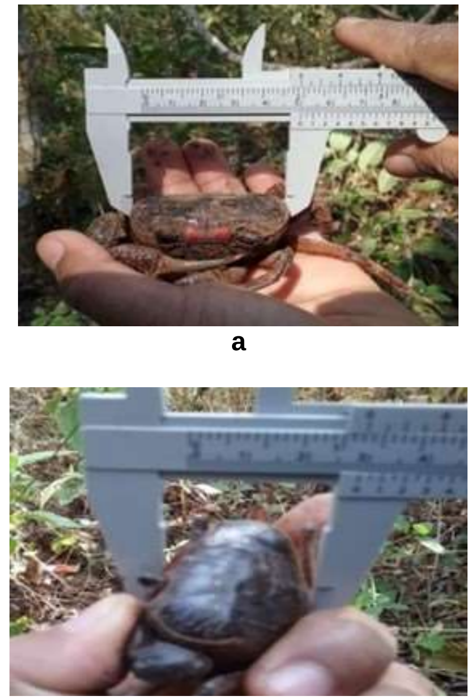
The research was conducted in the laboratory by randomly selecting postlarval crabs, two days after hatched from 10 females (20 crab/female), totaling 200 crabs. These crabs were moved to circular plastic tray (30 cm in diameter, 29 cm in height) at a density of 1 crab per tray (from May 2018 to June 2019). They were fed with instant shrimp food pellets two times a day at 06.00 a.m. and 08.00 p.m. to maintain water quality, 100% of water was changed every seven days. Body weight (W; g) was determined using a digital scale of 2 decimal places, while carapace width (CW; cm) and carapace length (CL; cm) were measured via Vernier Caliper (0.01 mm resolution) as shown in Figure 1. Molting data were checked and recorded daily, while W, CW and CL were determined three days after molting.

The weight and length relationships in terms of W-CW and W-CL of crab was determined as the method of Narasimham (1970) for both sexes, male and female represented $W = aCW^b$ and $W = aCL^b$ or their logarithmic equation represented

$$\mathrm{Log}W = \log a + b \log CW \quad \text{and}$$

$$\log W = \log a + b \log CL$$

Where, W = Body Weight (g), CW = Carapace Width (cm), CL = Carapace Length (cm), a = the intercept (condition factor), and b = the slope (growth coefficient).



a

b

**Figure 1: Methods of measuring a) carapace width (CW), b) carapace length (CL) were investigated.**

**Data analysis**

Data on W, CW, and CL, and growth rate from molting crabs were presented as average ± standard deviation. Also, a statistical difference was analyzed by the t-test method at 95 percent reliability level by using Microsoft Excel.

## RESULTS AND DISCUSSION

**Habitat**

The results indicated that *P. wanonniwat* crab's habitat ranged from the adjacent forests to rice-fields. These areas possessed plenty of groundwater and water flowing through in the rainy season, with trees and sunlight shining through. The size of the crab's hole had an average diameter of 5.7±1.20 cm with the depth at an average of 28±0.80 cm and 82 ±0.12 cm for lateritic and loamy clay soil, respectively. The average slope was 66.16±10.64 degrees. These parameters depend on the crab's size and sex. It was found that the size of the female hole was larger than the male hole. Similarly, Tongnunui et al. (2013) reported that big crab had a larger hole than small crab, and the female had a bigger hole than males at the same size. The hole's depth depended on several conditions, such as the soil and moisture in the soil. The crab hole was deep in the high moisture soil; whereas, the dried soil had much shallow hole. This was consistent with Kaewkrom et al. (2014), who reported that the hole's depth depended on the season. During the rainy season, the holes are shallow, but the depth is greater in summer, depending on the depth level of groundwater.

**Stomach content**

Food compositions in stomach contents of 180 crabs, consisting of 60 crabs in the rainy season with the average CW of 4.49 ± 0.44 cm, 60 crabs in winter with the average CW of 3.98±0.75 cm and 60 crabs in summer with the average CW of 4.23±0.62 cm. The Stomach content compositions were classified into six groups, such as insect, plant, snail, earthworm, phytoplankton, and unidentified particle. Percentage frequency type of each food in the stomach of crabs in three seasons was frequency type of each food in the stomach of crabs in three seasons was 100±0.00, 37.22±13.47, 46.67±10.84, 31.88±33.29, 35.00±53.89 and 20.63±6.01 for insect, plant, snail, earthworm, phytoplankton, and unidentified particle, respectively (Figure 2).

The percentage of numerical compositions of insect, plant, snail, earthworm, phytoplankton and unidentified particle was 56.90±15.54, 12.60±8.14, 7.36±3.57, 4.48±4.51, 14.06±24.35 and 4.60±2.01, respectively (Figure 3).

*P. wanonniwat* ate both plants and animals (Omnivorous species), and insect was the main food, which was found in three seasons of the year. Other food types were more or less found, depending on the season. For example, phytoplankton food was found only rainy season, while this group was not found in crab's stomach during the winter and summer season. Likewise, according to Williner and Collins (2013) and Sharifian and Kamrani (2017), the differences in the proportions of crab's stomach content in various areas were due to the differences in the type and density of victims in each place, including the season and time of the day as well. Furthermore, the crabs ate other animals of the same type (cannibalism).

Big crabs ate smaller crabs or while molting or having just molted when the carapace is still soft, and the movement is slow (Doolgindachbaporn et al., 2002). Crab consumes plant foods, such as grass and aquatic plant. Most crabs looked for food at night, when their stomach were full of food. Several factors that affects the food type and proportion of crabs, such as temperature, salinity, and molting phase.

It has been well documented that temperature below 7°C resulted in reduced agility of the crabs and less eating (Kidjit, 1979 cited by Mornkam, 2007). However, lower salinity of the water was found to not effect on eating, even one's slough (Pakdeenarong et al., 2009). After molted 12-24 hrs., crabs were strong, and they could eat their slough. This was consistent with the report of Promdam and Sumontha (2008), who indicated that they ate their slough and may be eaten by other crabs apart from slough owners. Eating own slough is the common behavior of crabs that do not live in the sea to preserve minerals important for the strength of carapace in order not to be wasted.

**Mating season and Spawning**

A field survey found that they mated in their holes in twenty mating pairs (November to December 2017). In the laboratory, fifteen cases were observed, and the results indicated that crabs mated from November to December of the second year (2018).

The courtship behavior, male and female, were aggressive by raised the chelates to attract each other. When they were ready to mate, the male gradually moved closer by slowly tipping over. Then, the male inserted gonopods into gonopores of females for 30-60 min. After that, the male used walking legs to caress female for 8 hrs. or more, and some pairs took 3-7 days. Likewise, according to the report of López-Victoria and Werding (2008) *Johngarthia malpilensis* took an hour or more for mating, while *Potamon fluviatile* took 30 min to 21 hrs. for their mating (Micheli et al. 1990).

Females carried their eggs on the abdomen, which were found from January to May, and most frequently in February. These crabs started to hatch around the middle of May. The size of the eggs was approximately 4 mm in diameter.

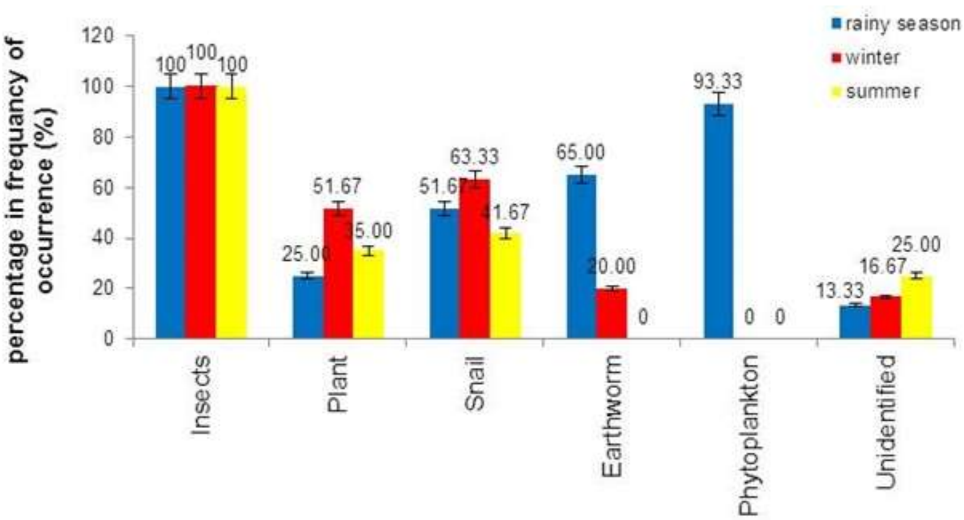



**Figure 2: Percentage in frequency of occurrence of major food groups of *P. wanonniwat***

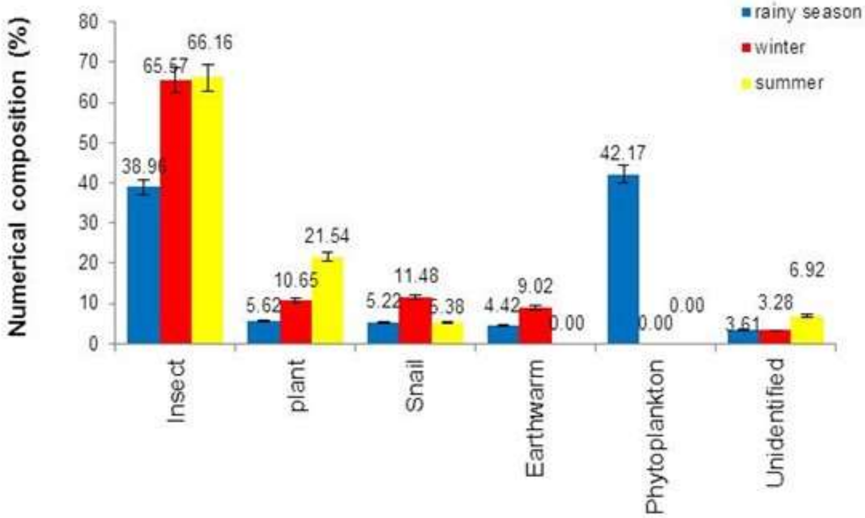



**Figure 3: Percentages of numerical composition of major food groups of *P. wanonniwat***

The average of CW, CL, and fecundity of thirty crabs was 5.01±0.80 and 3.92±0.48 cm and 68.18±23.18 eggs, respectively. Likewise, *Paratelphusa spinigera*, *Travancoriana schirnerae* were mated and spawned once a year (monovoltine species). However, *P. spinigera* has berried females until hatching (postlarva) by taking about 30-35 days. *Sinopotamon yangtsekiense* took 77 days (Kaur et al., 2006; Wu et al., 2010; Sudha Devi and Smija, 2013) for berried females to hatch. For *Geosesarma krathing* species, they began to have berried females from January to April. The smallest size of berried females was 10.06 mm, and the average number of eggs per crab was 50 eggs (Damrongrojwattana et al. 2009). Liu and Li (2000) reported that *Candidiopotamon rathbunae* berried females with CW of about 30 mm could produce 9-117 eggs, only a few could produce more than 100 eggs.

For *Travancoriana schirnerae*, female crab with CW of approximately 3.7 cm could produce 115 eggs, while a bigger size (CW of 5.3 cm) could give more eggs (approximately 281 eggs per female) (Sudha Devi and Smija, 2013). Rice-field crab was also found to mate from May to July with water as a key factor. The fecundity of the eggs was around 26 to 1,067 eggs per female. The spawning period was from February to July (Doolgindachbaporn et al., 2002). The fecundity is associated with the size of the mother crab. Wehrtmann et al. (2010) compiled information on the fecundity of freshwater crab and found that female crab could produce 100 to 300 eggs.

However, fecundity may vary depending on several factors. Freshwater crabs have a small amount of eggs compared to sea crabs because freshwater crab's eggs are large and yolks sac are bigger sea crabs. Kaur et al. (2006) found that freshwater crabs' fecundity was much lower than that of sea crabs. Their eggs directly develop into postlarvae. The berried females of *P. wanonniwat* or early fertilized eggs divided cells continuously, and continued growth into the next phase. Egg development could be observed via color changes. The color of eggs changed according to the development phases of embryos that increase due to the reduction in the amount of yolk sac used as food in embryo development and the increase in black pigment inside. Also, the volume of eggs was increased according to the development stages due to the growth of embryos' tissues inside the eggs (Tangkrock-olan and Champati, 2007; Wu et al., 2010).

**Growth performance**

The study on the growth of 200 crabs found that, after two days of hatching (both sexes of crabs), the average weight, CW, and CL were 0.29±0.03 g, 0.87±0.02, and 0.76±0.02 cm, respectively. One hundred crabs survived after one year. The average W, CW, and CL were 21.29±3.35 g, 3.91±0.51 and 3.14±0.16 cm. The growth rate percentage indicated that W, CW and CL were 74.44±27.83, 20.67±5.33 and 19.81±4.79 percent, respectively.

The relationship and difference between males and females in terms of growth performance were studied. It was found that male (n=84) had the average W, CW and CL of 0.29±0.23 g, 0.87±0.01 and 0.76±0.02 cm, respectively, whereas female (n=89) had W, CW and CL approximately 0.29±0.23 g, 0.87±0.02 and 0.76±0.02 cm, respectively. The average W, CW, and CL of 1-year old male crabs (n=57) were 21.32±3.79 g, 3.86±0.25 and 3.12±0.18 cm, respectively. Female (n=43) had the average W, CW and CL of 21.29±2.74 g, 3.87±0.20 and 3.16±0.14 cm, respectively. As to percentage of growth rate, the average W, CW, and CL of the males were 74.76±27.74, 20.83±5.58 and 19.69±5.00 percent, respectively and that of females were 74.39±30.66, 20.91±5.62 and 19.53±5.19 percent, respectively (Figure 4). However, no significant differences in growth performance in W, CW, and CL were observed between males and females throughout the study ($P > 0.05$).

Molting data indicated that the crabs could molt eight times per year. The results were inconsistent with previous in rice-field crab, which they could molt 13-15 times per year (Doolgindachbaporn et al., 2001). Sudha Devi and Smija (2015) indicated that *Travancoriana schirnerae* is could molt fourteen times per year. This result supports the finding of Doolgindachabaporn et al. (2002), Koedprang and Songrak (2011), Tamtin et al. (2014) and Sudha Devi and Smija (2015).These results revealed that the frequency of molting was higher in the young crab compared to grow older.

The result indicated that a crisis was also found while molting. During the 3rd and 4th molting, the imperfect molting characteristics were found, namely carapace deformities. This suggested that the CW was narrower than the CL, including death crab, characterized by the inability to shed the old slough. Before molting, the crabs stopped eating food for about 1-2 days. It was also found that about 2-3 hrs. after molting, the crabs ate their slough as food to preserve minerals important for the strength of carapace and maintain water quality. It has been advocated that crustaceans, including crab have a relatively high amount of ash up to 15.90% in their bodies. Also, a high amount of chitin and chitosan was found as the main structural components of the exoskeleton of crustaceans (de Andrade et al., 2012; Ifuku, 2014). This result supports the finding of Zanotto et al. (2004) and Panalikul et al. (2017a), who indicated that calcium and calcium carbonate was necessary for each molting period of the growing crabs and it is needed as a significant proportion for exoskeleton development. Chitosan was also played an essential role in promoting the growth of the crabs (Panalikul et al., 2017b).

The relationships between W and CW; and W and CL of both sexes were in the same direction. The results suggested that weight gain led to an increase in CW and CL. The relationships between W and CW (Figure 5), and W and CL are shown in Figure 6 and Table 1.

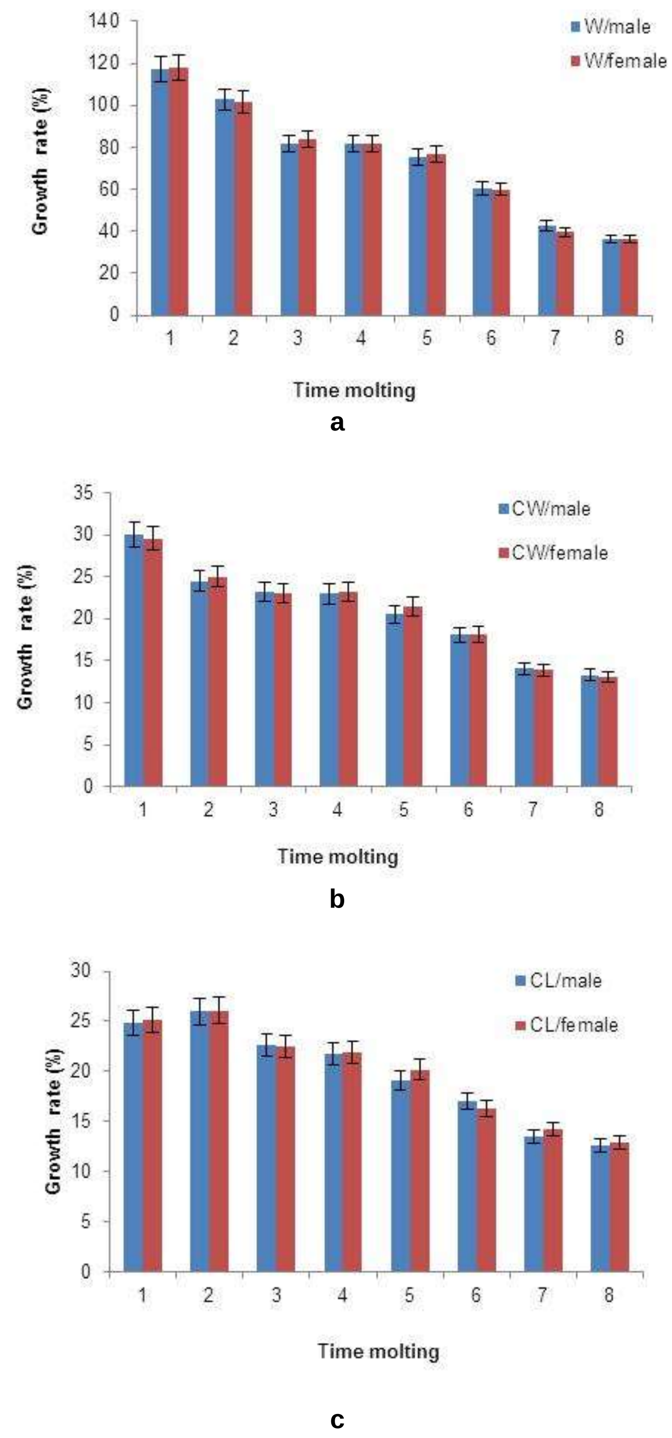



**Figure 4: Male and female of *P. wanonniwat* were compared after the 8$^{th}$ molting. a) Body weight b) Carapace width and c) Carapace length**

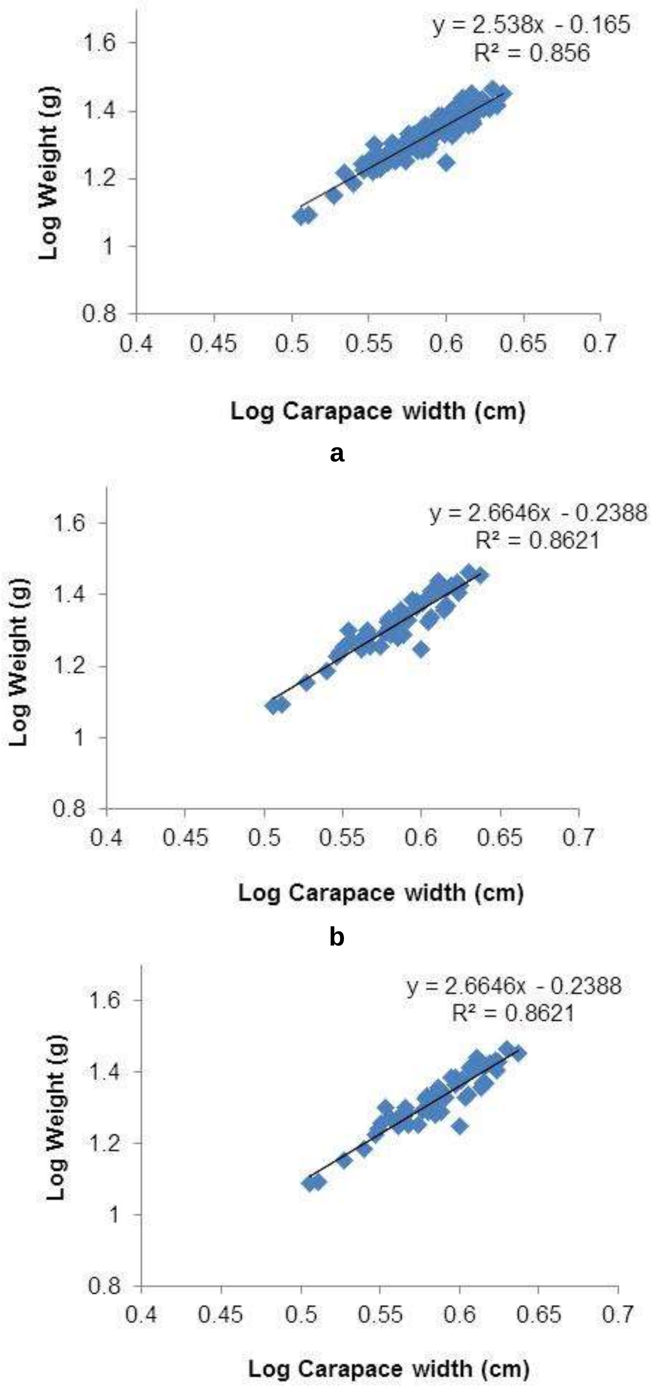
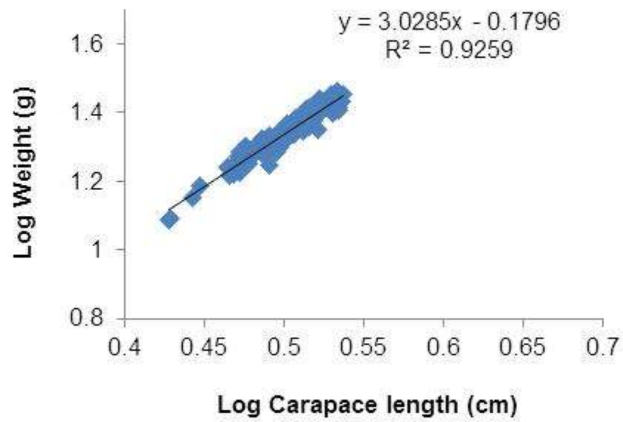
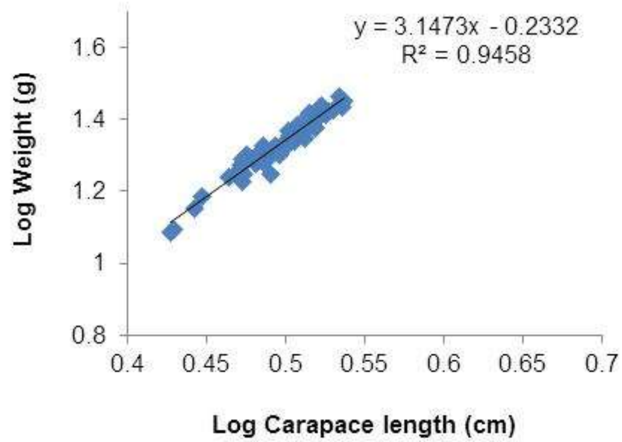



**Figure 5: Logarithmic relationships between weight and carapace width of *P. wanonniwat* were analyzed after the 8$^{th}$ molting. a) Both sexes b) Male and c) Female**




**a**

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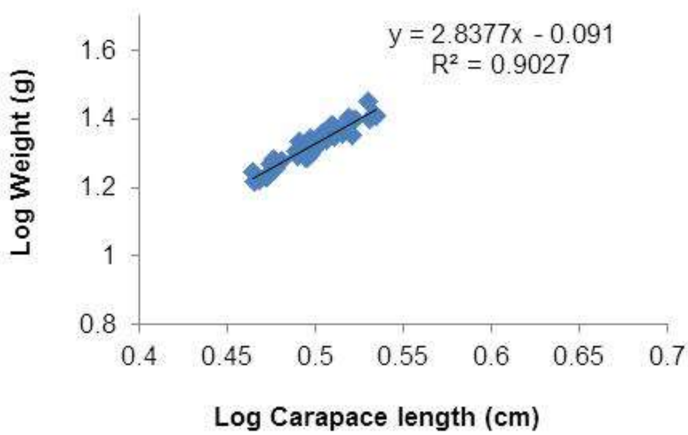


**c**

**Figure 6: Logarithmic relationships between weight and carapace length of *P. wanonniwat* were analyzed after the 8th molting.**
**a) Both sexes b) Male and c) Female**

**Table 1: Regression analyses between weight (W) and carapace width (CW), and weight (W) and carapace length (CL) (B; both sexes, M; Male, F; Female)**

| Valiable | Sex | N | Power Equation $Y = aX^b$ | logY = log a + b logX | $R^2$ |
|---|---|---|---|---|---|
| W:CW | B | 100 | $W = 1.462CW^{2.538}$ | logW = 2.538+0.165logCW | 0.857 |
| | M | 57 | $W = 1.734CW^{2.665}$ | logW = 2.665+0.239logCW | 0.862 |
| | F | 43 | $W = 1.028CW^{2.278}$ | logW = 2.278+0.012logCW | 0.857 |
| W:CL | B | 100 | $W = 1.514CL^{3.028}$ | logW = 3.028+0.180logCL | 0.926 |
| | M | 57 | $W = 1.710CL^{3.147}$ | logW = 3.147+0.233logCL | 0.946 |
| | F | 43 | $W = 1.233CL^{2.838}$ | logW = 2.838+0.091logCL | 0.903 |

The result indicated that no significant differences in these parameters were observed ($P > 0.05$).

## CONCLUSION

Terrestrial crab, *Pudaengon wanonniwat* was omnivorous species. Its food included insect, plant, snail, earthworm, phytoplankton, and some unidentified particles, which insect group was major food. This crab lived in the range from adjacent forests to rice-fields, were always watery in the rainy season. Mating season was in November to December. Then spawning began in January to May, and fertilized eggs hatched in May. Fecundity was approximately 68.18±23.18 eggs. Crab growth performance in terms of averages of W, CW, and CL was 21.33 ± 3.36 g, 3.91±0.51 cm, and 3.14±0.16 cm, respectively, after the 8$^{th}$ molting in one year. The growth performance of W and CW, and W and CL relationships represented $W = 1.462CW^{2.538}$ ($R^2$ = 0.857), and $W = 1.514CL^{3.028}$ ($R^2$ = 0.926).

## CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

## ACKNOWLEDGEMENT

This research was supported by Department of Fisheries, Faculty of Agriculture, Khon Kaen University and Fishery Program, Faculty of Agriculture and Technology, Nakhon Phanom University, Nakhon Phanom Province for facilities.

## AUTHOR CONTRIBUTIONS

VA designed and performed the experiments, data analysis and also wrote the manuscript. SD and SW designed experiments and reviewed manuscript. All authors read and approved the final version.



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