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# Impact of Different Pond Bottom Soil Substrates on Blue Swimming Crab (*Portunus pelagicus*) Culture

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**Abstract**

Pond bottom soil quality is an important factor in aquaculture, but studies on the soil quality in crab ponds are very limited. The objectives of this experiment were to investigate the impact of pond bottom soils on blue swimming crab, *Portunus pelagicus*. Experimental crabs were reared under simulated earthen pond conditions in 0.5×2.5×0.4 $m^3$ fiberglass tanks with different soil substrates. The 4 treatments used were crabs reared with sandy loam (T1), sand (T2) and clay (T3) as substrates (10 cm thick) and without soil substrate (T4). Crabs were fed with shrimp feed at 5 % of body weight per day (twice a day at 0900 h and 1500 h), for 90 days. Each treatment was done in triplicate. The results showed that the average daily growth of crabs reared with the sandy loam or sand as substrates, and without soil substrate were not significantly different ($p > 0.05$) but these were significantly higher ($p < 0.05$) than that of using clay as a substrate. The survival rates of crabs reared with different soil substrates were not significantly different ($p > 0.05$). The feed conversion ratio of crabs reared with clay or sandy loam as substrates were also not significantly different ($p > 0.05$), but those reared with clay soil were significantly higher ($p < 0.05$) than that of using sand as a substrate or without soil substrate. In addition, the levels of organic matter, pH and ammonia in the soil of crabs reared with the clay as substrate were higher than those with sandy loam or sand as substrates. This study demonstrated that soil substrate and changing properties due to levels of organic matter, pH and ammonia in the soil affected the growth rate and feed conversion ratio of *P. pelagicus* crabs.

**Keywords:** Blue swimming crab, *Portunus pelagicus*, pond bottom soil substrate

## Introduction

The blue swimming crab, *Portunus pelagicus* (Linnaeus, 1758), a commercially important species, is distributed throughout coastal waters in the tropical regions of the western Indian Ocean and the eastern Pacific Ocean. Due to overfishing and increased market demand, the production of this crab in the marine environment have shown a decreasing trend since 2009 [1,2]. Currently, many countries are actively involved in *P. pelagicus* crab culture and associated research, e.g. the Philippines, Indonesia, India, Australia, Malaysia and Thailand [3,5]. Nevertheless, rearing technologies for this crab have not been established in commercial farms [6-10] because of their obtaining low productivity (for example, to 26.7 to 140.5 kg/1,600 $m^2$ at harvest) [5], which is a major bottleneck to the development of this crab's aquaculture. Factors that contribute to the low survival rate of the crab rearing in grow-out ponds (2.97 to 59.59 %) have been identified as cannibalism [11,12], nutritional quality of feed [13,14] and pond bottom soil quality [15]. In general, pond bottom soil quality is an important factor in aquaculture, with the water quality in aquaculture ponds influenced by the exchange of substances between soil and water. The 4 most important features of soils with regard to aquaculture production are the texture, organic matter concentration, pH and nutrient concentration [16]. However, studies on the effect of bottom soil quality on this crab are very limited. To improve income from crabs, optimal rearing conditions must be determined. Thus, the objective of this study was to improve rearing techniques by investigating the impact of pond bottom soil substrates on crab growth and survival. The knowledge gained from the research will be useful for *P. pelagicus* crab production and the development of the crab farming.

## Materials and methods

### Study site and the crabs used for the experiment

The experiment of this study was conducted at the hatchery of the Klongwan Fisheries Research Station, Prachuap Khiri Khan province, Thailand. The females of *P. pelagicus* crab with dark grey eggs were caught and collected by native fishermen using small-scale crab traps in the coastal area of Prachuap Bay, Prachuap Khiri Khan province, Thailand. After that, they were transferred to the hatchery and placed in 200-L fiberglass tanks to allow them to release eggs for hatching. They were not fed during this period. The newly hatched larvae of the crabs were transferred to 2,000-L fiberglass tanks for nursing at a density of 100 crabs/L. At this stage, they were initially fed with rotifer (*Branchionus* sp.) and diatom (*Chaetoceros* sp). From the Zoea 2 stage onwards, they were fed with *Artemia* nauplii until larval metamorphosis to the first crab stage. Then crabs were transferred to 400 $m^2$ earthen ponds (20 m width × 20 m length × 1 m depth) at a density of 5 crabs/$m^2$. Crabs were fed with shrimp feed according to Oniam *et al*. [8] until the experiment commenced.

### Experimental design and set-up

Experimental crabs were reared in 400 $m^2$ earthen ponds for 30 days. Then, the crabs were randomly sampled to be reared in simulated earthen pond conditions in 12 fiberglass rectangular tanks (0.5 m width × 2.5 m length × 0.4 m depth) with different soil substrates. The 4 treatments were sandy loam (T1), sand (T2) and clay (T3) as substrates at 10 cm thick (**Figure 1**), and without soil substrate (T4). Each treatment had 3 replicates. Each fiberglass tank was separated to 5 compartments with nets and lined with the substrates as mentioned above. The crabs were reared individually in each compartment. Thus the total number of crabs was 60. Mean initial carapace width (CW) and body weight (BW) of the crabs were 3.75 - 4.00 cm and 8.01 - 8.97 g, respectively. The crabs were fed with shrimp feed (pellet size about 3.5 mm, 38 % protein) at 5 % of body weight per day (twice a day at 0900 h and 1500 h). The experiment was carried out for 90 days.

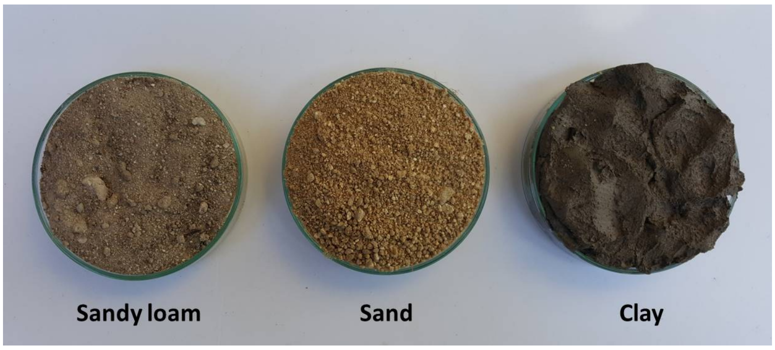


**Figure 1** Physical characteristics of different soil substrates in simulated earthen pond conditions during the culture period: sandy loam (75.1 - 80.9 % sand, 6.7 - 12.8 % silt and 12.0 - 16.0 % clay), sand (92.9 - 95.1 % sand, 2.7 - 4.8 % silt and 2.0 - 2.2 % clay), and clay (17.2 - 22.9 % sand, 2.7 - 6.7 % silt and 72.2 - 78.0 % clay).

**Data collection**

The total number of crabs from each treatment was used to evaluate their growth and survival every 30 days. For each crab, the CW (cm) and BW (g) were recorded. The CW was measured between the tips of the epibranchial spines using a Vernier caliper. The BW was measured using a set of digital weighing scales. At the end of the experiment, the average daily growth (ADG) and feed conversion ratio (FCR) were calculated.

During the experiment, 50 % of the water volume was exchanged once a week. The water quality parameters i.e. salinity, pH, temperature, dissolved oxygen, total ammonia, nitrite and alkalinity were analyzed biweekly. Salinity was measured with a refractometer, pH was measured with a portable pH meter, temperature and dissolved oxygen concentration (DO) were measured with an oxygen meter, and total ammonia, nitrite and alkalinity were determined by the indophenol blue method, the colorimetric method, and the titration method, respectively (Association of Official Analytical Chemists methods) [17].

In addition, the bottom soil quality was analyzed once a month by collecting bottom soil samples from 2 places in each simulated earthen pond using a 5 cm diameter, clear plastic, core liner tube. The upper 5 cm segment of each core was removed as described by Munsiri *et al*. [18], and 2 core segments from each pond were combined to provide a composite sample for analysis. Tests were undertaken for organic matter concentration by the ignition loss method [19]. Soil pH was measured by the method described by Thunjai *et al*. [20] and total ammonia concentration was determined by the method described by Chuan and Sugahara [21].

**Statistical analysis**

Data on the growth, survival, ADG and FCR, including water and bottom soil qualities of all treatments were analyzed using one-way ANOVA and difference between the means was tested using Duncan's multiple range test at the 95 % level of confidence. All data were analyzed using the IBM SPSS Statistics for Windows software package (Version 21.0; IBM Corp., Armonk, NY, USA).

## Results and discussion

**Growth and survival of the crabs**

The results showed that the mean CW and BW of crabs reared with sandy loam (8.72 ± 3.72 cm and 82.20 ± 35.38 g, respectively) and sand (9.98 ± 3.04 cm and 91.26 ± 31.22 g, respectively) as substrates and without soil substrate (8.80 ± 3.65 cm and 84.26 ± 31.18 g, respectively) were not significantly different ($p > 0.05$) among the treatments but these were significantly higher ($p < 0.05$) than that of used clay (6.82 ± 4.31 cm and 62.00 ± 11.30 g, respectively) as a substrate (**Figure 2**).

The mean survival rates of crabs reared with sandy loam, sand or clay as substrates, and without soil substrate were not significantly different ($p > 0.05$) (**Table 1**). In addition, the ADG of crabs reared with sandy loam or sand as substrate, and without soil substrate were not significantly different ($p > 0.05$) but these were significantly higher ($p < 0.05$) than that of using clay as a substrate. The FCR of crabs reared with clay or sandy loam as substrates were not significantly different ($p > 0.05$) but for the crabs reared with clay as a substrate, the FCR was significantly higher ($p < 0.05$) than that of using sand as a substrate and without soil substrate (**Table 1**).

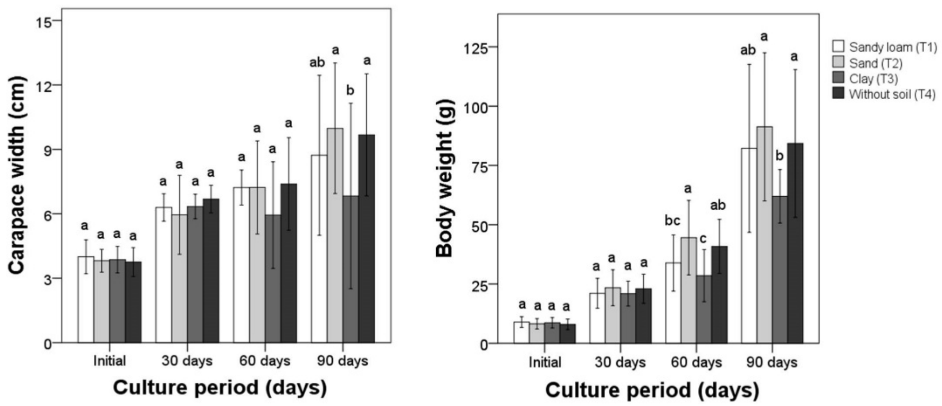


**Figure 2** Growth of blue swimming crab, *Portunus pelagicus*, raised under simulated earthen pond conditions with different soil substrates. Different lower case letters above bars indicate a significant difference at $p < 0.05$. Error bars indicate mean ± SD.

**Table 1** Survival rate, average daily growth and feed conversion ratio of blue swimming crab, *Portunus pelagicus*, raised under simulated earthen pond conditions with different soil substrates, for 90 days (mean ± SD).

| Parameter | Treatment (substrate) | | | |
|---|---|---|---|---|
| | Sandy loam | Sand | Clay | Without soil |
| Survival rate (%) | $93.33 \pm 11.54^{a}$ | $93.33 \pm 11.54^{a}$ | $80.00 \pm 20.00^{a}$ | $90.00 \pm 13.48^{a}$ |
| Average daily growth (g/day) | $0.78 \pm 0.18^{ab}$ | $0.92 \pm 0.14^{a}$ | $0.58 \pm 0.03^{b}$ | $0.88 \pm 0.37^{a}$ |
| Feed conversion ratio | $2.81 \pm 0.48^{ab}$ | $2.29 \pm 0.11^{b}$ | $3.16 \pm 0.21^{a}$ | $2.37 \pm 0.36^{b}$ |

Means within the same row with different lowercase superscripts are significantly different ($p < 0.05$).

**Water and soil qualities**

The average values of the water quality are shown in **Table 2**. The results showed that the different bottom soil substrates significantly affected some water quality parameters i.e. DO, pH, ammonia and nitrite ($p < 0.05$; **Table 2**), but did not significantly affect temperature, salinity and alkalinity levels ($p > 0.05$; **Table 2**).

In this experiment, the mean values of organic matter concentration, pH and ammonia concentration in the crab pond bottom soil with sandy loam, sand or clay as substrates are shown in **Figure 3**. At the end of the experimental period, the organic matter (14.36 ± 3.81 %), pH (7.69 ± 0.19) and ammonia (17.54 ± 4.03 mg/kg) in the bottom soil with clay substrate were significantly higher ($p < 0.05$) than those of sandy loam (7.45 ± 1.65 %, 7.03 ± 0.10 and 7.93 ± 1.42 mg/kg, respectively) and sand (2.29 ± 0.87 %, 6.37 ± 0.17 and 6.83 ± 1.72 mg/kg, respectively; **Figure 3**).

**Table 2** Water quality parameters during the experiment of blue swimming crab, *Portunus pelagicus*, raised under simulated earthen pond conditions with different pond bottom soil substrates, for 90 days (mean ± SD).

| Parameter | Treatment (substrate) | | | |
|---|---|---|---|---|
| | Sandy loam | Sand | Clay | Without soil |
| Dissolved oxygen (mg/L) | $4.10 \pm 0.56^{b}$ | $4.11 \pm 0.12^{b}$ | $3.74 \pm 0.07^{c}$ | $4.88 \pm 0.14^{a}$ |
| Temperature (°C) | $28.30 \pm 0.10^{a}$ | $28.26 \pm 0.11^{a}$ | $28.26 \pm 0.05^{a}$ | $28.30 \pm 0.10^{a}$ |
| pH | $7.81 \pm 0.02^{b}$ | $7.87 \pm 0.04^{ab}$ | $7.80 \pm 0.14^{b}$ | $8.00 \pm 0.04^{a}$ |
| Salinity (ppt) | $32.66 \pm 0.05^{a}$ | $32.70 \pm 0.10^{a}$ | $32.66 \pm 0.05^{a}$ | $32.63 \pm 0.05^{a}$ |
| Total ammonia (mg-N/L) | $0.11 \pm 0.02^{b}$ | $0.11 \pm 0.03^{b}$ | $0.40 \pm 0.09^{a}$ | $0.11 \pm 0.03^{b}$ |
| Nitrite (mg-N/L) | $0.03 \pm 0.01^{b}$ | $0.03 \pm 0.01^{b}$ | $0.12 \pm 0.02^{a}$ | $0.05 \pm 0.01^{b}$ |
| Alkalinity (mg/L as $CaCO_3$) | $121.76 \pm 5.58^{a}$ | $125.03 \pm 2.51^{a}$ | $120.80 \pm 4.10^{a}$ | $122.66 \pm 6.17^{a}$ |

Means within the same row with different lowercase superscripts are significantly different ($p < 0.05$).

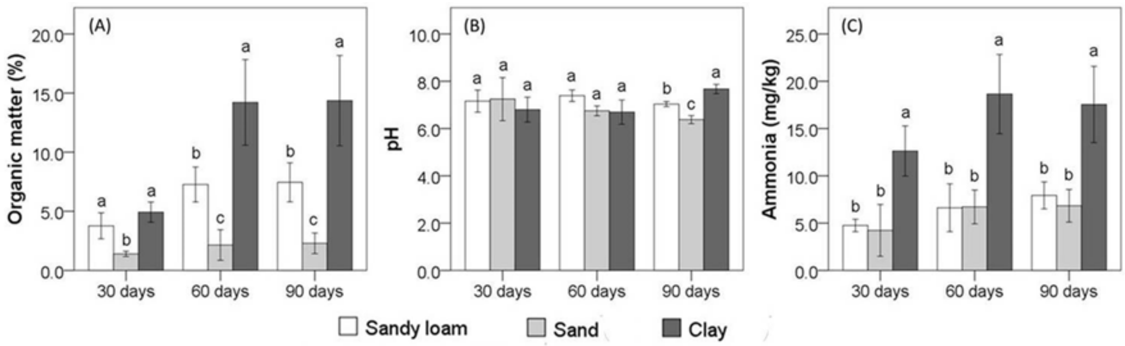


**Figure 3** Organic matter (A), pH (B) and ammonia (C) in soil of blue swimming crab, *Portunus pelagicus*, rearing ponds with different soil substrates. Different lower case letters above each bar indicate a significant difference at $p < 0.05$. Error bars indicate mean ± SD.

In the current experiment, pond bottom soil quality was considered to be an important factor affecting *P. pelagicus* crab culture. The treatments with optimum soil substrates should result in a high growth rate and a low FCR. The properties of the crab pond bottom soil (sandy loam, sand or clay as substrates or without soil substrate) affected the crabs reared and in particular, the crabs reared with clay as the substrate had a lower growth rate (0.58 g/day) and higher FCR (3.16) compared to those of the crabs reared with sand as a substrate (0.92 g/day and 2.29, respectively) and without soil substrate (0.88 g/day and 2.37, respectively). This can be explained by the fact that the clay soil has a smaller particle size and a higher porosity [16], which results in higher values of organic matter and ammonia retention in it than sandy loam and sand which affect the growth rates of the crabs reared. Therefore, it could be concluded that the pond bottom soil substrate has an impact on *P. pelagicus* crab.

For *P. pelagicus* crabs in grow-out ponds, Oniam *et al*. [12] reported that the factor contributing the most mortality of this crab was cannibalism, especially during 30 - 45 days of culture period. However, this decreases as the crab's age increases [12]. Oniam [15] also reported that especially after 90 days of culture, the pond bottom soil quality would be a greater cause of crab mortality than cannibalism. In the current study, the level of organic matter and the ammonia concentration in the pond bottom soil (with either sandy loam, sand or clay as substrates) were positively related to the culture period and these affect the productivity (**Figure 3**). Similar results have been reported in marine shrimp culture [22,23]. Boyd [16] stated that fertilizer and formulated feed were important sources of nitrogen in ponds which among the 3 major nitrogenous compounds (ammonia, nitrite and nitrate), ammonia-N was generally the most toxic to aquatic animals. This is supported by the results of this study where the growth rate of the crab from 60 days onwards was influenced by the changing levels of organic matter and the ammonia concentrations in the bottom soil. The pH level in the bottom soil with clay as a substrate (7.69) was higher than that of sandy loam (7.03) or sand (6.37) as substrates. The pH level has both direct and indirect effects on other environment variables. For example, the proportion of total ammonia nitrogen ($NH_4^+$ + $NH_3$) existing in the toxic, un-ionized form ($NH_3$) increases as the pH increases [16]. Thus, the mean level of toxic ammonia in the soil with clay as a substrate was higher than in the soil with sandy loam or sand as substrates, and this affected the crabs reared. Similarly, Oniam [15] reported that *P. pelagicus* crabs reared in pond soil with high levels of pH (7.88 ± 0.17) and ammonia (24.62 ± 20.36 mg/kg) had lower productivity (102.4 ± 15.3 kg/1,600 $m^2$) and higher FCR (4.01 ± 0.11) compared to those in pond soil with lower levels of pH (7.04 ± 0.41) and ammonia (6.72 ± 4.84 mg/kg) which had a productivity at 173.5 ± 36.2 kg/1,600 $m^2$ and an FCR at 3.41±0.23. In this experiment, we found that the death characteristics of the crab due to changing properties in soil were similar to "gill discoloration disease" in mud crabs, *Scylla* spp. [24] and other crustacean species, e.g. the black tiger shrimp *Penaeus monodon* and the Pacific white shrimp *Litopenaeus vananmai.* [25]. The occurrence of gill discoloration is usually associated with environmental failure due to improper pond preparation and accumulation of organic load due to uneaten and excess feed or changing properties of bottom soil (i.e., higher values of organic matter and ammonia retention). Aquatic animals will be infected by bacteria, fungi or protozoa, thereby affecting growth and survival [18,24,25]. In addition, the water quality in aquaculture ponds is influenced by the exchange of substances between the soil and water [16,22,23], and supported by the results in the current study, where the water quality in terms of DO, pH, ammonia and nitrite of crabs reared with different pond bottom soil substrates were significantly different. Although, these parameters were still in optimal ranges for this crab growth (DO higher than 3 mg/L, pH ranged from 7.5 to 8.5 and total ammonia lower than 1.65 mg-N/L) [4,5,12,15,26,27]. However, there were no data about the effects of nitrite on *P. pelagicus* crab. There is only a study by Romano and Zen [28] who reported that at 3 mg-N/L of nitrite decreased survival and reduced growth of early *P. pelagicus* juveniles crabs within only 20 days of subchronic exposure.

From another point of view, the *P. pelagicus* crabs reared under the simulated earthen pond conditions without soil substrate were able to survive and grow. In addition, they had a higher growth rate and a lower FCR compared to crabs reared with clay as a substrate. This indicates that we may improve crab culture technique by rearing in ponds without substrate e.g. concrete ponds, plastic-lined or High-density polyethylene (HDPE)-lined ponds. These types of pond can prevent bottom deterioration and also obtain higher growth rates as reported in other commercial crustacean species, e.g. giant tiger prawn, *Penaeus monodon* and Pacific white shrimp, *Litopenaeus vannamei* cultures [29,30]. This is an interesting point for future study on increasing *P. pelagicus* crab production.

## Conclusions

The pond bottom soil substrate affected the growth rate and FCR of blue swimming crabs, *P. pelagicus*, but did not affect the survival rate of this crab. The crabs reared with sandy loam or sand as substrates or without soil substrate had higher growth rates than those using clay as a substrate. The FCR of the crabs reared with clay as a substrate was higher than with sand as a substrate or without soil substrate. It could be concluded that changing the levels of organic matter, pH and ammonia in the soil affected the crabs reared. This study demonstrated that pond bottom soil quality is an important factor in *P. pelagicus* crab culture and this crab can grow and survive without any soil substrate. Thus, more extensive research is needed to investigate ways to improve pond bottom soil quality or rearing without soil substrate to increase crab production.

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