

## Research Article

## Impact of Seaweed Species and Density on Mitigating Cannibalism in Blue Swimming Crab (*Portunus pelagicus*) Juvenile

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### ABSTRACT

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Cannibalism poses a major challenge in the aquaculture of crustaceans, particularly during the early nursery stages of the blue swimming crab (*Portunus pelagicus*), an economically important species that often experiences high mortality rates due to this behavior. This study investigated the effects of varying densities of *Caulerpa lentillifera* (CL), and *Chaetomorpha crassa* (CC), two green seaweed species, on reducing cannibalism in juvenile C2-stage crabs during their nursery phase. A two-factor experimental design was applied to crabs reared in controlled-environment indoor tanks for 30 days, based on seaweed density (0.5, 1, and 2 kg/m<sup>2</sup>) and species presence, with a negative control group lacking shelter. Based on the findings, while the seaweed species did not significantly influence growth parameters, the density of 0.5 kg/m<sup>2</sup> promoted higher growth rates, although these were not significantly different from the negative control. Conversely, the 2 kg/m<sup>2</sup> density of CC resulted in a substantial reduction in cannibalism, particularly from day 20 onward, with the lowest mortality rates observed under this treatment. These results underscore the importance of optimizing seaweed density and species type to enhance survival rates in crab juveniles, suggesting that CC at 0.5 kg/m<sup>2</sup> was optimal for growth, while CC at 2 kg/m<sup>2</sup> was most effective in minimizing cannibalism.

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## 1. Introduction

Global food and nutrition security depend on the growth and development of aquacultural production systems because fisheries and aquaculture contribute approximately 17% of the total animal-source protein amounts of the major animal groups, with crustaceans being the second largest from capture fisheries and aquaculture (Boyd *et al.*, 2022). Cai and Galli (2021) reported that marine crabs accounted for 447,372 t of the 120 million t of aquaculture produced worldwide in 2019—a 3.74% increase over 2018. Unfortunately, overexploitation has led to decreasing crab populations, which has resulted in a decline in *P. pelagicus* landings and exports over the past few decades. Thus, blue swimming crab cultivation has grown in importance. In 2023, Thailand had 5,176 productive sea crab farms, an increase of 13 farms or 0.25 percent from 2022. The value of

sea crab farming was 1,257.74 million baht, an increase of 301.58 million baht or 31.54 percent from 2022 (Department of Fisheries, 2024). Although research and knowledge regarding the cultivation of crabs are constantly expanding, the most frequent difficulty with caring for crablets is their low survival rate, caused by cannibalism (Leearam *et al.*, 2023).

Often, cannibalism constitutes the largest cause of mortality, affecting up to 90% of the young crabs (Oniam *et al.*, 2011; Leearam *et al.*, 2023). One of the many strategies applied to reduce cannibalism in the nursery phase has been the provision of appropriate shelters or substrates, which minimize physical encounters and, consequently, cannibalism. Since shelters provide an abiotic element, they are essential for lowering cannibalism-related mortality and reducing biota stress levels (Ly Van *et al.*, 2020). Several studies have found that providing the provision of both natural shelters and artificial ones during

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the nursery grow-out phases increased survivability and decreased cannibalism (Mirera and Moksnes, 2013; Oniam *et al.*, 2015; 2020; Ly Van *et al.*, 2020; Leearam *et al.*, 2023).

In aquaculture farms, seaweeds used as shelters for juvenile crabs have had strongly positive effects on crab survival due to seaweed generally being a natural food source and providing habitat, shelter, and a nursery ground (Mirera and Moksnes, 2013; Al-Hafedh *et al.*, 2015; Mantri *et al.*, 2020; Kang *et al.*, 2021). The green seaweed *Caulerpa* spp., belonging to the family Caulerpaceae, is common along the Andaman coast and the Gulf of Thailand. It is economically important as human food, as well as for health, cosmetics, bio-remediation, and commercial aquaculture production (Kamleshbhai *et al.*, 2022). *Chaetomorpha* sp., the green filamentous seaweed inhabitant of stagnant coastal waters of central Thailand. It was abundant throughout the year that serving as a shelter for settling blue swimming crabs during the first instar stage. This genus has high biofiltration capacity, which can improve water quality and increase aquaculture productivity through integrated aquaculture systems. (Arumugam *et al.*, 2018; Xu *et al.*, 2018; Roleda and Hurd, 2019; Zubia *et al.*, 2019; Mahamad *et al.*, 2023).

It has been suggested that the integration of aquaculture with green seaweed positively reduced cannibalism rates among blue swimming crab during the juveniles. Therefore, the goal of the present study was to assess the effects of different density levels of *Caulerpa lentillifera* and *Chaetomorpha crassa* on the mortality and growth of blue swimming crab (*P. pelagicus*) in the nursery stage using indoor rearing tanks.

## 2. Materials and Methods

### 2.1 Sources of experimental crab and seaweed

Samples of the blue swimming crab (BSC), *Portunus pelagicus*, in the juvenile were obtained from the hatchery at the Klongwan Fisheries Research Station (KFRS), Prachuap Khiri Khan province, Thailand. *Caulerpa lentillifera* and *Chaetomorpha crassa* are fast-growing, complex green seaweed that provide shelter and reduce cannibalism in crab larvae, as shown in Figure 1, were harvested from an improved earthen pond within the KFRS.



**Figure 1** Characteristics of two green seaweeds as shelter for rearing BSC (*Portunus pelagicus*) in juveniles: (a) *Caulerpa lentillifera*; (b) *Chaetomorpha crassa*.

### 2.2 Experimental design and management

A two-factor experiment was randomly established with three densities of seaweed (0.5, 1, and 2 kg/m<sup>2</sup>) and without any seaweed as shelter (negative control) in separate tanks each having a bottom area of 0.13 m<sup>2</sup> (The dimensions are 0.32 × 0.47 × 0.30 meters) water volume 40 liters, combined with two species of seaweed *Caulerpa lentillifera* and *Chaetomorpha crassa*; denoted as CL and CC, respectively). Each treatment contained three replicates. Uniformly sized crabs were selected for the experiment, with mean ± standard deviation (SD) values for the initial carapace width (CW) and body weight (BW) of 0.31 ± 0.09 cm, and 0.03 ± 0.01 g, respectively. In this paper, seaweed densities of 0.5, 1, and 2 kg/m<sup>2</sup> are designated as D0.5, D1, and D2, respectively.

The rearing system was established at the KFRS, with all fiber tanks exposed to a natural photoperiod. The C2 crabs were reared in the filtered tanks at a density of 300 individuals/m<sup>2</sup> (40 crabs/tank) with constant aeration. Frozen Artemia biomass was used as food for the crabs who were fed twice daily between 09:00 and 16:00 hours, at 20% of their biomass/day (Ly Van *et al.*, 2020). Water exchange occurred in each tank every 5 days, replacing approximately 50% of the water volume. The crabs were raised for a total of 30 days. Every 15 days, the harvested seaweed biomass is weighed and measured. After using a cloth to wipe away extra water, the wet weight was recorded using a precision balance accurate to 0.1 g.

### 2.3 Data collection and analysis

Water quality was analyzed every 5 days. Salinity was measured using a refractometer (Master-S10 alpha; Atago, Japan), pH using a pH meter (pH Testr30; Eutech; Singapore), temperature, and dissolved oxygen (DO) concentration using an oxygen meter (Pro20i; YSI; USA), total ammonia based on Koroleff's indophenol blue method, nitrite using the colorimetric method, and alkalinity using the titration method according to APHA, AWWA, and WEF (2023).

The initial CW and BW statistics of the crabs were determined by randomly sampling 50 individuals from the acclimation tank. The weight of each crab was measured using a precision balance with an accuracy of 0.01 g, while CW was measured using calipers. The growth and mortality of each BSC were recorded every 10 days, during which four crabs were randomly sampled from each tank to assess their BW and CW. After measurement, each BSC was returned to its original tank. At the conclusion of the experiment, all surviving crabs in each tank were measured. Before weighing the crabs, excess water was removed using cloth, and the wet weight of the crabs was recorded to calculate growth rates. The average daily growth (ADG) in terms of BW (ADGBW) and CW (ADGCW) were calculated, along with the specific growth rate (SGR) for BW (SGRBW) and CW (SGRCW), using formulas (1)–(4), respectively. And show the size variation (CV) in terms of BW (CVBW) and CW (CVCW), using formula (5).

$$\text{ADGBW (g/day)} = \frac{(\text{final BW} - \text{initial BW})}{t} \quad (1)$$

$$\text{ADGCW (cm/day)} = \frac{(\text{final CW} - \text{initial CW})}{t} \quad (2)$$

$$\text{SGRBW (\%/day)} = \frac{(\ln \text{final BW} - \ln \text{initial BW})}{t} \times 100 \quad (3)$$

$$\text{SGRCW (\%/day)} = \frac{(\ln \text{final CW} - \ln \text{initial CW})}{t} \times 100 \quad (4)$$

$$\text{Coefficient of variation (\%)} = \frac{\text{SD}}{\text{Mean}} \times 100 \quad (5)$$

where  $t$  = culture period (days), and  $\ln$  indicates the Napierian logarithm.

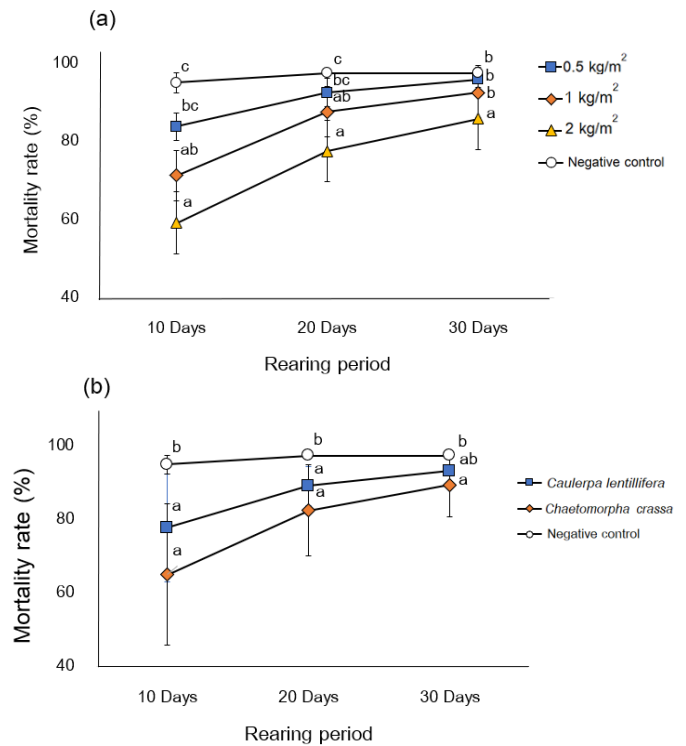
#### 2.4 Statistical analysis

The experimental data were examined for normal distribution using the Kolmogorov-Smirnov test. When the data were found to be normally distributed, the variance of the data was examined using the Levene's test. Data meeting these assumptions were analyzed using two-way ANOVA (F test,  $p < 0.05$ ) to identify the influence of species and density of seaweed and their interaction on growth performance and mortality rate of cannibalism on crabs. Duncan's multiple range test at a 95 percent confidence level was applied to evaluate significant differences among treatments utilizing the IBM SPSS Statistics for Windows software (Version 24.0; IBM Corp.; Armonk, NY, USA). Data were presented as mean  $\pm$  standard deviation values.

### 3. Results and Discussion

#### 3.1 Cannibalism of BSC juveniles

The utilization of the seaweeds, *Caulerpa lentillifera* (CL) and *Chaetomorpha crassa* (CC) as shelters significantly decreased the cannibalism mortality of the BSC juveniles during the first 20 days only ( $p < 0.05$ ), with the mortality rate due to cannibalism being notably reduced from day 10 of the rearing onward compared to those reared without shelter (negative control), as shown in Figure 2a. In addition, the density of seaweed per unit area had a direct effect on the reduction of cannibalistic mortality, with the shelter at a density of 2 kg/m<sup>2</sup> having the best reduction in mortality compared to the other densities ( $p = 0.003$ ) at over 30 days (Figure 2b).



**Figure 2** Mortality rates of BSC (*Portunus pelagicus*) juveniles reared with *Caulerpa lentillifera* and *Chaetomorpha crassa* (a) and across different seaweed densities used as shelter (b), compared to juveniles reared without any seaweed shelter (negative control). Error bars represent the mean  $\pm$  SD, and different lowercase letters at the symbols indicate significant differences ( $p < 0.05$ ).

Furthermore, examination of the interaction between seaweed species and densities on the mortality rate due to cannibalism in BSC juveniles indicated that these two factors collectively resulted in the significant reduction of cannibalism from day 20 onwards ( $p < 0.05$ ). The juveniles reared with UL as shelters at a density of 2 kg/m<sup>2</sup> had the lowest cannibalistic mortality rate ( $79.16 \pm 7.21\%$ ) at 30 days, of all treatments (Table 1).

**Table 1** Mortality rate (MR), carapace width (CW), and body weight (BW) of BSC (*Portunus pelagicus*) juveniles reared in shelters with *Caulerpa lentillifera* (CL) and *Chaetomorpha crassa* (CC) at various densities, as well as in negative control group without shelter. Different superscript letters within same column indicated significant differences among treatments ( $p < 0.05$ ).

| Treatment<br>code                | Species                      | Density<br>(kg/m <sup>2</sup> ) | MR (%)                   |                         |                         | CW (cm/day)            |                        |                        | BW (g/day)             |                        |                        |
|----------------------------------|------------------------------|---------------------------------|--------------------------|-------------------------|-------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|
|                                  |                              |                                 | 10 Days                  | 20 Days                 | 30 Days                 | 10 Days                | 20 Days                | 30 Days                | 10 Days                | 20 Days                | 30 Days                |
| Individual treatment means (n=3) |                              |                                 |                          |                         |                         |                        |                        |                        |                        |                        |                        |
| Negative control                 |                              |                                 |                          |                         |                         |                        |                        |                        |                        |                        |                        |
| CL + D0.5                        | <i>Caulerpa lentillifera</i> | 0.5                             | 95.00±2.50 <sup>a</sup>  | 97.50±0.00 <sup>a</sup> | 97.50±0.00 <sup>a</sup> | 0.79±0.31 <sup>a</sup> | 1.54±0.34 <sup>a</sup> | 1.74±0.00 <sup>a</sup> | 0.10±0.10 <sup>a</sup> | 0.54±0.22 <sup>a</sup> | 0.69±0.14 <sup>a</sup> |
| CC + D0.5                        | <i>Chaetomorpha crassa</i>   | 0.5                             | 84.16±14.64 <sup>a</sup> | 93.33±1.44 <sup>a</sup> | 95.00±0.00 <sup>a</sup> | 0.50±0.22 <sup>a</sup> | 0.89±0.36 <sup>a</sup> | 1.34±0.08 <sup>b</sup> | 0.09±0.03 <sup>a</sup> | 0.26±0.08 <sup>a</sup> | 0.44±0.01 <sup>b</sup> |
| CL + D1                          | <i>Caulerpa lentillifera</i> | 1                               | 83.33±5.20 <sup>a</sup>  | 91.66±6.29 <sup>a</sup> | 96.66±1.44 <sup>a</sup> | 0.34±0.02 <sup>a</sup> | 1.15±0.40 <sup>a</sup> | 2.12±0.38 <sup>a</sup> | 0.11±0.11 <sup>a</sup> | 0.49±0.10 <sup>a</sup> | 1.26±0.27 <sup>a</sup> |
| CC + D1                          | <i>Chaetomorpha crassa</i>   | 1                               | 74.16±12.8 <sup>a</sup>  | 87.50±6.61 <sup>a</sup> | 92.50±6.61 <sup>a</sup> | 0.44±0.23 <sup>a</sup> | 0.77±0.09 <sup>a</sup> | 1.35±0.63 <sup>b</sup> | 0.07±0.02 <sup>a</sup> | 0.14±0.05 <sup>a</sup> | 0.47±0.35 <sup>b</sup> |
| CL + D2                          | <i>Caulerpa lentillifera</i> | 2                               | 68.33±13.76 <sup>a</sup> | 87.50±2.50 <sup>a</sup> | 92.50±0.00 <sup>a</sup> | 0.32±0.15 <sup>a</sup> | 0.71±0.08 <sup>a</sup> | 1.04±0.08 <sup>b</sup> | 0.04±0.01 <sup>a</sup> | 0.16±0.01 <sup>a</sup> | 0.32±0.02 <sup>b</sup> |
| CC + D2                          | <i>Chaetomorpha crassa</i>   | 2                               | 75.00±19.84 <sup>a</sup> | 86.66±5.20 <sup>a</sup> | 92.50±4.33 <sup>a</sup> | 0.37±0.03 <sup>a</sup> | 0.84±0.07 <sup>a</sup> | 1.14±0.09 <sup>b</sup> | 0.08±0.01 <sup>a</sup> | 0.19±0.04 <sup>a</sup> | 0.43±0.17 <sup>b</sup> |
| Two-way ANOVA P-values           |                              |                                 |                          |                         |                         |                        |                        |                        |                        |                        |                        |
| Seaweed species (1)              |                              |                                 | 0.044                    | 0.025                   | 0.063                   | 0.331                  | 0.936                  | 0.749                  | 0.741                  | 0.193                  | 0.114                  |
| Seaweed density (2)              |                              |                                 | 0.013                    | 0.001                   | 0.003                   | 0.913                  | 0.115                  | 0.002                  | 0.651                  | 0.004                  | 0.001                  |
| Interaction (1) × (2)            |                              |                                 | 0.099                    | 0.025                   | 0.013                   | 0.728                  | 0.282                  | 0.009                  | 0.852                  | 0.091                  | 0.001                  |

D0.5 = seaweed densities of 0.5 kg/m<sup>2</sup>; D1 seaweed densities of 1 kg/m<sup>2</sup>; D2 = seaweed densities of 2 kg/m<sup>2</sup>.

### 3.2 Growth rate of BSC

The seaweed species had no significant impact on CW and BW throughout the rearing period. Seaweed density at 1 and 2 kg/m<sup>2</sup> had a significant influence on both CL and BW, which was evident on day 30, with the significantly lowest growth of the C-stage BSC juveniles. Although seaweed species did not significantly affect CW or BW, there was a significant interaction effect between species and density on day 30 for CW and BW. Furthermore, CC at a density of 0.5 kg/m<sup>2</sup> produced CW and BW values of  $2.12 \pm 0.38$  cm/day and  $1.26 \pm 0.27$  g/day, respectively, which were greater than the other treatments. In addition, CC at 0.5 kg/m<sup>2</sup> was not significantly different from the negative control (Table 1).

The seaweed species did not significantly affect ADG and SGR based on all parameters of the BSC juveniles throughout the culture period. However, the seaweed density influenced the growth; compared to the other densities, the crabs reared at 0.5 kg/m<sup>2</sup> had higher growth for ADGCW, ADGBW, SGRCW, and SGRBW with values of  $0.05 \pm 0.01$  cm/day,  $0.02 \pm 0.01$  g/day,  $5.13 \pm 0.73$  %/day, and  $10.81 \pm 1.89$  %/day, respectively. In addition, there were significant interaction effects between

species and density in both ADG and SGR for all parameters. CC at a density of 0.5 kg/m<sup>2</sup> produced higher growth than in the other experimental treatments. However, there was no significant difference for CC at 0.5 kg/m<sup>2</sup> in ADG and SGR in CW compared to the negative control (Table 2).

The coefficient of variation (CV) of CW and BW was used to assess growth uniformity among treatments at 10, 20, and 30 days (Table 3). Overall, the CV values for both CW and BW tended to decrease during the rearing period in most treatments, indicating improved size uniformity as the crabs grew. The lower CV values were found in tanks containing CC at 0.5-1 kg/m<sup>2</sup>. By day 30, the lowest size variation in CW (6.46%) and BW (2.88%) was recorded in the CL at 0.5 kg/m<sup>2</sup>, suggesting enhanced growth uniformity under this condition. In contrast, higher CV values were detected in the CC at 2 kg/m<sup>2</sup> with 31.71% for CW and 65.11% for BW, indicating greater size disparity at higher seaweed density. These results suggest that moderate seaweed coverage (0.5 kg/m<sup>2</sup>) promotes more uniform growth, whereas excessive density may lead to competition or reduced space availability, resulting in higher variation among individuals.

**Table 2** Average daily growth (ADG) and specific growth rate (SGR) of BSC (*Portunus pelagicus*) juveniles reared in shelters with *Caulerpa lentillifera* (CL) and *Chaetomorpha crassa* (CC) at various densities, as well as in negative control group without shelter. Different superscript letters within same column indicated significant differences among treatments ( $p < 0.05$ ).

| Treatment code                     | Species                      | Density (kg/m <sup>2</sup> ) | ADG <sub>CW</sub> (cm/day) | SGR <sub>CW</sub> (%/day) | ADG <sub>BW</sub> (g/day) | SGR <sub>BW</sub> (%/day) |
|------------------------------------|------------------------------|------------------------------|----------------------------|---------------------------|---------------------------|---------------------------|
| Individual treatment means (n = 3) |                              |                              |                            |                           |                           |                           |
| Negative control                   |                              |                              | 0.06±0.00 <sup>a</sup>     | 5.22±0.00 <sup>a</sup>    | 0.02±0.00 <sup>b</sup>    | 10.56±0.71 <sup>b</sup>   |
| CL + D0.5                          | <i>Caulerpa lentillifera</i> | 0.5                          | 0.04±0.00 <sup>b</sup>     | 4.54±0.16 <sup>b</sup>    | 0.01±0.00 <sup>b</sup>    | 9.14±0.09 <sup>b</sup>    |
| CC + D0.5                          | <i>Chaetomorpha crassa</i>   | 0.5                          | 0.07±0.01 <sup>a</sup>     | 5.72±0.52 <sup>a</sup>    | 0.04±0.01 <sup>a</sup>    | 12.48±0.74 <sup>a</sup>   |
| CL + D1                            | <i>Caulerpa lentillifera</i> | 1                            | 0.04±0.02 <sup>b</sup>     | 4.44±1.15 <sup>bc</sup>   | 0.01±0.01 <sup>b</sup>    | 8.86±2.30 <sup>b</sup>    |
| CC + D1                            | <i>Chaetomorpha crassa</i>   | 1                            | 0.03±0.00 <sup>b</sup>     | 3.93±0.19 <sup>bc</sup>   | 0.01±0.00 <sup>b</sup>    | 8.19±0.24 <sup>b</sup>    |
| CL + D2                            | <i>Caulerpa lentillifera</i> | 2                            | 0.04±0.00 <sup>b</sup>     | 4.16±0.19 <sup>bc</sup>   | 0.01±0.00 <sup>b</sup>    | 8.95±1.19 <sup>b</sup>    |
| CC + D2                            | <i>Chaetomorpha crassa</i>   | 2                            | 0.02±0.01 <sup>b</sup>     | 3.37±0.64 <sup>c</sup>    | 0.00±0.00 <sup>b</sup>    | 6.89±1.89 <sup>b</sup>    |
| Two-way ANOVA P-values             |                              |                              |                            |                           |                           |                           |
| Seaweed species (1)                |                              |                              | 0.821                      | 0.876                     | 0.214                     | 0.740                     |
| Seaweed density (2)                |                              |                              | 0.005                      | 0.002                     | 0.003                     | 0.004                     |
| Interaction (1) × (2)              |                              |                              | 0.008                      | 0.016                     | 0.002                     | 0.007                     |

D0.5 = seaweed densities of 0.5 kg/m<sup>2</sup>; D1 seaweed densities of 1 kg/m<sup>2</sup>; D2 = seaweed densities of 2 kg/m<sup>2</sup>.

**Table 3** Size variation of BSC (*Portunus pelagicus*) juveniles reared in shelters with *Caulerpa lentillifera* (CL) and *Chaetomorpha crassa* (CC) at various densities, as well as in negative control group without shelter.

| Treatment code                   | Species                      | Density (kg/m <sup>2</sup> ) | Coefficient of variation (%CV) <sub>CW</sub> |         |         | Coefficient of variation (%CV) <sub>BW</sub> |         |         |
|----------------------------------|------------------------------|------------------------------|----------------------------------------------|---------|---------|----------------------------------------------|---------|---------|
|                                  |                              |                              | 10 Days                                      | 20 Days | 30 Days | 10 Days                                      | 20 Days | 30 Days |
| Individual treatment means (n=3) |                              |                              |                                              |         |         |                                              |         |         |
| Negative control                 |                              |                              | 39.53                                        | 22.49   | 0       | 83.23                                        | 41.41   | 21.02   |
| CL + D0.5                        | <i>Caulerpa lentillifera</i> | 0.5                          | 45.3                                         | 41.17   | 6.46    | 39.22                                        | 28.94   | 2.88    |
| CC + D0.5                        | <i>Chaetomorpha crassa</i>   | 0.5                          | 7.35                                         | 35.21   | 18.29   | 98.66                                        | 21.21   | 21.85   |
| CL + D1                          | <i>Caulerpa lentillifera</i> | 1                            | 54.5                                         | 12.24   | 46.67   | 25.13                                        | 37.58   | 75.42   |
| CC + D1                          | <i>Chaetomorpha crassa</i>   | 1                            | 47.24                                        | 12.61   | 8.48    | 39.95                                        | 6.15    | 7.93    |

**Table 3** (Continuous)

| Treatment code | Species                      | Density (kg/m <sup>2</sup> ) | Coefficient of variation (%CV) <sub>CW</sub> |         |         | Coefficient of variation (%CV) <sub>BW</sub> |         |         |
|----------------|------------------------------|------------------------------|----------------------------------------------|---------|---------|----------------------------------------------|---------|---------|
|                |                              |                              | 10 Days                                      | 20 Days | 30 Days | 10 Days                                      | 20 Days | 30 Days |
| CL + D2        | <i>Caulerpa lentillifera</i> | 2                            | 8.97                                         | 8.11    | 7.86    | 24.66                                        | 20.88   | 40.65   |
| CC + D2        | <i>Chaetomorpha crassa</i>   | 2                            | 36.08                                        | 27.44   | 31.71   | 26.75                                        | 49.33   | 65.11   |

D0.5 = seaweed densities of 0.5 kg/m<sup>2</sup>; D1 seaweed densities of 1 kg/m<sup>2</sup>; D2 = seaweed densities of 2 kg/m<sup>2</sup>.

### 3.3 Water quality factors

During the 30 days of raising the crabs, the salinity, DO, temperature, pH, total ammonia, nitrite, and alkalinity levels in the culture tanks fluctuated in the ranges 31.3–33.7 ppt, 3.5–4.1 mg/L, 25.8–28.9 °C, 7.4–7.7, 0.04–0.24 mg-N/L, 0.00–0.22 mg-N/L, and 173–203 mg/L CaCO<sub>3</sub>, respectively. These ranges in water quality parameters did not affect the growth or survival rate of BSC as they were within the ranges reported as suitable for crablet culture (Syafaat *et al.*, 2021).

Reducing cannibalism in crustaceans, particularly crabs, is crucial to the success of commercial aquaculture, as cannibalism, especially in the BSC, has emerged as a major cause of failure during cultivation and this behavior has resulted in low yields that were deemed unprofitable (Oniam *et al.*, 2011). Other studies have shown that the use of shelter for rearing BSC resulted in a lower mortality rate compared to conditions without shelter (Haemasaton and Pisuttharachai, 2017; Fatihah *et al.*, 2017; Oniam *et al.*, 2020; Leearam *et al.*, 2023). In addition, the physical characteristics of the shelter influenced survival rates (Oniam *et al.*, 2015; Zhang *et al.*, 2021). Specifically, the use of plants as shelter is not only decreased mortality but also improved water quality and food availability (Arumugam *et al.*, 2018; Roleda and Hurd, 2019).

Based on the results of the present study, throughout the experiment, the seaweed species did not significantly affect cannibalism in BSC; however, the seaweed density of 2 kg/m<sup>2</sup> clearly produced the best reduction in crab mortality from 20 days onward. Notably, from 20 days onward, considering the interaction between species and density of seaweed, the crab mortality rate in the CA-cultured crabs was higher than for the UL-cultured crabs for the density of 2 kg/m<sup>2</sup>. This difference could be attributed to the distinct morphologies of the two species. For example, the genus *Caulerpa* features a simple structure consisting of long branching horizontal stolons and numerous simple or branched erect portions (Zubia *et al.*, 2019; Lagourgue *et al.*, 2024). Whereas *Chaetomorpha* is a common green seaweed genus characterized by it consists of uniseriate, unbranched filaments growing to 50–60 cm when fully developed. Lateral rhizoidal filaments are not observed at any time during the year, which provides better shelter (Xu *et al.*, 2018; Mahamad *et al.*, 2023). The present results were consistent with the findings of Toi *et al.* (2023), where all *Cladophora* sp. treatments had lower crab survival rates, biomass, and productivity than those in *Gracilaria tenuistipitata* treatments, likely due to differences in morphology and

structural complexity of these seaweeds used as shelter. There was a reduced mortality rate for the BSC reared with a seaweed density of 2 kg/m<sup>2</sup>. Other studies have reported that increasing the shelter complexity in crab culture could add surface area and interstitial spaces, thereby reducing encounter rates and cannibalism (Daly *et al.*, 2009; Oniam *et al.*, 2020; Leearam *et al.*, 2023). Mirera and Moksne (2013) reported that cannibalism rates could be further reduced by providing shelters that diminished encounter rates among crabs, with such shelters being particularly important in mitigating cannibalism by offering refugia to smaller or molting crabs.

The seaweed species did not affect the crab growth throughout the culture experiment. However, at the end of the experiment, the seaweed densities of 1 and 2 kg/m<sup>2</sup> resulted in the lowest growth performance of BSC compared with the density of 0.5 kg/m<sup>2</sup> and the negative control. Budzafek *et al.* (2021) reported that Macroalgae in marine environments; Ulvophyceae, Chlorophyta (green algae), Florideophyceae, Rhodophyta (red algae), and Phaeophyceae, Ochrophyta (brown algae), with confirmed allelopathic activity against other heterotrophic organisms, which are synthesized as a defense strategy against coexisting aquatic competitors (Cnidaria) and herbivores (Annelida, Echinodermata, Arthropoda, Mollusca, and Chordata). Considering the interaction between species and density, UL with 0.5 kg/m<sup>2</sup> as a cover provided better growth for BSC than the other treatments and was not significantly different from the negative control. Therefore, the use of seaweed as a shelter has provided a positive effect on crabs, i.e., the crabs received additional food from the decaying algae, and a negative effect was that if the algae density was high, allelopathic compounds were synthesized, which affected the growth performance. In this experiment, the growth of crabs in terms of CW and BW was related to survival. For example, there were no significant differences between experiments in the size and growth rate of the BSC during the first 10 days because there was low mortality during this period in all treatments. Conversely, providing too much shelter might block the crabs from consuming primary foods (*Artemia* sp.). Daly *et al.* (2009) found that providing artificial substrate for the red king crab (*Paralithodes camtschaticus*) increased their survival but reduced the growth of juveniles, likely due to reduced cannibalism and increased time spent foraging. Arumugam *et al.* (2018), and Roleda and Hurd (2019) reported that seaweeds helped to improve water quality and have been used commonly in integrated mariculture systems to absorb inorganic nutrient

wastes. Thus, seaweed is a simple bio-filter, low-footprint aquaculture species that can be used for wastewater treatment and creating a habitat conducive habitat for crab development. Chaiyawat *et al.* (2009) revealed that crab fed only red seaweed (*Gracilaria edulis*) had the lowest percentage of body weight; however, as a dietary supplement for crabs, it increased the astaxanthin, red color, seaweed odor, and flavor of the meat and this was suitable for improving the meat quality of these crabs during short feeding periods. Shelley and Lovatelli (2011) reported that crabs were opportunistic omnivores so their aggression and associated cannibalism resulted in those surviving crabs in the tank eating both the experimental food and any recently molted crabs (Leearam *et al.*, 2023). This was similar to the finding of Islam *et al.* (2018), who revealed that the cannibalistic nature of *Scylla paramamosain* gave them additional nutrition, which increased their growth rate. As shown by the results in the present study, the seaweed not only provided cover for crabs, but was also a food source. Chaiyawat *et al.* (2009) also reported on cultivating crabs (*P. pelagicus*) using red seaweed (*Gracilaria edulis*). According to Phinrub *et al.* (2019), crabs are animals that consume both plants and animals, both living and non-living. In their study, crabs were raised beside seagrass and it was reported that the crabs consumed the seagrass leaves, while those in green seaweed (*Caulerpa lentillifera*) shelters had the best growth performance and survival. This improvement was likely attributable to the supplementary food sources provided, as well as the algae that grew on the shelter structures, which served as an additional dietary component. Furthermore, as reported by Shelley and Lovatelli (2011), juvenile *Scylla serrata* consumed seagrass and algae, constituting approximately 13.05% of their total diet.

#### 4. Conclusion

The use of seaweed shelters, particularly *Chaetomorpha crassa* (CC) at a density of 2 kg/m<sup>2</sup>, significantly reduced cannibalism-induced mortality in the C-stage BSC juveniles of the BSC (*Portunus pelagicus*), especially after day 10 of rearing. The seaweed species did not significantly affect growth parameters. The seaweed density of 0.5 kg/m<sup>2</sup> promoted higher growth rates in terms of average daily growth and specific growth rate in the juveniles, although these results were not significantly different from the negative control. Notably, the interaction between species and density of seaweed further enhanced these outcomes, indicating that optimal combinations exist. To balance growth performance with cannibalism prevention, we recommend applying CC at 0.5 kg/m<sup>2</sup> when the goal is to improve BSC larval growth, and increasing CC to 2 kg/m<sup>2</sup> when the priority is to reduce mortality and boost survival. Using these two density regimes according to production stage can substantially increase the success rate of BSC larval seaweed cultivation.

#### 5. References

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