

Article

Artificial Reefs Reduce Morbidity and Mortality of Small Cultured Sea Cucumbers *Apostichopus japonicus* at High Temperature

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Abstract: Summer mortality and morbidity are serious environment-related problems in cultured sea cucumbers (*Apostichopus japonicus*). Air exposure probably worsens the impact of high temperature on cultured sea cucumbers. In this present study, two laboratory experiments were designed to investigate the effects of artificial reefs on mortality, morbidity, crawling, feeding, and adhesion behaviors of small sea cucumbers (~1 g of wet body weight) after air exposure and disease outbreaks at 25 °C, respectively. Significantly lower mortality and morbidity occurred in the group with artificial reefs compared with those in the group without artificial reefs in the two experiments. This present study found that the stressed sea cucumbers cultured inside artificial reefs showed a significantly higher adhesion index, feeding behavior, and crawling frequency than those cultured without artificial reefs. In disease challenge assays, small sea cucumbers cultured inside the artificial reefs showed a significantly higher adhesion index and crawling frequency than those cultured without artificial reefs. In disease challenge assays, small sea cucumbers cultured inside the artificial reefs showed a significantly higher adhesion index and crawling frequency than those cultured without artificial reefs. Feeding, crawling, and adhesion behaviors of sea cucumbers cultured outside artificial reefs were not significantly different from those cultured without artificial reefs. The experimental results indicate that sea cucumbers with good fitness-related behaviors may be less affected by the disease and more likely to move into the crevices of artificial reefs. Fitness-related behaviors were poor in sea cucumbers cultured outside artificial reefs, so we considered them as affected individuals. Thus, artificial reefs provide a place to reduce the physical contact between unaffected and diseased/affected individuals, showing a potential to reduce disease transmission. Our present study establishes a cost-effective approach to increasing the survival of small sea cucumbers in seed production at high temperatures.

Keywords: sea cucumbers; morbidity; mortality; artificial reef; high temperature



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1. Introduction

The sea cucumber (*Apostichopus japonicus*) is popular internationally because of its high nutritional and medicinal value [1]. High market demand stimulated the development of sea cucumber aquaculture [2,3]. The annual production of sea cucumber aquaculture was 222,707 tons in 2022 in China, which was 13.3% higher than that in 2021 [4]. Seed production is a common process in aquaculture, in which small sea cucumbers (~1 g of wet body weight) are intensively cultured in land-based factories until they reach the size available for the following pond culture and stock enhancement [5]. During this process, sea cucumbers are commonly challenged by high temperatures in summer [5]. Sea cucumbers are most suitable for survival in the temperature range between 10 and 21 °C, and the physiology and growth of sea cucumbers are greatly affected when water temperature is higher than 23 °C [6,7]. High temperature (>25 °C) damages the immune system and gut microbiota [6,7] and, consequently, causes mass mortality and morbidity

of sea cucumbers in summer. It is very expensive to decrease the water temperature in seed production in summer. Therefore, it is essential to establish a cost-effective method to reduce the mortality and morbidity of sea cucumbers at high temperatures.

Changing seawater and removing feces are necessary in seed production. Thus, sea cucumbers are inevitably exposed to the air during this process. Air exposure leads to tissue damage and complicated oxidative stress [8,9], which increases the risk of death and disease in sea cucumbers [10]. Sea cucumbers are more severely affected when high temperature and air exposure happen together [11], which is probably responsible for the high mortality and morbidity of sea cucumbers in seed production in summer. In addition, skin ulcer syndrome (SUS) is one of the most common and severe diseases that occur to sea cucumbers in aquaculture, resulting in high mortality due to the high infectivity [12]. High temperature decreases the immunity of sea cucumbers and increases the transmission of pathogenic bacteria [13]. Therefore, sea cucumbers are more susceptible to disease in summer. Diseased sea cucumbers probably spread the disease to other individuals and increase the frequency of disease outbreaks [14,15], resulting in mass mortality and morbidity in aquaculture [16]. Therefore, it is of primary importance to reduce the mortality and morbidity of sea cucumbers after air exposure and disease outbreaks.

Although there is no obvious symptom (e.g., skin ulceration) in the early stages of the disease, sea cucumbers show decreased feeding, movement, and adhesion [16,17]. Thus, fitness-related behaviors are helpful for determining whether sea cucumbers are affected. Decreasing disease transmission is key to reducing mortality and morbidity during the disease outbreak. Our previous study suggests that separation effectively decreases the disease transmission of the sea urchin *Strongylocentrotus intermedius* [18]. However, it is costly and difficult to separate diseased/affected from unaffected individuals in aquaculture. We attempted to reduce mortality and morbidity by reducing the frequency that unaffected individuals have contact with diseased/affected ones [19]. Sea cucumbers are naturally attracted to artificial reefs [20], and they hide into crevices formed by rocks or reefs at high temperatures [21]. Diseased and affected individuals probably struggle to move to crevices because of their poor movement ability [16]. Tian et al. [11] found that artificial reefs improve fitness-related behaviors of stressed sea cucumbers at high temperatures. It is necessary to explore the key role of artificial reefs, which is beneficial to reduce the mortality and morbidity of sea cucumbers at high temperatures. Thus, we hypothesized that artificial reefs reduce mortality and morbidity by reducing physical contact between diseased/affected and unaffected sea cucumbers in seed production at high temperatures.

The main aim of the present study is to investigate whether artificial reefs improve sea cucumber survival in summer, as detailed below: (1) whether artificial reefs reduce the morbidity and mortality of sea cucumbers exposed to the air at high temperatures; (2) whether artificial reefs reduce the morbidity and mortality of sea cucumbers under a disease outbreak at high temperatures; and (3) whether artificial reefs improve the fitness-related behaviors of sea cucumbers exposed to both air exposure and disease outbreak at high temperatures.

2. Materials and Methods

2.1. Experimental Animals

Small green *A. japonicus* (~1 g of wet body weight) were transported from Yinharma Aquatic Products Co., Ltd. (121°54' E, 39°38' N) to the Key Laboratory of Mariculture and Stock Enhancement in the North China's Sea and Ministry of Agriculture and Rural Affairs at Dalian Ocean University (121°56' E, 38°87' N) on 2 March 2022. Small sea cucumbers were cultured in fiberglass tanks (length × width × height: 115 cm × 75 cm × 60 cm) at a density of 400 g/m² [22,23] and with aeration. Water temperature was increased 1 °C each day from 8 °C to 25 °C (ambient temperature) by using a temperature-controlled system (Huixin Co., Dalian, China). Sea cucumbers were maintained at 25 °C for two weeks to be acclimatized to the experimental conditions. Sand-filtered seawater was replaced

every three days with feces and uneaten food removed. Salinity, pH, and dissolved oxygen were tested weekly using a water quality detector (YSI Incorporated, Yellow Springs, OH, USA). The results were 31.36 ± 0.19 , 8.01 ± 0.03 and 9.34 ± 0.21 mg/L, respectively. Small sea cucumbers were fed *ad libitum* with sea mud and commercial feed (Anyuan Industrial Co., Ltd., Yantai, China) at a ratio of 1:4 until the experiments started in April 2022.

2.2. Experiment I

This experiment was carried out to investigate whether artificial reefs improve the survival and fitness-related behaviors (crawling, feeding, and adhesion) of sea cucumbers exposed to the air at 25 °C. The presence or absence of artificial reefs was the experimental factor. An artificial reef (length \times width \times height: 50 mm \times 60 mm \times 55 mm, Figure 1A) consists of seven plastic cylinders (diameter \times length: 15 mm \times 50 mm, Figure 1A), in which only one sea cucumber is accessible. The plastic boxes (top length \times bottom length \times height: 20 cm \times 16.5 cm \times 14 cm) with and without artificial reefs were named group O and group C, respectively ($n = 6$). Twelve plastic boxes were placed in two temperature-controlled tanks (length \times width \times height: 115 cm \times 75 cm \times 60 cm) to maintain the water temperature at 25 °C. Two groups of sixteen sea cucumbers (~ 1 g of wet body weight) without ulcerated body walls were randomly selected and placed in each box with aeration (Figure 1B). Groups C and O each had six parallel groups. Sea cucumbers in each box were fed 5 g of a mixture of sea mud and commercial feed (Anyuan Industrial Co., Ltd. Yantai, China) at a 1:4 ratio every day. Sand-filtered seawater was changed daily in plastic boxes. Sea cucumbers were exposed to the air for 30 min after the seawater was removed from each box. Sea cucumbers were cultured in the above experimental conditions for 5 days.

We identified the sea cucumber with ulcerated skin as a diseased individual (Figure 1D). The numbers of dead and diseased sea cucumbers in each group were counted after the experiment. Sea cucumbers without ulcerated body walls (Figure 1D) were placed in plastic boxes (top length \times bottom length \times height: 20 cm \times 16.5 cm \times 14 cm) in group C. Consistently, individuals without ulcerated body walls in group O were divided into two groups, among which sea cucumbers in one group were inside the artificial reefs and sea cucumbers in the other group were outside the artificial reefs. Then, the two groups were placed in two different cubic plastic boxes (top length \times bottom length \times height: 20 cm \times 16.5 cm \times 14 cm). Finally, we obtained three groups of sea cucumbers without ulcerated body walls, which were the ones from group C, the ones inside the artificial reefs in group O (group O—In), and the ones outside the artificial reefs in group O (group O—Out). Three sea cucumbers were randomly selected from each of the three groups for the measurement of fitness-related behaviors (feeding, crawling, and adhesion). Feeding behavior and crawling behavior of the sea cucumbers were recorded using a digital camera (FDR-AXP55, Shanghai Suoguang Electronics Co., Ltd., Shanghai, China) fixed above the plastic cubic boxes (length \times width \times height: 60 mm \times 47 mm \times 45 mm) within 1 h. Adhesion behavior was measured in cubic plastic boxes (length \times width \times height: 180 mm \times 140 mm \times 45 mm) with 18 compartments. We repeated this experiment 6 times using different sea cucumbers for each group ($n = 6$).

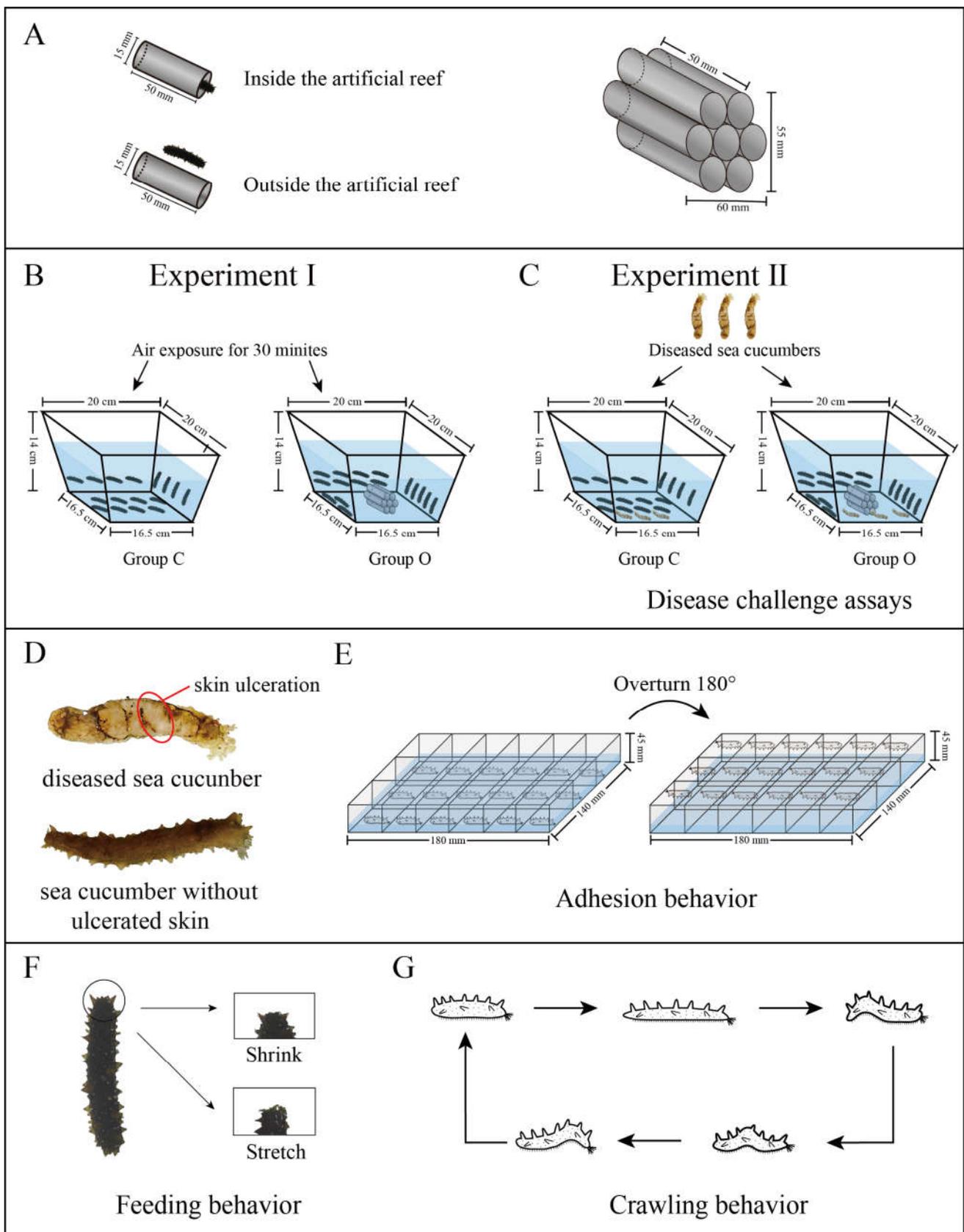


Figure 1. The diagrams show an artificial reef (A), the experiment designs for experiments I (B) and II (C), diseased and individuals without ulcerated skin (D), adhesion behavior (E), feeding behavior (F), and crawling behavior (G) of sea cucumbers.

2.3. Experiment II

We further investigated whether artificial reefs improve the survival and fitness-related behaviors (crawling, feeding, and adhesion) of sea cucumbers at 25 °C when disease outbreaks. The same artificial reefs as experiment I were consistently used in experiment II. Sixteen sea cucumbers without ulcerated body walls were placed in each box (Figure 1C). Diseased sea cucumbers were subsequently put in to create a disease outbreak. The plastic boxes (top length × bottom length × height: 20 cm × 16.5 cm × 14 cm) with and without artificial reefs were named as group O and group C, respectively ($n = 6$). Twelve plastic boxes (top length × bottom length × height: 20 cm × 16.5 cm × 14 cm) were placed in two temperature-controlled tanks (length × width × height: 115 cm × 75 cm × 60 cm). Each group was daily fed ad libitum with 5 g of a mixture of sea mud and commercial feed (Anyuan Industrial Co., Ltd., Yantai, China) at a 1:4 ratio. Sea cucumbers were cultured for three days without the seawater replacement in the same experimental conditions.

The numbers of dead and diseased sea cucumbers were counted after the experiment. Consistently, we obtained three groups of sea cucumbers without ulcerated body walls, which were the ones from group C, the ones inside the artificial reefs in group O (group O—In), and the ones outside the artificial reefs in group O (group O—Out). Crawling, feeding, and adhesion behaviors of sea cucumbers were recorded following the same procedures as described above for each group.

2.4. Mortality and Morbidity

Skin ulceration begins with one or more small white ulcerative spots, followed by the appearance of deep and enlarging ulcerative lesions, resulting in the exposure of the underlying muscles and spicules [24] (Figure 1D). Mortality and morbidity were calculated as follows:

$$M_t = \frac{D_e}{T} \times 100\%$$

$$M_b = \frac{D_i}{T} \times 100\%$$

where M_t is the mortality, M_b is the morbidity, D_e is the number of dead sea cucumbers, D_i is the number of diseased sea cucumbers, and T is the total number of sea cucumbers.

2.5. Crawling Behavior

Crawling behavior of sea cucumbers is composed of five stages (Figure 1G) [25]. A sea cucumber extends its body and then enters the contraction stage. The sea cucumber contracts from the back of the body to the middle and front in sequence. Finally, it returns to a static state [25]. Sea cucumbers complete each crawling cycle as described above, which is counted as one occurrence of crawling behavior. Sea cucumbers were randomly selected from each group and were placed into cubic plastic boxes (length × width × height: 60 mm × 47 mm × 45 mm). Two grams of mixture of sea mud and commercial feed at a ratio of 1:4 was put in the bottom of the box, and the number of occurrences of crawling behavior was counted within 1 h by using a digital camera (FDR-AXP55, Shanghai Suoguang Electronics Co., Ltd., Shanghai, China) ($n = 6$).

2.6. Feeding Behavior

Feeding behavior refers to the process of sea cucumbers ingesting food through its mouth tentacle activity [26]. Sea cucumbers stretch their tentacles out of their mouths, then extend their tentacles to grab the food, and finally capture the food into their mouths by contracting their tentacles [26]. One occurrence of tentacle activity was counted if sea cucumbers completed one tentacle activity cycle. Sea cucumbers were selected from each group and placed into cubic plastic boxes (length × width × height: 60 mm × 47 mm × 45 mm; Figure 1F). Two grams of mixture of sea mud and commercial feed at a ratio of 1:4 was put in the bottom of the box, and the duration of tentacle activity of all sea cucumbers

within one hour was recorded by using a digital camera (FDR-AXP55, Shanghai Suoguang Electronics Co., Ltd., Shanghai, China) ($n = 6$).

2.7. Adhesion Behavior

Movement and ingestion of sea cucumbers greatly relies on the adhesion of mouth tentacles and tube feet. Adhesion behavior was measured according to Tian et al. [11]. Eighteen sea cucumbers from each group were randomly selected and placed in cubic devices (length \times width \times height: 180 mm \times 140 mm \times 45 mm; Figure 1E) with eighteen compartments. Each sea cucumber was placed in one compartment. Seawater was added to the devices until the seawater level reached a height of 2 cm. The devices were slowly turned to 180° after 10 min. We recorded the time that sea cucumbers fell from the top of the devices, since their adhesion abilities were disabled to support their body weight. The adhesion time was recorded as 600 s if sea cucumbers still adhered to the top of the box after 10 min. Wet body weight (g) of each sea cucumber was measured by using an electronic balance (G & G Co., San Diego, CA, USA) after the recording ($n = 6$). Data are accurate to one decimal place. Adhesion index (A_i) was formulated, as described by Tian et al. [11]:

$$A_i = \frac{T}{W}$$

where A_i is the adhesion index, T is the adhesion time (s), and W is the wet weight of an individual sea cucumber (g).

2.8. Statistical Analysis

All data were subjected to the analysis of variance distribution and homogeneity of variance using the Kolmogorov–Smirnov test and the Levene test, respectively. The mortality and morbidity of experiments I and II were non-normally distributed and/or heterogeneity of variance. Thus, the data were compared by using the Mann–Whitney U test. Crawling frequency, tentacle activity frequency, and the adhesion index were analyzed by using Kruskal–Wallis H in both experiments. All statistical analyses were performed using SPSS 22.0 statistical software. The level of significance was considered as $p < 0.05$.

3. Results

3.1. Experiment I

3.1.1. Mortality and Morbidity

Mortality was significantly higher in group C ($43.06 \pm 2.56\%$) than that in group O ($5.56 \pm 4.12\%$; Mann–Whitney $U = 2.961$, $p = 0.003$; Figure 2A). Consistently, group C showed significantly higher morbidity ($48.61 \pm 1.39\%$) than group O ($6.94 \pm 3.98\%$; Mann–Whitney $U = 3.017$, $p = 0.003$; Figure 2B).

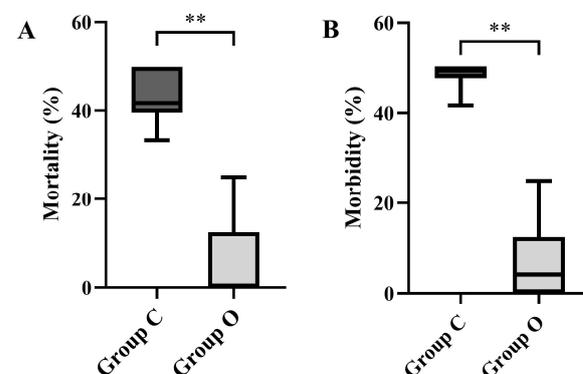


Figure 2. Mortality (A) and morbidity (B) of sea cucumbers between groups C and O. The asterisks ** mean $p < 0.01$ (mean \pm SE, $n = 6$). Group C: sea cucumbers without artificial reefs; Group O: sea cucumbers with artificial reefs.

3.1.2. Crawling Frequency

There was no significant difference in crawling frequency between sea cucumbers of group C (4.44 ± 0.88 times) and individuals outside the artificial reef of group O (3.22 ± 0.50 times; Kruskal–Wallis $H = 14.332$, $p = 1.000$; Figure 3A). Crawling frequency was significantly higher in sea cucumbers inside the artificial reefs of group O (10.83 ± 1.52 times) than that in sea cucumbers outside the artificial reefs of group O (Kruskal–Wallis $H = 14.332$, $p = 0.001$) and group C (Kruskal–Wallis $H = 14.332$, $p = 0.008$; Figure 3A).

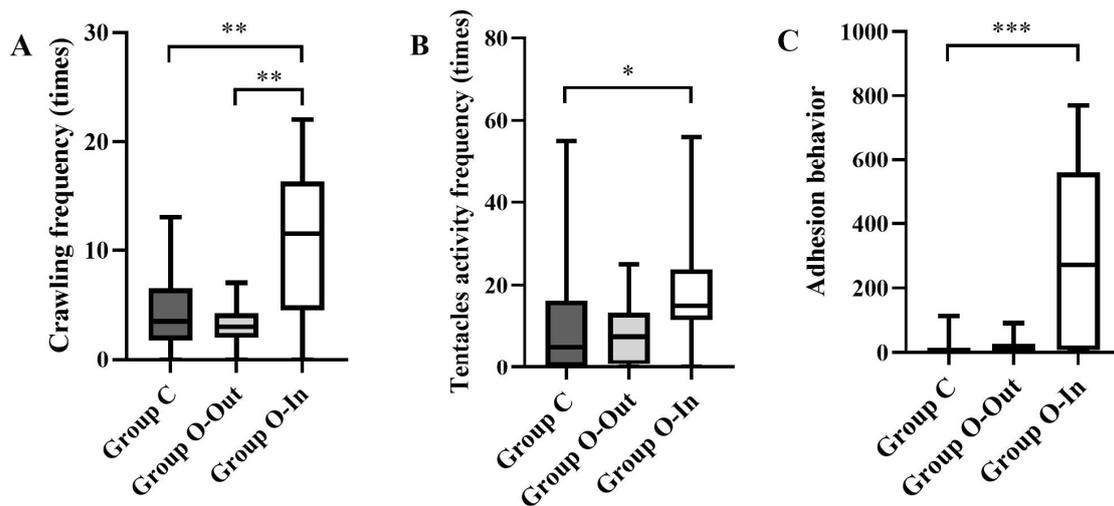


Figure 3. Crawling frequency (A), tentacles activity frequency (B) and adhesion behavior (C) of sea cucumbers. The asterisks *, **, *** mean $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively (mean \pm SE, $n = 6$). Group C: sea cucumbers without artificial reefs in group C; Group O-Out: sea cucumbers outside the artificial reefs in group O; Group O-In: sea cucumbers inside the artificial reefs in group O.

3.1.3. Feeding Behavior

There was no significant difference in tentacle activity frequency between sea cucumbers of group C (10.44 ± 3.51 times) and individuals outside the artificial reefs of group O (8.50 ± 1.87 times; Kruskal–Wallis $H = 7.705$, $p = 1.000$; Figure 3B). Consistently, tentacle activity frequency was not significantly different between sea cucumbers outside (8.50 ± 1.87 times) and inside (18.72 ± 3.26 times) the artificial reefs (Kruskal–Wallis $H = 7.705$, $p = 0.067$; Figure 3B). Tentacle activity frequency was significantly higher in sea cucumbers inside the artificial reefs in group O than that in individuals of group C (Kruskal–Wallis $H = 7.705$, $p = 0.036$; Figure 3B).

3.1.4. Adhesion Behavior

The adhesion index was not significantly different between sea cucumbers outside the artificial reefs of group O (16.66 ± 5.50) and individuals of group C (9.75 ± 6.28 ; Kruskal–Wallis $H = 0.561$, $p = 0.065$; Figure 3C). Consistently, the adhesion index was not significantly different in sea cucumbers outside and inside the artificial reefs (283.62 ± 61.4) in group O (Kruskal–Wallis $H = 0.561$, $p = 0.076$; Figure 3C). However, sea cucumbers inside the artificial reefs of group O showed significantly higher adhesion index than individuals of group C (Kruskal–Wallis $H = 0.561$, $p < 0.001$; Figure 3C).

3.2. Experiment II

3.2.1. Mortality and Morbidity

Mortality was significantly higher in group C ($30.56 \pm 3.51\%$) than that in group O ($4.17 \pm 2.84\%$; Mann–Whitney $U = 2.879$, $p = 0.004$; Figure 4A). Morbidity was significantly higher in group C ($43.06 \pm 2.56\%$) than that in group O ($6.94 \pm 4.52\%$; Mann–Whitney $U = 2.961$, $p = 0.003$; Figure 4B).

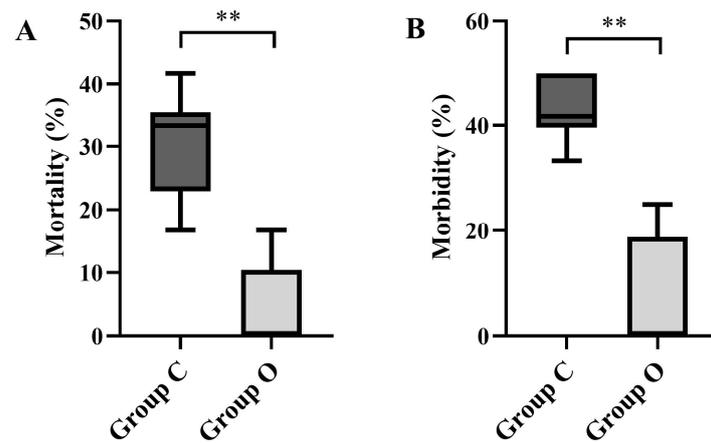


Figure 4. Mortality (A) and morbidity (B) of sea cucumbers between groups C and O. The asterisks ** mean $p < 0.01$ (mean \pm SE, $n = 6$). Group C: sea cucumbers without artificial reefs; Group O: sea cucumbers with artificial reefs.

3.2.2. Crawling Frequency

There was no significant difference in crawling frequency between sea cucumbers of group C (1.67 ± 0.35 times) and individuals outside the artificial reefs in group O (2.56 ± 0.56 times; Kruskal–Wallis $H = 6.908$, $p = 0.762$; Figure 5A). Consistently, crawling frequencies of sea cucumbers outside (2.56 ± 0.56 times) and inside (3.67 ± 0.60 times) the artificial reefs in group O (Kruskal–Wallis $H = 6.908$, $p = 0.417$; Figure 5A) were not significantly different in the experiments. Significantly higher crawling frequency occurred in sea cucumbers inside the artificial reefs in group O than individuals in group C (Kruskal–Wallis $H = 6.908$, $p = 0.026$; Figure 5A).

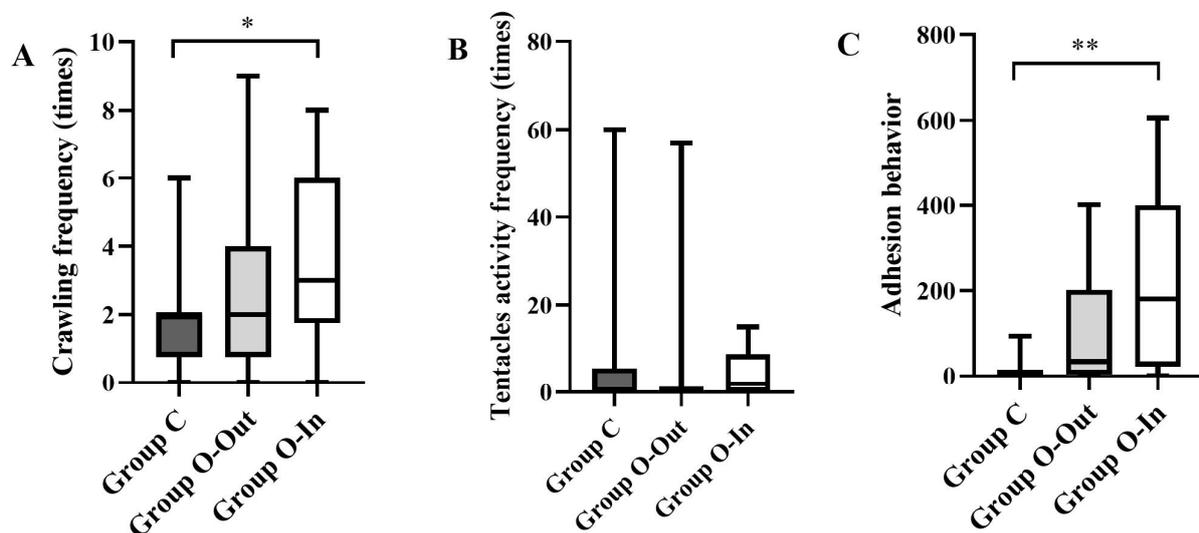


Figure 5. Crawling frequency (A), tentacle activity frequency (B), and adhesion behavior (C) of sea cucumbers. The asterisks *, ** mean $p < 0.05$ and $p < 0.01$, respectively (mean \pm SE, $n = 6$). Group C: sea cucumbers without the artificial reefs in group C; Group O-Out: sea cucumbers outside the artificial reefs in group O; Group O-In: sea cucumbers inside the artificial reefs in group O.

3.2.3. Feeding Behavior

There was no significant difference in feeding behavior of sea cucumbers cultured inside artificial reefs of group O, outside artificial reefs of group O, and in group C ($p > 0.05$; Figure 5B).

3.2.4. Adhesion Behavior

The adhesion index was significantly higher in sea cucumbers inside the artificial reefs of group O (206.44 ± 47.12) than that in the individuals of group C (11.41 ± 5.37 ; Kruskal–Wallis $H = 13.007$, $p = 0.001$; Figure 5C). The adhesion index of sea cucumbers of group C (11.41 ± 5.37) was not significantly different from that of those outside the artificial reefs in group O (105.28 ± 28.26) (Kruskal–Wallis $H = 13.007$, $p = 0.053$; Figure 5C). Consistently, no significant difference was found in the adhesion indexes between sea cucumbers outside and inside the artificial reefs in group O (Kruskal–Wallis $H = 13.007$, $p = 0.732$; Figure 5C).

4. Discussion

The survival of sea cucumbers is constrained by high temperatures. A temperature of $25\text{ }^{\circ}\text{C}$ is the threshold for severe heat stress in sea cucumbers, leading to a significantly increased risk of death and disease in sea cucumbers [6]. Air exposure is unavoidable in seed production, which not only causes mechanical damage [27] but also disrupts the immune system of sea cucumbers [9]. Furthermore, our previous study found that the negative effects of air exposure and high temperature on sea cucumbers were synergistic [11]. This present study found that artificial reefs significantly reduced mortality and morbidity of sea cucumbers exposed to the air at $25\text{ }^{\circ}\text{C}$. Specifically, in such conditions, the morbidity and mortality of sea cucumbers not cultured in artificial reefs were 7-fold and 8-fold the morbidity and mortality of those cultured in artificial reefs, respectively. This suggests that artificial reefs are an effective approach to improving the survival of sea cucumbers under the combined stress of high temperature and air exposure. Fitness-related behavior is a common indicator for evaluating the condition of sea cucumbers. The typical characteristics of sea cucumbers in poor conditions are anorexia, decreased adhesion capacity, and poor movements ability [17]. Tian et al. [11] found that artificial reefs improved fitness-related behaviors of sea cucumbers after they were exposed to high temperatures and the air. However, it is unknown how artificial reefs improve fitness-related behaviors. This present study found that sea cucumbers cultured inside the artificial reefs had significantly better feeding, crawling, and adhesion behaviors than those cultured without artificial reefs. Sea cucumbers exposed to the air and/or high temperatures reduce feeding or even stop feeding behavior [11,27,28]. Sea cucumbers cultured inside artificial reefs may acquire more energy by frequent feeding [28] and, consequently, may better cope with combined stressors. The adhesion of the tube feet of sea cucumbers affects the subsequent movement such as crawling behavior [29]. It is important to note that the fitness-related behaviors (feeding, crawling, and adhesion behaviors) of sea cucumbers cultured outside artificial reefs are not significantly different from those of sea cucumbers without artificial reefs. This suggests that the condition of the sea cucumbers inside the artificial reefs is better under the combined stressors. Therefore, artificial reef improves the survival and fitness-related behaviors of sea cucumbers after they are exposed to high temperatures and the air, thereby increasing the survival rate.

Skin ulcer syndrome is one of the common diseases with high infectivity and high mortality in seed production of sea cucumbers [17]. High temperatures reduce the immunity and disease resistance of sea cucumbers [30,31] and, thus, greatly increases the possibility of infection. The present study found that artificial reefs significantly reduced the mortality and morbidity of sea cucumbers during disease outbreaks at high temperatures. Specifically, during disease outbreaks at high temperatures, the morbidity and mortality of sea cucumbers not cultured in artificial reefs were 6-fold and 7-fold the morbidity and mortality of those cultured in artificial reefs. This suggests that artificial reefs improve the survival in disease environments and probably affect the spread of skin ulcer syndrome. In disease challenge assays, sea cucumbers cultured inside artificial reefs had significantly better adhesion and crawling behaviors, while there was no significant difference in the fitness-related behaviors between the sea cucumbers cultured outside and without artificial reefs. Decreased adhesion and movement ability are the symptoms of skin ulcer

syndrome [17]. Hence, we speculated that sea cucumbers cultured outside and without artificial reefs were more affected by diseased sea cucumbers, although their skins were not ulcerated. Chemosensory cues are important for a variety of fundamental behavioral processes [32,33]. Lobsters use chemical cues from diseased individuals to determine whether the shelter is safe, and healthy lobsters rarely share the shelters with diseased lobsters [34]. Diseased sea cucumbers emit certain chemical cues for warning [33,35]. Sea cucumbers show escaping behavior after receiving alarm cues from diseased individuals [35,36]. Artificial reefs are possible places for escape due to their attraction to sea cucumbers [20,37]. Frequent physical contact with diseased individuals increases the probability of disease transmission [19]. Diseased sea cucumbers probably release chemical cues that promote the escape of individuals with strong movement to the crevices of artificial reefs. In the limited space, sea cucumbers that did not enter the reef inevitably contacted with diseased individuals, which increased the extent to which they were affected. In the present study, each crevice is accessible to only one sea cucumber. Sea cucumbers cultured with artificial reefs probably showed less contact with diseased and affected individuals after staying in porous artificial reefs, which reduced the extent of the affection. Seed production is an intensive aquaculture mode, and isolation of diseased sea cucumbers is expensive. Therefore, the artificial reef is a cost-effective approach to increasing the possibility of the survival of sea cucumbers by reducing the frequency of contact with diseased and affected individuals in seed production. Notably, our experiment is based on a laboratory experiment. Therefore, conducting a further field experiment is necessary.

In conclusion, we encourage aqua-farmers to use artificial reefs to decrease disease transmission and, thus, reduce the mortality and morbidity of small sea cucumbers in seed production at high temperatures. The present novel finding provides valuable insights into the improved management for the seed production of sea cucumbers in summer.

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Conflicts of Interest: The authors declare no conflict of interest.

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