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A New Approach to Integrated Multi-Trophic Aquaculture System of the Sea Cucumber *Apostichopus japonicus* and the Sea Urchin *Strongylocentrotus intermedius*

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Abstract: The sea cucumber *Apostichopus japonicus* and the sea urchin *Strongylocentrotus intermedius* are two commercially important species and are widely cultured in China. Here, a laboratory experiment was conducted for 34 days to assess whether the survival, growth and behavior performances are better in the new commercially valuable integrated multi-trophic aquaculture (IMTA) system (group M, 90 *S. intermedius* and 37 *A. japonicus*/10,638 cm^3 of stocking density) than those in the control group for sea urchins (group U, 90 *S. intermedius*/10,638 cm^3 of stocking density) and the control group for sea cucumbers (group C, 37 *A. japonicus*/10,638 cm^3 of stocking density). We found that feeding behavior, crawling behavior, body length and body weight of sea cucumbers were significantly greater in group M than those in group C. These results suggest that the new IMTA system improves fitness-related behaviors and consequently leads to a better growth in *A. japonicus* while maintaining a high biomass. We further found that group M showed significantly larger body size and Aristotle's lantern reflex as well as significantly lower mortality and morbidity in sea urchins, compared to those in group U. This suggests that the new IMTA system greatly improves feeding behavior and body growth, and survival of cultured *S. intermedius*. This IMTA system is a promising candidate to promote the production efficiency of juvenile *A. japonicus* (as primary species) and *S. intermedius* (as subsidiary species) in China.

Keywords: echinoderm; IMTA; feeding behavior; growth; survival

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1. Introduction

Integrated Multi-Trophic Aquaculture (IMTA) system attracts wide attentions in recent years [1], because it brings higher profits and more diversification of commercial production with less environmental pollution [2–4]. The land-based IMTA of sea urchins (as primary product species) and sea cucumbers (as subsidiary product species) has been successfully developed due to their high commercial value and feeding habits [5–7]. For example, sea cucumbers *Holothuria tubulosa* ingested 54% of organic matters from the feces of sea urchins *Paracentrotus lividus* [8], which greatly reduces the total waste and increases additional value (i.e., sea cucumbers). However, the aquaculture production of sea cucumber is higher than that of sea urchins in China. The annual production, for example, is 196,564 metric tons in sea cucumbers and 7952 metric tons in sea urchins in China in 2020 [9]. This suggests that the above approach probably is not applicable to producing echinoderms in China. It is, therefore, essential to develop a new approach to IMTA with sea cucumbers, as the primary species, and sea urchins as the subsidiary species with a high stocking biomass in China.

The sea cucumber *Apostichopus japonicus* and the sea urchin *Strongylocentrotus intermedius* are two commercially important species and are widely cultured in China [10,11]. Seed productions of *A. japonicus* and *S. intermedius* are both feasible in May and June [12,13].

The body weight of *A. japonicus* reaches 1–2.5 g in November while the test diameter of *S. intermedius* reaches 6–15 mm at that time [12]. In the production of juvenile *A. japonicus*, polyethylene nets were commonly used as the substrate on the top of nursery tanks to increase culture biomass and avoid intraspecific competition [11]. In this context, many sea cucumber diets (mainly commercial powdered diets) fail to adhere to the nets and settle at the bottom of tanks, making it difficult to use the water space and deposited diets below the nursery tanks for *A. japonicus* situated on the nets. Sea urchins are omnivorous animals and their unique feeding organ, Aristotle's lantern, is adapted for omnivorous diets such as macroalgae, hard calcified surfaces, and soft sediments [14–16]. Many edible macroalgae for sea urchins, such as *Saccharina japonica* (the most commonly food used for sea urchin aquaculture), *Sargassum thunbergii*, *S. polycystum*, are made into powdered diets and used for sea cucumber aquaculture [17–19]. Thus, it is reasonable to assume that the powdered diets wasted in nursery tanks can be effectively used by *S. intermedius*. However, the sufficient food source for *S. intermedius* is not enough to support an effective IMTA with *A. japonicus*. Physical interactions between sea urchins and sea cucumbers display negative effects on the fitness of both species. For example, Sun et al. [20] found that sea cucumbers showed significantly higher escaping speed when behavioral interactions existed between sea cucumbers and sea urchins. Mass mortality occurred in *A. japonicus* with the increased stocking density of *S. intermedius* when they were not cultured separately [21], which may be due to the injuries caused to the sea cucumbers by sea urchin spines. This suggests that a specialized culture facility is further required to separate *S. intermedius* from *A. japonicus* in the IMTA system. Our previous studies found that segregation in multi-layer culture significantly improved survival [22], food utilization and body growth [23] of *S. intermedius*, compared with those without the multi-layer culture. We assumed that a plastic box divided into three layers (each layer has many compartments) full of holes would represent a promising candidate for the *S. intermedius* aquaculture, because it not only allows the powdered diets to enter the facility through the holes for *S. intermedius* consumption, but also provides an additional substrate for *A. japonicus* in the IMTA system.

Here, the main purposes of the present study are to investigate: (1) whether juvenile *S. intermedius* show better survival, feeding behaviors and growth in the IMTA system; (2) whether the survival, fitness-related behaviors and growth of juvenile *A. japonicus* are better in the IMTA system; (3) what is the application potential of the new IMTA system with *A. japonicus* as the primary species and *S. intermedius* as the subsidiary species.

2. Materials and Methods

2.1. Experimental Animals

Juvenile *S. intermedius* (7.4 ± 1.0 mm of test diameter, 0.2 ± 0.1 g of wet body weight, mean ± SD) (n = 20) and *A. japonicus* (green type, 30.1 ± 3.5 mm of relaxed body length, 1.1 ± 0.4 g of wet body weight, mean ± SD) (n = 10) were randomly chosen from an aquafarm of Lvshun, Dalian (121°13′ E, 38°88′ N) on 13 April 2021 and Dalian Zhuang Yuanhai Ecological Seedling Industry Co., Ltd. (122°69′ E, 39°27′ N) on 16 April 2021, respectively. They were subsequently maintained in fiberglass tanks (length × width × height: 1150 × 750 × 600 mm) with aeration in the Key Laboratory of Mariculture & Stock Enhancement in north China's Sea, Ministry of Agriculture and Rural Affairs at Dalian Ocean University (121°56′ E, 38°87′ N). The incandescent light intensity was ~30 lx with the photoperiod (12 light: 12 dark), according to the culture management commonly used for seed production in China. Sea urchins and sea cucumbers were fed the leaf blade of fresh kelp *S. japonica* and a commercial sea cucumber powdered diet (mainly composed of algal powder, the grain size of 0.125 mm) (Anyuan Industrial Co., Yantai, China.), respectively. Two-thirds of seawater was changed daily. Water temperature and salinity were monitored daily using a portable water quality monitoring meter (Xylem Co., OH, USA). They were 15.4 ± 0.2 °C and 30.8 ± 0.1‰, respectively.

Experimental animals were fasted for three days to standardize their nutritional status, and their initial body sizes were subsequently assessed at the beginning of the trial.

2.2. Experimental Design

There were three groups in this study: the control group for *A. japonicus* (group C, Figure 1A), the control group for *S. intermedius* (group U, Figure 1B) and the integrated multi-trophic aquaculture of *A. japonicus* and *S. intermedius* (group M, Figure 1C). The stocking density of sea urchins and sea cucumbers in group M was consistent with those in groups U and C, respectively. In group C, three pieces of polyethylene nets (length × width: 100 ×100 mm for each net; 1 mm diameter of mesh size) were tied as substrate under a plastic ball (to float those nets) in a cylindrical plastic bucket (diameter × height: 220 × 280 mm; 37 *A. japonicus*/10,638 cm^3 of stocking density). Thirty-seven *A. japonicus* were randomly chosen and placed in the plastic bucket according to the culture management commonly used for the seed production of sea cucumbers. In group U, 90 juvenile *S. intermedius* (~7 mm of test diameter) were randomly selected and maintained in the plastic cage (length × width × height: 150 × 150 × 130 mm, 3 mm diameter of mesh size) in a cylindrical plastic bucket (diameter × height: 220 × 280 mm; 90 *S. intermedius*/10,638 cm^3 of stocking density) according to the culture practice for the seed production of sea urchins. In group M, culture areas existed for *A. japonicus* on top and *S. intermedius* at the bottom in the plastic bucket (diameter × height: 220 × 280 mm; 90 *S. intermedius* and 37 *A. japonicus* /10,638 cm^3 of stocking density). A dismountable plastic box (length × width × height: 150 × 150 × 130 mm, 30 holes of 4 mm diameter/100 cm^2), divided into three layers and six compartments in each layer (Figure 1D), was put into the culture areas of *S. intermedius*. Five *S. intermedius* were randomly selected and put into each compartment of the plastic box (90 *S. intermedius* in total for each box).

Each group had 6 replicates under ~30 lx of incandescent light intensity with the photoperiod (12 h light: 12 h dark) in this study from 24 April 2021 to 28 May 2021. The leaf blade of wild fresh kelp *S. japonica* collected from Heishijiao, Dalian (121°58′ E, 38°87′ N) was provided ad libitum to *S. intermedius* in group U. Animals in groups C and M were fed a commercial powdered diet (Anyuan Industrial Co., Yantai, China). The feces and dead individuals were removed daily for all the groups. Water temperature was not controlled, ranging from 14.3 to 16.6 °C (ambient temperatures). Salinity was 30.7 ± 0.7 ‰, according to the weekly measurement by a portable water quality monitoring meter (Xylem Co., Yellow Springs, OH, USA). All the experimental tanks were kept in still water with aeration, because this does not cause the loss of powdered diet. Two-thirds of the seawater was renewed daily to avoid deterioration of water quality.

2.3. Mortality and Morbidity

Black-mouth disease, which is characterized by the blackened peristomial membrane (Figure 1E), is one of the most serious diseases in *S. intermedius* aquaculture [24]. The performance of sea urchins without disease is shown in Figure 1E. Sea cucumbers with skin ulceration syndrome are indicated by the white spots on the integument, and these spots quickly fill the whole integument and consequently leads to death [25] (Figure 1F). The performance of sea cucumbers without disease is shown in Figure 1F. Mortality and morbidity were evaluated at the end of the experiment.

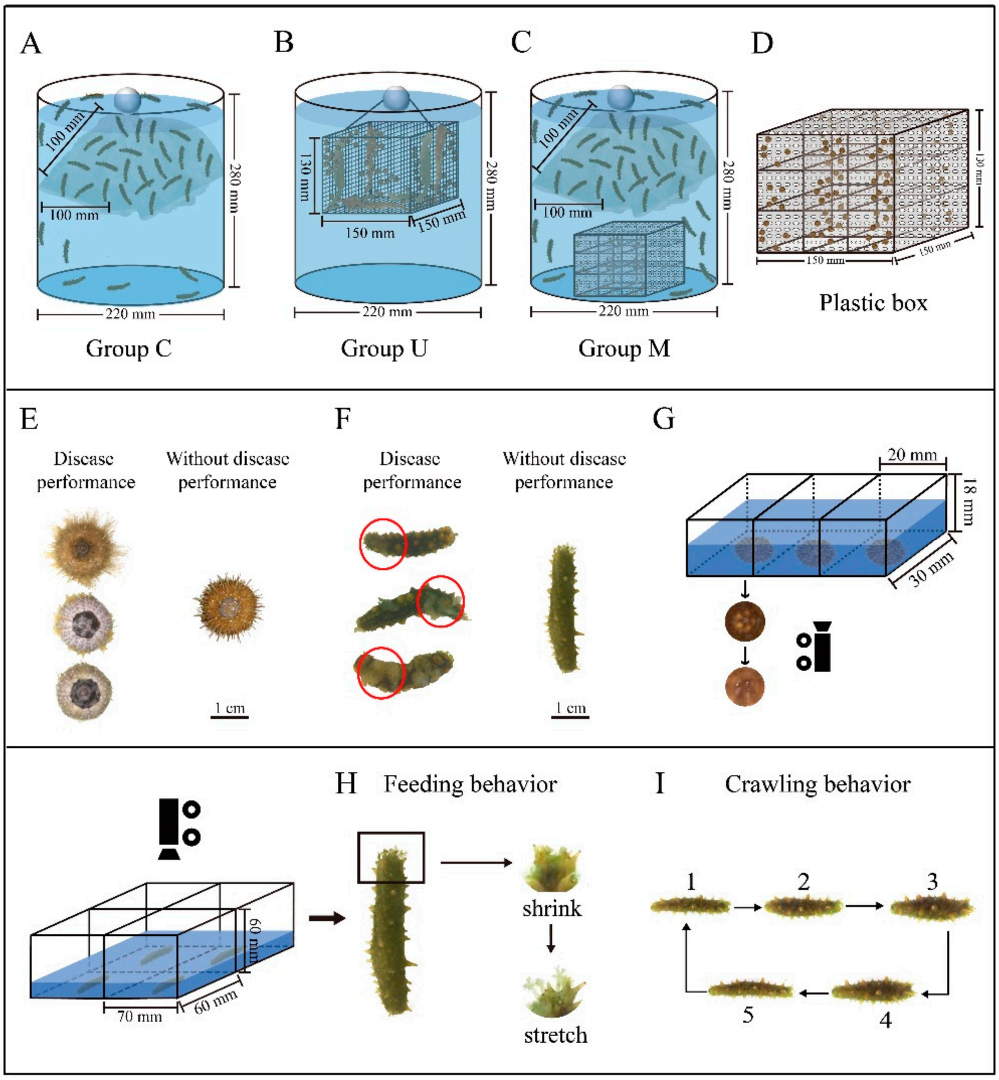

Figure 1. Experimental facilities used for group C (**A**), group U (**B**), and group M (**C**). Group C: the control group for sea cucumbers. Group U: the control group for sea urchins. Group M: the integrated multi-trophic aquaculture of sea cucumbers and sea urchins. A dismountable plastic box divided into three layers and six compartments in each layer was used to culture sea urchins in group M (**D**). Sea urchins with the performance of black-mouth disease and without the disease performance (**E**). Sea cucumbers with the performance of skin ulceration syndrome and without the disease performance (**F**). Red circles indicate the ulcerative skin in sea cucumbers (**F**). Aristotle's lantern reflex of sea urchin (**G**). Feeding behavior of sea cucumbers refers to the process, by which the tentacles reach the food sediments and deliver them to the mouth (**H**). Crawling behavior indicates the movement ability of sea cucumbers, and it was divided into five stages (**I**).

2.4. Growth

Final growth traits were assessed at the end of the experiment. Sea cucumbers (both inside and outside nets) from groups C and M were randomly selected for the subsequent measurement. Sea urchins of group M were randomly selected from ten haphazardly chosen compartments for the following measurement [22]. Test diameter and lantern length of *S. intermedius* were measured using a digital vernier caliper (Mahr Co., Ruhr, Germany). Body and lantern were weighted wet by an electric balance (G & G Co., San Diego, CA, USA). To evaluate the growth traits of *A. japonicus*, they were randomly selected and placed in a small tank (length × width × height: 227 × 157 × 61 mm) filled with fresh seawater according to Broeke et al. [26]. The software ImageJ 1.51 n was used to measure their naturally relaxed body length as a polygonal line after being photographed by a digital video (Canon Co., Shenzhen, China). Wet body weight was assessed using an electric balance (G & G Co., San Diego, CA, USA). The average of all the ten animals was considered as one value for each of the six replicates ($n = 6$).

2.5. Aristotle's Lantern Reflex of S. intermedius

Aristotle's lantern reflex is defined as one cycle of the teeth from opening to closing, indicating the ability to obtain food in *S. intermedius* [24]. A simple device with three compartments (length × width × height: 30 × 20 × 18 mm for each compartment) with agar film (2 g kelp powder with 3 g agar powder for feeding of sea urchins) at the bottom was used to assess Aristotle's lantern reflex according to Ding et al. [27] (Figure 1G). Five *S. intermedius* were randomly selected from each group and put into the experimental device at the end of the rearing experiment. The number of Aristotle's lantern reflex within 10 min were counted using a digital camera (Canon Co., Shenzhen, China). The average of all the five *S. intermedius* was considered as one value for each of the six replicates ($n = 6$).

2.6. Feeding and Crawling Behaviors of A. japonicus

Feeding behavior, which is closely related to the food intake of *A. japonicus* [28], was evaluated according to Sun et al. [29]. It refers to the process by which the tentacles reach the food sediments and deliver them to the mouth in *A. japonicus* [29] (Figure 1H). Completing one cycle was recorded as one tentacle activity frequency in this study.

Crawling behavior indicates the movement ability of *A. japonicus* [30,31]. Crawling behavior includes the processes below according to Lin [32]: (1) sea cucumbers remain at the quiescent condition; (2) they contract from the anus to the back; (3) the contraction gradually transits to the ostium; (4) the contraction subsides, and sea cucumbers remain at the quiescent condition (Figure 1I). Completing one cycle was recorded as one crawling frequency in this study.

Four *A. japonicus* of each replicate were randomly selected and individually put into experimental tanks (length × width × height: 70 × 60 × 60 mm) filled with a commercial powdered diet (Anyuan Industrial Co., Yantai, China) at the end of the experiment. Tentacle activity frequency and crawling frequency were recorded within one hour using a digital camera (Canon Co., Shenzhen, China). The average of all the four *A. japonicus* was considered as one value for each of the six replicates ($n = 6$)

2.7. Statistical Analysis

The normal distribution and homogeneity of variance were analyzed using the Kolmogorov–Smirnov test and Levene test, respectively. Body weight and Aristotle's lantern reflex of *S. intermedius*, body weight and tentacles activity frequency of *A. japonicus* were analyzed using the Mann–Whitney *U* test, because of the non-normal distribution and/or heterogeneity of variance. Differences in the rest traits between groups were compared using the independent sample *t*-test. The replicate means were calculated for all variables. All statistical analyses were performed using SPSS 19.0 statistical software. $p < 0.05$ was considered statistically significant.

3. Results

3.1. Mortality and Morbidity

The mortality (53.3 ± 5.0%) and morbidity (54.4 ± 5.5%) of the group M were significantly lower than those in the group U (67.8 ± 12.1%, $t = 7.349$, $p = 0.022$ for mortality, Figure 2A) (68.0 ± 12.0%, $t = 6.297$, $p = 0.031$ for morbidity, Figure 2B). However, there were no significant differences in mortality (14.0 ± 13.0% for group M and 21.6 ± 11.8% for group C) and morbidity (15.3 ± 15.0% for group M and 23.9 ± 12.5% for group C) between groups M and C ($t = 1.141$, $p = 0.310$ for mortality, Figure 2C) ($t = 1.156$, $p = 0.307$ for morbidity, Figure 2D).

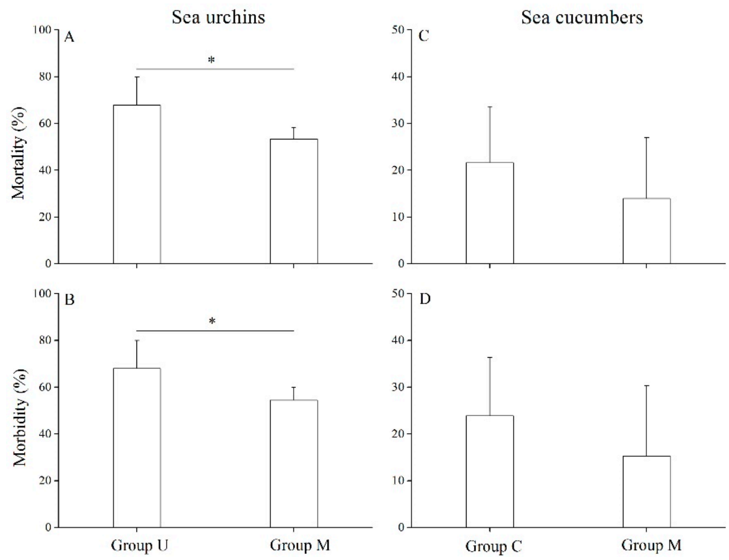

Figure 2. Mortality of *Strongylocentrotus intermedius* (**A**) and *Apostichopus japonicus* (**C**) as well as the morbidity of *S. intermedius* (**B**) and *A. japonicus* (**D**) between groups (mean ± SD, n = 6). Group C: the control group for sea cucumbers. Group U: the control group for sea urchins. Group M: the integrated multi-trophic aquaculture of sea cucumbers and sea urchins. The asterisk * means $p < 0.05$.

3.2. Growth

Test diameter (12.8 ± 2.2 mm) and body weight (0.8 ± 0.3 g) of *S. intermedius* in group M were significantly higher than those in group U (10.4 ± 2.1 mm, t = 6.197, $p < 0.001$ for test diameter, Figure 3A) (0.5 ± 0.2 g, Mann–Whitney U = 669, $p < 0.001$ for body weight, Figure 3B). Further, body length (38.2 ± 3.1 mm) and body weight (1.4 ± 0.8 g) of *A. japonicus* in group M were significantly larger than those in group C (33.9 ± 1.3 mm, t = 9.500, p = 0.012 for body length, Figure 3C) (1.0 ± 0.4 g, Mann–Whitney U = 1260, p = 0.005 for body weight, Figure 3D).

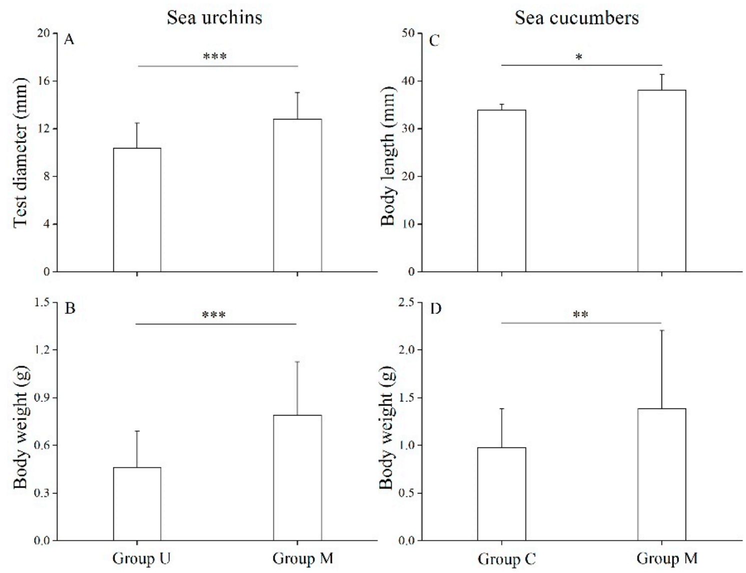

Figure 3. Test diameter (**A**) and wet body weight (**B**) of *Strongylocentrotus intermedius* between groups U and M. Body length (**C**) and wet body weight (**D**) of *Apostichopus japonicus* between groups C and M (mean ± SD, n = 6). Group C: the control group for sea cucumbers. Group U: the control group for sea urchins. Group M: the integrated multi-trophic aquaculture of sea cucumbers and sea urchins. The asterisks *, ** and *** mean $p < 0.05$, $p < 0.01$, $p < 0.001$, respectively.

No significant difference in lantern length/test diameter was found between groups U (0.033 ± 0.006) and M (0.037 ± 0.005) (t = 0.002, p = 0.999, Figure 4A)

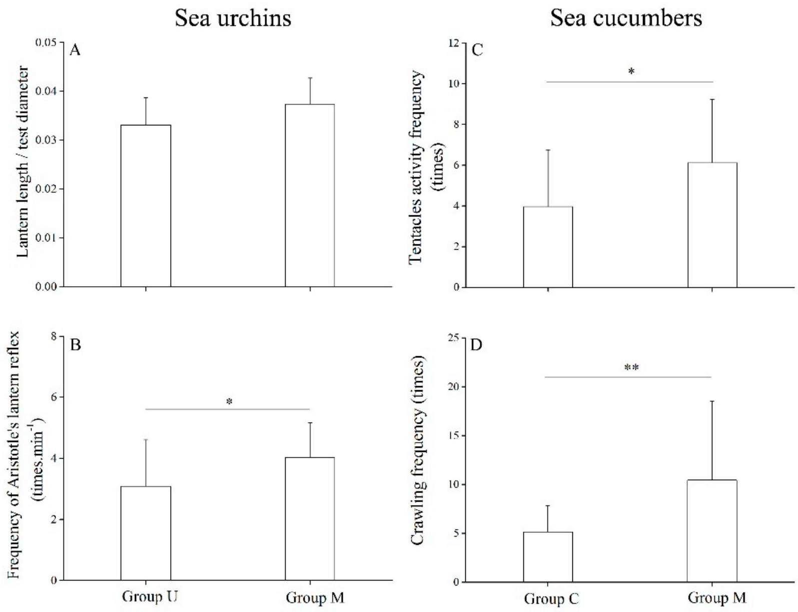

Figure 4. Lantern length/test diameter (**A**) and Aristotle's lantern reflex (**B**) of *Strongylocentrotus intermedius* between groups U and M (mean ± SD, n = 6). Tentacles activity frequency (**C**) and crawling frequency (**D**) of *Apostichopus japonicus* between groups C and M (mean ± SD, n = 6). Group C: the control group for sea cucumbers. Group U: the control group for sea urchins. Group M: the integrated multi-trophic aquaculture of sea cucumbers and sea urchins. The asterisks * and ** mean $p < 0.05$ and $p < 0.01$, respectively.

3.3. Aristotle's Lantern Reflex of S. intermedius

Significantly higher Aristotle's lantern reflex was observed in group M (4.0 ± 1.2 times·min^{-1}) than that in group U (3.1 ± 1.5 times·min^{-1}) (Mann–Whitney U = 611.5, p = 0.017, Figure 4B).

3.4. Feeding and Crawling Behaviors of A. japonicus

The tentacles activity frequency (6.1 ± 3.1 times) and crawling frequency (10.5 ± 8.1 times) of group M were both significantly higher than those of group C (4.0 ± 2.8 times, Mann–Whitney U = 176, p = 0.020 for tentacles activity frequency, Figure 4C) (5.2 ± 2.6 times, t = 3.045, p = 0.005 for crawling frequency, Figure 4D).

4. Discussion

4.1. Growth Performance of A. japonicus at a High Biomass

The sea cucumber *A. japonicus* (>1 g of body weight) are seeded into the bottom of the sea in the pond culture and stock enhancement in China [33]. This suggests that juvenile *A. japonicus* require an intermediate culture in land-based nursery tanks. However, slow growth and serious food wastage occur in the production of juvenile *A. japonicus* (<1 g of body weight), which greatly hampers the development of the aquaculture industry. Significantly better body size and body weight were found in group M (90 *S. intermedius* and 37 *A. japonicus*/10,638 cm^3) than those in group C (37 *A. japonicus*/10,638 cm^3). High stocking density is generally considered to display negative impacts on the growth of cultured animals [34,35]. Reducing density is therefore a general practice for the trade-off between animal welfare and economic benefits [36,37]. This study suggests an effective approach that greatly improves body growth while maintaining a high biomass. More research should be conducted to support the potential extension to large-scale aquaculture using this new IMTA system.

4.2. Fitness-Related Behaviors and Growth in A. japonicus

Feeding and crawling are essential fitness-related behaviors, displaying a strong relationship with the growth performance of *A. japonicus* [38]. Feeding behavior is defined as the process of sea cucumbers collecting diets using the tentacles around their mouth [39,40]. The tentacles activity commonly reflects food consumption in the sea cucumber *Cucumaria frondosa* [28]. Crawling behavior is the movement pattern that sea cucumbers crawl to a place where is suitable for their survival [30,31]. Significantly greater tentacles activity frequency and crawling frequency occurred in group M than those in group C, which probably leads to better body growth of *A. japonicus*. It has been well documented that juvenile *A. japonicus* lose their balance when they move to the silt or sand [41,42], because their ambulacral feet are adapted for attaching to a large enough surface [43]. The surface of the plastic box used in group M was smooth and covered with powdered diets, which probably provided an optimal shelter and habitat for *A. japonicus*, and thus improved their fitness-related behaviors. This indicates that the production efficiency of *A. japonicus* can be further improved in the existing nursery tanks. For example, adding artificial reefs to the bottom of nursery tanks could represent a promising candidate to promote the growth performance of *A. japonicus*.

4.3. Feeding Behavior and Body Size of S. intermedius

Sea urchin seeds (>10 mm of test diameter) are further used for longline culture and stock enhancement [12]. It takes about three months for juvenile *S. intermedius* (3–4 mm of test diameter) to become qualified seeds (10–20 mm of test diameter) by feeding fresh kelp in land-based nursery tanks [10]. The slow growth of *S. intermedius* along with the high cost of diets greatly hinders the development of sea urchin aquaculture. The present study found that group M showed a significantly larger body size compared with those in group U after 34 days, suggesting that the IMTA system displays a great potential for seed production (>10 mm of test diameter) in large quantities. A significantly higher rate of Aristotle's lantern reflex was consistently found in group M than that in group U in this study. Aristotle's lantern reflex, which refers to the process of grasping and jawing food using the teeth [44], is commonly used to represent the ability of food consumption in sea urchins [27]. For example, Hu et al. [24] found that superior Aristotle's lantern reflex led to significantly higher food consumption in sea urchins. Thus, it is probable that the improved feeding behavior of *S. intermedius* contributed to the better utilization of the powdered diets in this new IMTA system.

Furthermore, although fresh kelp can be used for *S. intermedius* culture, this approach is inefficient and not environmentally sustainable [45], because it displays a low level of energy protein and varies seasonally in nutrients [46,47]. Formulated feed (e.g., sea cucumber diet), which can be supplied stably with high nutrients [48,49], greatly improved somatic growth during the juvenile stages of *S. intermedius* in this study. Most importantly, *S. intermedius* can utilize wasted sea cucumber diets deposited in the bottom of tanks. Thus, there is no need for additional supplementation of fresh kelp in the culture period. This greatly decreases the cost in *S. intermedius* aquaculture.

4.4. Survival of S. intermedius

The present study found that mass mortality and morbidity of *S. intermedius* occurred in both groups M and U. However, group M showed significantly lower mortality and morbidity compared to those in group U. Mass mortality and morbidity of sea urchins are consistent with Lawrence et al. [10], in which 80–90% of *S. intermedius* died due to the increasing water temperature. Black-mouth disease, which is caused by opportunistic bacteria, such as the genus *Bacillus firmus* [50], is one of the most serious diseases threatening the survival of *S. intermedius* in aquaculture [24]. The optimum temperature for the growth of both the pathogenic bacteria *B. firmus* and sea urchins *S. intermedius* is ~15 °C [12,50], which consequently results in the disease outbreak during the production season of sea urchins. Our previous study documented that eliminating interactions in multi-layer cultures greatly improved the survival after disease challenge assays in cultured *S. intermedius* [22]. However, it remains unclear whether this approach is applicable in the IMTA system. Here, the present results indicate that segregation in multi-layer culture is essential for *S. intermedius* culture in the IMTA system. It greatly contributes to improving the survival of *S. intermedius* when disease outbreaks, although the potential differences were not evaluated in pathogenic bacteria between groups in the present study.

In addition, there were various seaweeds in the powdered diets of sea cucumbers [17]. Dietary supplementation with seaweed promotes the activities of immunocytes and immunologic factors [51], because seaweeds have antiviral and antimicrobial activities [52,53]. For example, dietary supplementation with seaweeds *Sargassum whitti* and *Ulva prolifera* facilitates the levels of lysozyme in fishes *Mugil cephalus* and *Scophthalmus maximus*, respectively [54,55]. Therefore, another explanation is that sea urchins being fed a sea cucumber diet is probably beneficial to their survival in the new IMTA, which further highlights the superiority of this new system.

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Data Availability Statement: The data presented in this study are available on request from the corresponding author.

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

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