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Development of a nursery upweller for young oysters in tropical mangrove estuaries

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Abstract
In this study, the aim was to prototype a small intermediate cultivation device using commercially available materials based on the passive flow-type upwelling system. Young oysters were housed in the device on a farm, and a test run was conducted. To evaluate the effect on cultivation, net cage and cage + aeration groups were established for comparison, and problems associated with the field operation of the device were analyzed. The device promoted growth, as indicated by the increased levels of phytopigments in the midgut gland tissues and new shell formation in the oysters. However, regular cleaning for antifouling measures was necessary at least once a week to use this device in regions with brackish mangrove water. Further simplification of the device structure can aid in its widespread use for fishermen.

Keywords
Oyster, Nursery upweller, *Mangrove estuary*, *Shell formation*, *Phytopigments*

Introduction

The high mortality rate of young oysters poses a significant challenge for effective oyster aquaculture (King *et al.*, 2006; EFSA, 2015; Carrasco *et al.*, 2017). The body volume of young oysters is low, and they are susceptible to environmental changes. Artificially cultured diatoms are fed to oysters in land-based tanks at seed production sites; however, extensive efforts are necessary to cultivate these oysters to an appropriate size that can be transfer to the hanging culture (Laing and Chang, 1998; Barillé *et al.*, 2003). In recent years, the floating upweller system (FLUPSY), which uses naturally occurring microalgae as food, eliminating the need for artificially cultured algae and promoting stable growth, has been developed as an intermediate rearing method for young oysters (Campbell and Hall, 2019; Campbell, 2020; Tarnecki *et al.*, 2023). This method involves the installation of the upwelling system using a pump in a tank with a float and the introduction of natural seawater containing microalgae, which serve as food for the oysters, into the oyster breeding tank. This method increases the seawater exchange rate in the tank and maintains an optimal feeding environment and water quality, thereby promoting the growth of young oysters. FLUPSY is widely implemented in the United States, Australia, Europe, and Japan (Yanmar, 2012; Chessa *et al.*, 2013; Campbell and Hall, 2019; Powell, 2022; Pumps, 2023). However, owing to its large size and association with a culture raft, this device is ex-

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pensive (over 1 million JPY/unit) (Yanmar, 2012). Despite the growing popularity of oyster farming along the west coast of the Malaysian Peninsula, only a few seed producers are capable of consistently supplying the artificial oyster seedlings to farmers. Therefore, development of efficient intermediate cultivation techniques to enhance the seedling survival rates is necessary to expand the scale of local oyster farming. However, local oyster farming is predominantly a small-scale farmer (Tan *et al.*, 2014) that lacks the financial resources to purchase commercially available intermediate growth equipment. Therefore, new intermediate growth equipment is needed for young oysters that meets the needs of local farmers. In this study, the authors constructed an intermediate growth device of useful size, which can be operated and maintained by the farmers, for installation on a raft. These devices are broadly divided into two categories: passive and forced flow devices (Leavitt, 2010). Then, they selected the passive-flow device as a reference, purchased commercially available materials, and assembled a prototype small intermediate growth device. Subsequently, they used the device to cultivate young oysters in a farm and conducted a test run to evaluate the effect on growth and identify any issues with onsite operation.

Materials and Methods

Oysters (*Crassostrea* cf. *iredalei*) with a shell length of 17 ± 3 mm (mean ± standard deviation) were used in the cultivation experiment. They were collected from Setiu Lagoon, Terengganu in June, 2023 (Yurimoto *et al.*, 2024). The following items were used in the intermediate cultivation apparatus: round shape garden bag (radius: 46 cm; height: 40 cm; capacity: 60 L), plastic square trash box (length: 35 cm; width: 26 cm; height: 45 cm), submersible pump (PC500; DoPhin, Malaysia), and plastic seedling tray (length: 34 cm; width: 25 cm; height: 5 cm; mesh size: 0.25 cm; Fig. 1). In this study, three groups were established: (1) net cage group, in which a weight was attached to the bottom of the net cage (width: 34 cm; depth: 34 cm; mesh size: 0.3 cm), (2) cage + aeration group, in which an air stone was attached to the bottom of the net cage, and aeration was provided during examination, and (3) upweller group as the experimental group (Fig. 2). This study was conducted for 10 d during the dry season between June and July, 2023 in the upper reaches of the Merbok River Estuary in Peninsular Malaysia (Fig. 3). During the experiment, various measurements were taken at regular intervals to determine the prevailing water temperature, salinity, chlorophyll-a concentration, turbidity, and dissolved oxygen concentration.

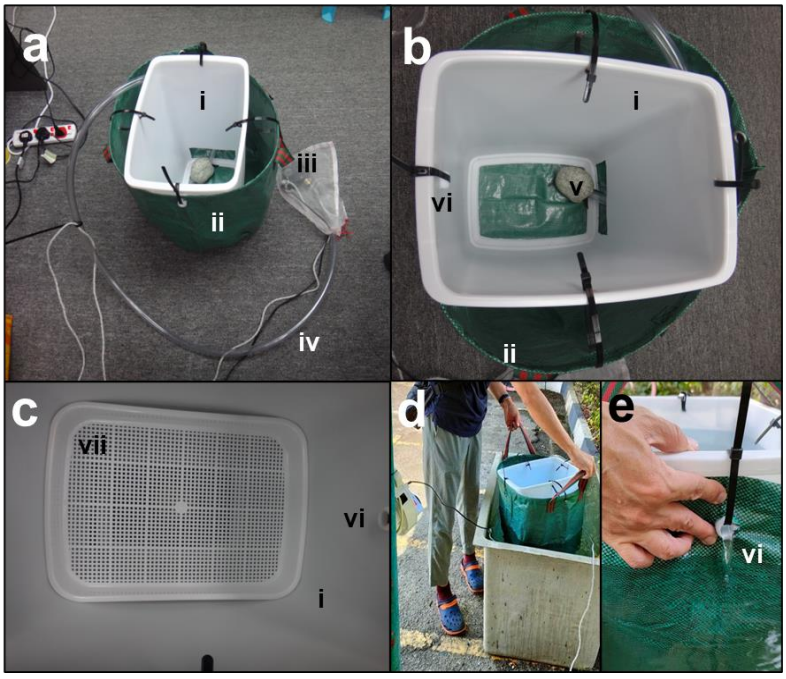

Figure 1
Assembly and test runs of upwelling device vessel using commercial products. a: Vessel part of the spat device. b: inside the vessel. c: storage tray installed in the vessel. d: test-running device in the tank. e: water supplied by the submersible pump flowing out of the device. i: square trashcan with the bottom and lower sides cut off, ii: garden bag, iii: mesh bag containing small submersible pump, iv: vinyl hose connecting tank to pump, v: weight to sink the hose to the bottom of the tank, vi: water outlet, vii: young oyster storage tray made from the mesh part of a seedling tray (holes drilled in the sides and fixed with cable ties).

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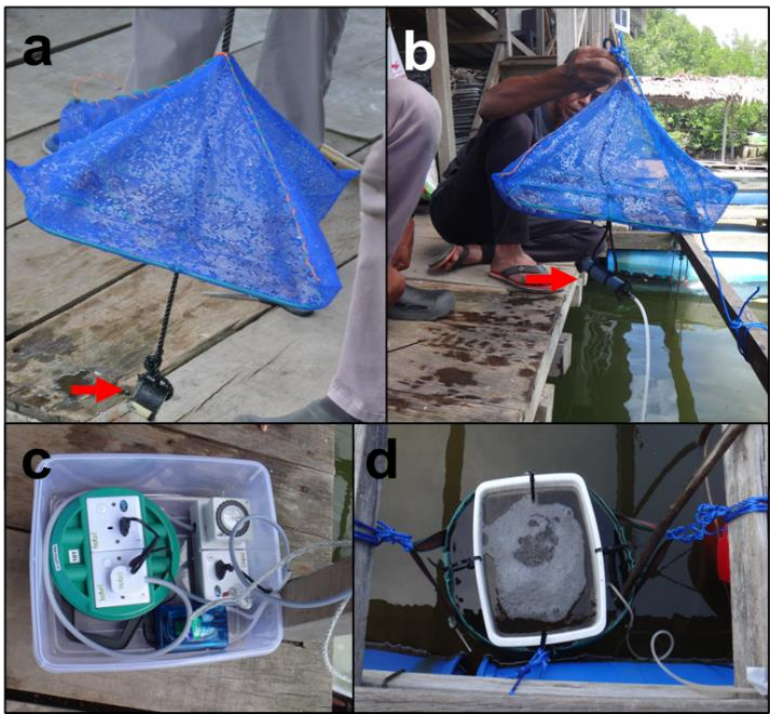

Figure 2
Net cages and upwelling device vessel were used for the experiment. a: net cage with a weight attached (net cage group), b: net cage with air stone attached (cage + aeration group), c: power supply unit of the upwelling device vessel (extension cord, aeration pump, and timer that controls the operation of the submersible pump), d: vessel part of the upwelling device. The arrows in (a) and (b) indicate the weight and the air stone, respectively.

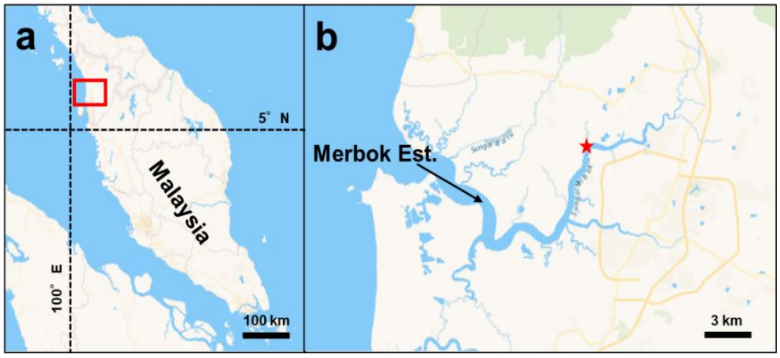

Figure 3
Location of oyster farming area (b) in the upper estuary of the Merbok River in Peninsular Malaysia (a) where the test was conducted. The red frame in (a) indicates the location of the Merbok Estuary area. B: The star in (b) indicates the location of the oyster farming area where the cultivation test was conducted. Maps were obtained from the Sentinel-hub EO Browser (https://apps sentinel hub com/ dashboard).

All measurements were performed using a water quality sensor (AAQ-RINKO; JFE-Advantech Co., Ltd., Japan). Precipitation levels during the experimental period were assessed using a weather meter (WS-2902; Ambient Weather, USA) on the culture raft. After the test period, the presence or absence of new shell at the margin was evaluated, and the shell length was measured using a Vernier caliper to clarify the shell length distribution in the tested oysters. Additionally, some oysters were opened and their midgut gland tissues were dissected and extracted in 6 mL of 90% v/v acetone for over 12 h in a cool and dark place. Pigment concentrations were measured using a spectrophotometer (Helios Zeta; Thermo Fisher Scientific Inc., USA) (Parsons and Strickland, 1963; Numaguchi, 1985; Numaguchi, 2001; Yurimoto *et al.*, 2007; Nagasoe *et al.*, 2011a; Nagasoe *et al.*, 2011b; Yurimoto *et al.*, 2021). Pigment concentration per mass weight of the midgut gland tissue was calculated from the sum of the measured values (µg/g) of the chlorophyll-a and pheophytin concentrations. Results of all groups were compared using the Tukey's multiple-comparison test, and statistical significance was set at $p < 0.05$.

Results

Dirt on the cage and upwelling device

Figure 4 illustrates the conditions of the cage and upwelling device after the experimental period. Despite the physical removal of detritus from the net cage on the intermediate day, the net soon became dirty and was coated with algae and suspended solids. The amount of dirt in the net cage group was comparable to that in the cage + aeration group. On the other hand, their upwelling device exhibited con-

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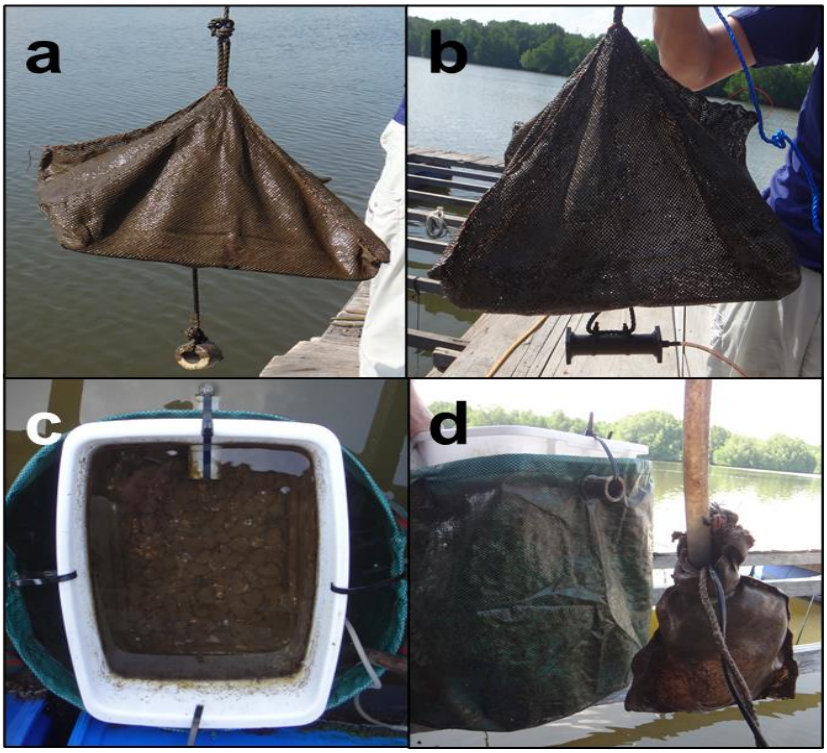

Figure 4
Condition of dirt in the mesh cages and the intermediate cultivation vessel at the end of test a: net cage group, b: cage + aeration group, c: inside view of the upweller, d: outside view of the upweller.

tamination both within and outside the tank. However, the water supply was not compromised by the clogging of the submersible pump due to regular cleaning, and the pump operated effectively until the conclusion of the test.

Fishing ground environment during the test period

Figure 5 shows a graphical representation of the data collected from the fishing ground environment du-

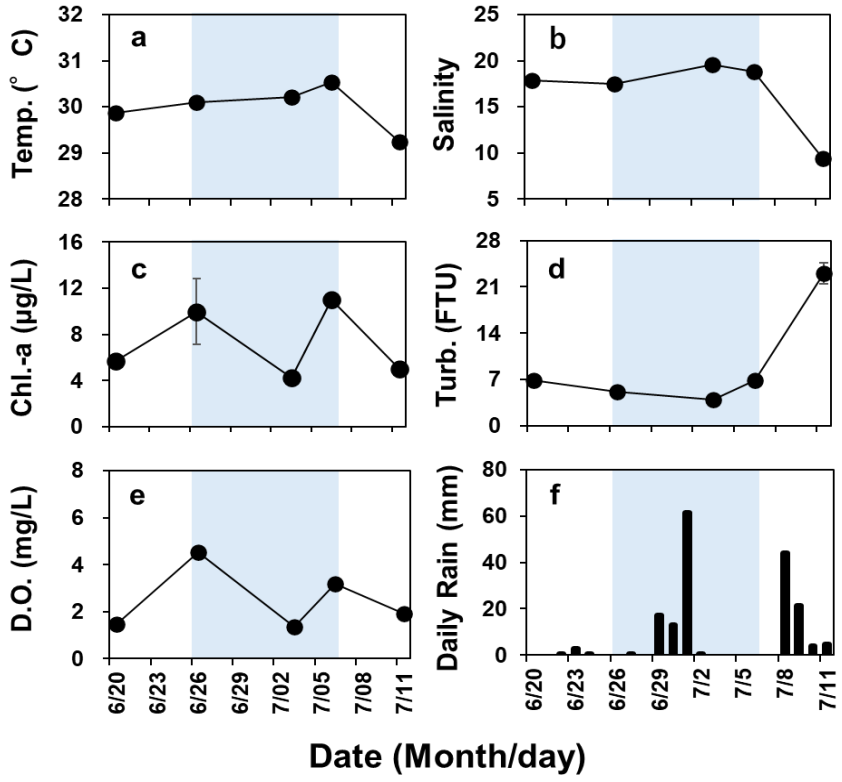

Figure 5
Water quality at the 0.5-1.2 m depth layer and rainfall during the experimental period (marked with light blue). a: water temperature (°C), b: salinity, c: chlorophyll-a concentration (µg/L), d: turbidity (FTU: formazan turbidity unit), e: dissolved oxygen concentration (mg/L), f: daily rainfall (mm). Each plot in Figures a-e shows the mean ± SD from 8-layer data of the 0.1 mm pitch.

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ring the test period. Water temperature changed to approximately 30 °C, with a salinity of approximately 18. From July 6, salinity decreased, reaching 9.4 on the 11th day (Figs. 5a and b). During the test period, chlorophyll-a concentration was 4.2–11 µg/L and turbidity was 3.9–6.9 FTU, which increased to 23.1 FTU on 11th July (Figs. 5c and d). Dissolved oxygen concentration was 1.4–4.6 mg/L, and precipitation levels were approximately 60 mm on 1st July and 40 mm on 8th July (Figs. 5e and 5f).

Oyster survival and growth

At the end of the experiment, no oyster death was observed in all groups. As shown in Figure 6, in addition to individuals showing no clear changes from the beginning of the experiment (Fig. 6a), many individuals formed new shells (Fig. 6b). The percentage of oysters that formed new shells was 14% in the net cage group, 49% in the cage + aeration group, and 64% in the upweller group (Fig. 7).

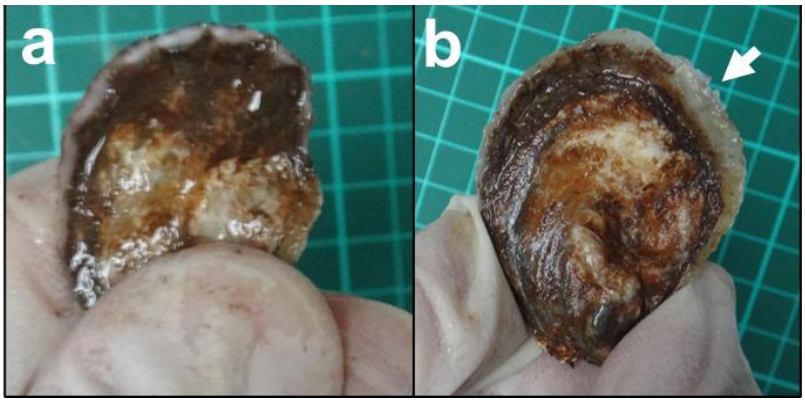

Figure 6
New shell formation in test oysters. a: individuals that are the same as at the start of the test; b: individuals in which new shell formation can be observed (arrow).

Histogram of shell length

Results of the shell length measurements were plotted as a histogram (Fig. 8). The proportion of larger individuals increased in all plots compared to that at the beginning of the experiment (Fig. 8a). However, in the net cage group, although 25–30 mm individuals were observed and the proportion of 20–25 mm individuals increased, the mode remained at 15–20 mm (Fig. 8b).

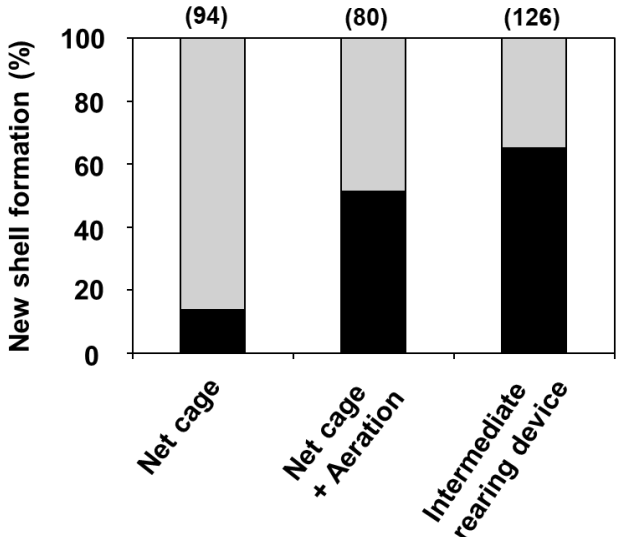

Figure 7. *Percentage of individuals in which new shell formation was observed. Numbers in parentheses indicate the number of specimens observed in each group.*

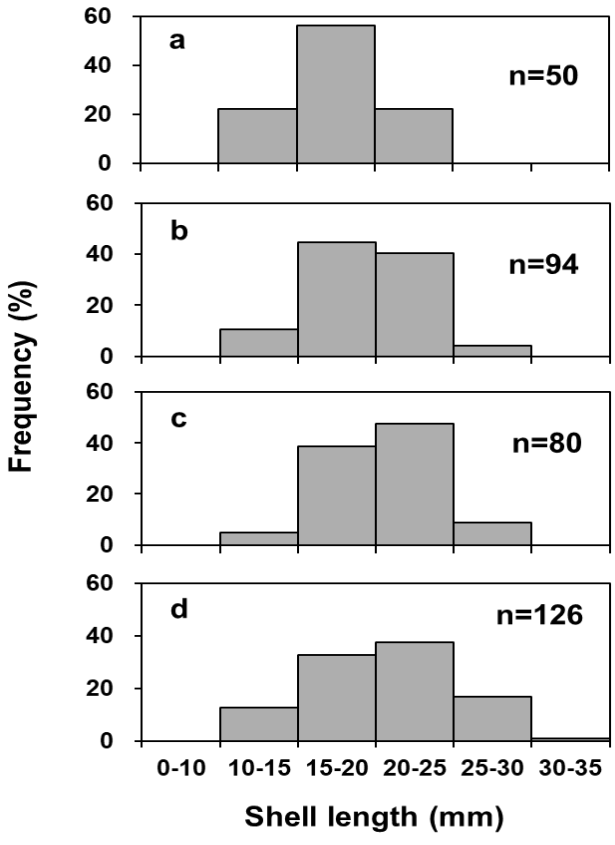

Figure 8. *Shell length composition of oysters used at the start and end of the experiment. a: initial group, b: net cage group, c: cage + aeration group, d: upweller group.*

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In contrast, in the cage + aeration and upweller groups, the change in shell length was more pronounced, with a mode of 20–25 mm (Figs. 8c and d). The proportion of 25–30 mm oysters was higher in the upweller group than in the other groups, and 30–35 mm oysters were also detected (Fig. 8d).

Estimation of feeding status

Results of the comparative analysis of phytopigments concentrations in the midgut gland tissues of the tested shellfish are shown in Fig. 9. The feeding value for each group was 122–329 µg/g, with no significant difference between the cage and cage + aeration groups. However, the upweller group exhibited a significantly higher feeding value ($p < 0.05$) than the other groups.

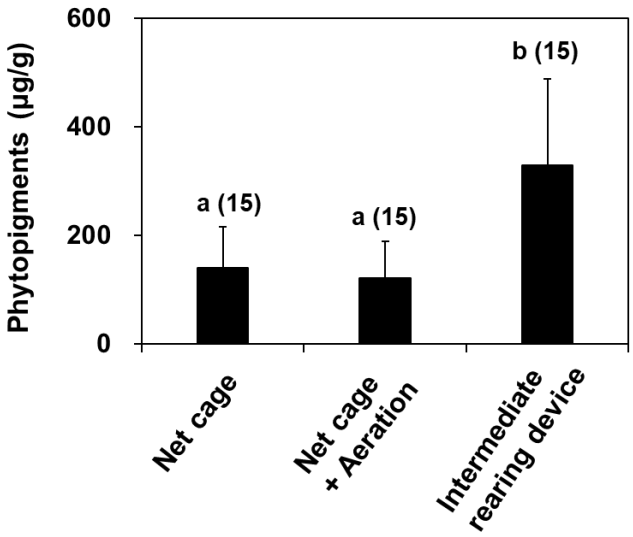

Figure 9. *Comparison of phytopigments concentrations in the midgut glands of examined oysters. The mean ± SD, the number in parentheses represents the number of samples analyzed, and the different letters indicate significant differences between each group according to Tukey's test ($p < 0.05$).*

Discussion

Environment of the test area

During the experimental period, the water temperature was maintained at approximately 30 °C, with a salinity of approximately 18 (Figs. 5a and b). Turbidity was less than 7 FTU (Fig. 5d), and rainfall from June 29 to July 2 had a negligible impact on the experiment (Fig. 5f). Levels of chlorophyll-a, an indicator of phytoplankton abundance, exceeded 4 and 8 µg/L during the study (Fig. 5c), suggesting that the phytoplankton density was sufficient to support bivalve growth. However, dissolved oxygen concentration was sometimes less than 2 mg/L (Fig. 5c). Dissolved oxygen concentration < 2 mg/L affects the feeding and filtering behaviors, even in bivalves capable of tolerating hypoxia, due to the ciliary movement of the gills (Baker and Mann, 1994; Higano, 2004; Xie at al., 2023). Here, dissolved oxygen concentration was measured at a depth of approximately 1 m, and the total water depth in the study area was approximately 4 m. Therefore, hypoxic conditions were observed despite the study area being close to the sea surface. This may be due to the presence of tropical mangrove forests in the vicinity of the aquaculture ground. Mangrove forests accumulate a significant amount of soil organic matter, which is reduced and converted to peat in some regions (Forrest *et al.*, 2009; Okamura *et al.*, 2010; Gedan *et al.*, 2017; Mattone and Sheaves, 2017; Dubuc *et al.*, 2019; Ma *et al.*, 2024). The tidal movement of water between the aquaculture grounds and mangrove forest results in the transfer of water between these two ecosystems. It is hypothesized that the reduced mud in the mangrove forest absorbs dissolved oxygen in the water, causing hypoxic water to flow into aquaculture grounds.

Effects of the upwelling device

Three groups were established: net cage, cage + aeration, and upweller groups. No aeration was performed in the net cage group; therefore, the dissolved oxygen concentration in the cage remained the same as natural, with a little increase in food intake and shell formation (Figs. 6 and 7). In contrast, the cage + aeration group showed no improvement in food intake but a significant increase in shell formation. Additionally, the upweller group exhibited increased food intake and shell formation (Figs. 7 and 9). The secretion of calcium carbonate from the mantle is crucial for shell formation in bivalves (Grégoire, 1972; Wilbur and Saleuddin, 1983; Wheeler, 2020). In response to hypoxic conditions, shellfish close their shells, thus inhibiting new shell formation (Gobler *et al.*, 2014; Song *et al.*, 2024). Therefore, it was believed that the quantity of food intake is related to both shell formation and filtration activities. Subsequently, the distribution of shell length in each group was examined by plotting a histogram, which revealed some elongation compared to the initial length, even in the net cage group. However, the mode remained within the 15–20 mm range, indicating that the number of individuals exhibiting shell elongation

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was limited (Figs. 8a and b). In contrast, histograms for the cage + aeration and upweller groups showed a mode of 20–25 mm, indicating the presence of individuals with shell lengths > 25 mm. However, this size difference was not observed in the initial. The data indicate that aeration resulted in shell elongation in both the cage + aeration and upweller groups. Therefore, these results suggest that regular cleaning of the upwelling device and effective maintenance of seawater exchange and aeration by the pumps promote feeding and shell formation in oysters.

Possible improvements to the device

In the study, a passive flow-type upwelling device was developed. The device functioned without any problems. Seawater was circulated using underwater and aeration pumps, improving the feeding environment and dissolved oxygen concentration in the tank and promoting feeding conditions and shell formations of oysters. However, considering the on-site conditions, the device structure is complex, and it is laborious to pull the device out of the raft and reinstall it after cleaning at least once a week. In addition, as the tank is a garden bucket, the bottom part is closed; therefore, simple cleaning procedures during operation may not be enough for the bottom of the bucket. Therefore, in brackish mangrove water with high flux, the developed device would require effort for maintenance, making it impractical for regular use. In comparison, the forced flow-type device is known structurally simpler and easier to use by fishermen, with better maintenance in brackish mangrove water (Leavitt, 2010). These aspects must be improved to facilitate the widespread use of the device. In the future, they aim to establish a prototype forced flow-type device and verify its effect on oyster growth.

Conclusions

They constructed and installed a prototype passive flow-type intermediate growth device on a raft. A trial run was conducted on a farm with young oysters, and the effect of the device on oyster growth was examined. Additionally, issues related to on-site operations were identified. Although the developed device promoted growth, regular cleaning and protection from dirt were necessary for its use in tropical mangrove areas. Therefore, further simplification of the device structure is required to facilitate its use by fishermen with minimal effort. Future studies should also assess the effects of a simple forced flow-type device and determine the efficient and practical device for oyster farming in mangrove estuaries.

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