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Effects of Temperature, Dissolved Oxygen Concentration, and Photosynthetic Photon Flux Density on the Growth of the Sea Bivalve *Tridacna crocea* in Combination with the Symbiotic Alga Zooxanthella

Yoshiaki Kitaya [1,*], Yasunori Iba [2], Toshio Shibuya [3] and Atsunori Masuda [4]

[1] Organization for Research Promotion, Osaka Metropolitan University, Gakuencho 1-1, Naka-ku, Sakai 599-8531, Osaka, Japan
[2] Forestry and Fisheries Department, Okinawa Prefecture Agriculture, Senzaki 1-2-2, Naha 900-8570, Okinawa, Japan; ibayasun@pref.okinawa.lg.jp
[3] Graduate School of Agriculture, Osaka Metropolitan University, Gakuencho 1-1, Naka-ku, Sakai 599-8531, Osaka, Japan; tshibuya@omu.ac.jp
[4] College of Agriculture, Tamagawa University, Tamagawa Gakuen 6-1-1, Machida 194-0041, Tokyo, Japan; amasuda@agr.tamagawa.ac.jp
* Correspondence: kitaya@omu.ac.jp

Abstract: The sea bivalve clam *Tridacna crocea* inhabiting the shallow sea of tropical and subtropical zones lives with the symbiotic alga zooxanthella in its mantle. Zooxanthellae algae perform photosynthesis and supply nutrients to *T. crocea*. Recently, the abundance of *T. crocea* has decreased rapidly due to overfishing in coastal areas in Okinawa, Japan. *T. crocea* culture systems for mass production will contribute to the conservation of *T. crocea* and thus marine ecosystems. Environmental control methods for *T. crocea* culture have not been established because of a lack of knowledge about the appropriate environmental conditions for *T. crocea* growth. The present study was initiated to obtain basic data for developing environmental control methods for *T. crocea* land-based aquaculture. The effects of water temperature, dissolved oxygen concentration, and photosynthetic photon flux density (PPFD) on the O_2 exchange rates of the symbiotic system of *T. crocea* and zooxanthella, which are indicators of photosynthesis and respiration in the system, and the effect of daily integrated PPFD on *T. crocea* growth were investigated. Basic knowledge was obtained for the development of optimal environmental control technology for *T. crocea* clam culture. The optimum water temperature and dissolved oxygen concentration for photosynthesis in this symbiotic system were 28 °C, 5–6 mgO_2 L^{-1} and 500 μmol m^{-2} d^{-1}, respectively. The optimum daily integrated PPFD for clam growth was 20 mol m^{-2} d^{-1}.

Keywords: aquaculture; coral reef; giant clam; light intensity; oxygen exchange rate; photosynthesis; symbiosis

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1. Introduction

The sea bivalve giant clam (*Tridacna* sp.) plays important roles in the ecosystems of the tropical shallow sea. They live in coral reefs, which are very important ecosystems where various organisms coexist [1].

Tridacna sp. is a hermaphrodite that spawns in the summer [2]. The fertilized eggs pass through the planktonic larval stage and then attach to rocks or corals. After settlement, they grow by drilling holes in rocks and corals [2], as shown in Figure 1a, and have a symbiotic alga called zooxanthellae, which is a general term for dinoflagellates that live symbiotically with animals in their mantle [3,4]. To provide waste and carbon dioxide to zooxanthellae, *Tridacna* sp. can use the photosynthetic products of zooxanthellae as a nutrient source [3,5–8].

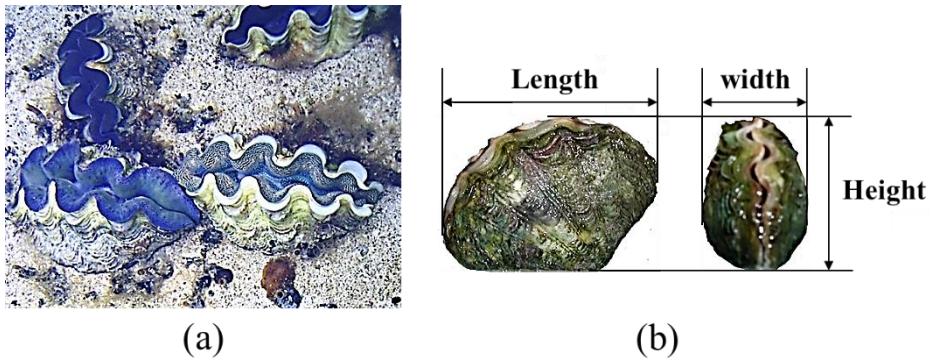

Figure 1. *Tridacna crocea* clams inhabiting the shallow sea (**a**) and measured shell size parameters (**b**).

Tridacna sp. plays important ecological roles in marine ecosystems [4]. The holes drilled by them affect the structure of the rocks and corals and expand the habitats of other organisms. Symbiotic algae that coexist with clams play important roles as primary producers of coral reefs. Many of them are small shellfish and food for other organisms. In particular, juvenile shellfish are a major food source for small fish, snails, crabs, and shrimp living in coral reefs. The birds that live around coral reefs also eat *Tridacna* sp. when they are exposed, especially at low tide. As mentioned above, *Tridacna* sp. is food for various organisms and plays a part in the food chain of coral reef ecosystems. The presence of organisms that feed on *Tridacna* sp. contributes to maintaining coral reef biodiversity and further to stabilizing the ecosystem of the entire coral reef. *Tridacna* sp. is therefore known as an indicator organism in coral reefs that are sensitive to environmental changes.

Tridacna sp. is also important human food and is subject to fisheries [9–12], and the overfishing and destruction of its habitat has a negative impact on the entire coral reef ecosystem. Owing to their attractive appearance, *Tridacna* sp. has attracted attention as a tourist resource [13,14], and their protection also contributes to the local economy.

As mentioned above, they play important roles in preserving coral reef ecosystems, and the protection of these organisms is necessary to maintain the ecosystems of coral reefs. *Tridacna* sp. clams have been fished out to extinction in many Indian Ocean and Pacific waters [15]. In Japan, *Tridacna* sp. is currently overfished for food and other purposes, leading to its depletion in Okinawa Prefecture [7,9,15]. The local government prohibits the harvest of *Tridacna* sp. with a shell length of less than 8 cm, but owing to the high demand for clams with a shell length of approximately 6–7 cm, many clams of regulated sizes are currently in circulation in markets. Therefore, supplying *Tridacna* sp. of this size through aquaculture is likely important for conserving *Tridacna* sp. and subsequently coral reef ecosystems.

In recent years, *Tridacna* sp. clam culture has been practiced in the Pacific Islands [16]. It covers the conservation and restocking of clams but with the potentially contributes to the rehabilitation of coral reefs degraded by bleaching induced by climate change, as well as food production and the development of remunerative local industries for local communities [16].

Tridacna sp. relies on the photosynthetic products of zooxanthellae for the energy it needs to live. For example, Ishikura et al. [5] reported that 46–80% of the fixed carbon in the photosynthate was translocated from zooxanthellae to host tissues in experiments in which carbon isotopes were used. The effects of environmental factors on the photosynthesis of zooxanthellae are partly known. Among these environmental factors, light intensity [17], water temperature [18], and dissolved oxygen concentration [19], which are relatively easy to control in land-based aquaculture, are reported to have strong effects on the photosynthesis of zooxanthellae.

The purpose of the present study was to develop land-based aquaculture techniques with the final goal of preserving the aquatic ecosystem around Okinawa Prefecture, Japan, by preventing the fishery harvest of *Tridacna* sp. clams from natural reefs. To obtain funda-

mental knowledge for establishing environmental control techniques for the land-based aquaculture of *Tridacna* sp., the effects of water temperature, dissolved O_2 concentration, and photosynthetic photon flux density (PPFD) on the O_2 exchange rate of the symbiotic system of *T. crocea* and zooxanthella, which is an indicator of photosynthesis and respiration in the system, and the effects of daily integrated PPFD on *T. crocea* growth were investigated. The experiments were conducted through the following procedures:

(1) Relationships between shell size and fresh biomass, dry biomass, and mantle area of *T. crocea* clams.

The fresh biomass excluding the shell (hereafter referred to as fresh mass), the dry biomass excluding the shell (hereafter referred to as dry mass), which are indicators of growth rates, and the mantle tissue area, which is related to photosynthetic activity, can be estimated from the product of the shell length, shell width, and shell height of each clam. The relationships among them were subsequently assessed.

(2) Effect of the dissolved oxygen concentration on the oxygen exchange rates of *T. crocea clams.*

The oxygen exchange rate of clams is considered to be an indicator of the net photosynthetic rate of the system consisting of zooxanthellae and *T. crocea*, which is carbon source gain activity in clams. The dissolved oxygen concentration affects the photosynthesis of zooxanthellae [19]. Terefore, the effect of the dissolved oxygen concentration on the oxygen exchange rate was investigated.

(3) Effects of light intensity and water temperature on the oxygen exchange rates of *T. crocea clams.*

The photosynthetic rate is affected by light intensity and temperature. Zooxanthellae are sensitive to water temperature [18]. Therefore, the combined effects of light intensity and water temperature on the oxygen exchange rate of *T. crocea* clams were investigated.

(4) Effect of daily integrated PPFD on the relative growth rates of *T. crocea* clams.

When cultivating *T. crocea* clams, understanding the effect of daily light intensity on their actual growth is important. Therefore, the effect of light intensity integrated daily on the growth of *T. crocea* clams was investigated.

2. Methods

T. crocea clams, estimated to be 30 months old, were obtained from the Yaeyama Branch of the Okinawa Prefectural Fisheries Research Center located at 124.2° east longitude and 24.5° north latitude. They were cultured in artificial seawater (Red Sea Salt, Red Sea Fish Pharm Ltd., Herzilia Pituach, Israel). As a symbiotic system with zooxanthellae had already been established, the clams were not artificially fed. For environmental conditions other than the factors tested in the following experiments, the water flow velocity was approximately 30 mm s^{-1}, the pH was 8.0, and the salinity ranged from 3.0 to 3.5%.

2.1. Relationships Between Shell Size and the Fresh Biomass, Dry Biomass, and Mantle Area of T. crocea Clams

The length, width, and height of the *T. crocea* clam shell were defined as shown in Figure 1b. The values for each clam shell were measured using an electronic caliper with a measurement accuracy of 0.1 mm. The fresh mass was measured after the image of the fully expanded mantle (hereafter referred to as the mantle tissue area) was captured, and then the dry mass was measured after the sample was kept in an oven at 80 °C for three days. The fresh and dry masses were measured using an electronic balance with a measurement accuracy of 0.01 g. The fully expanded mantle area was measured with the image analysis method using the software "ImageJ" after each image was captured. Then, equations expressing their relationships were obtained.

2.2. Effect of the Dissolved Oxygen Concentration on the Oxygen Exchange Rates of T. crocea Clams

Three clams (50.6 ± 7.2 (mean ± SD) mm in shell length, 26.5 ± 4.1 mm in shell width, and 34.6 ± 3.5 mm in shell height) were individually tested. For each individual, the same measurements were repeated three times. The system used to measure the oxygen exchange rates is shown in Figure 2. A transparent plastic container with a dissolved oxygen sensor was placed on a magnet stirrer. The individual clams were placed in containers. The container was fully filled with seawater that had been used to keep the sample clam and sealed. Fluorescent lamps were placed on top of the system as the lighting source.

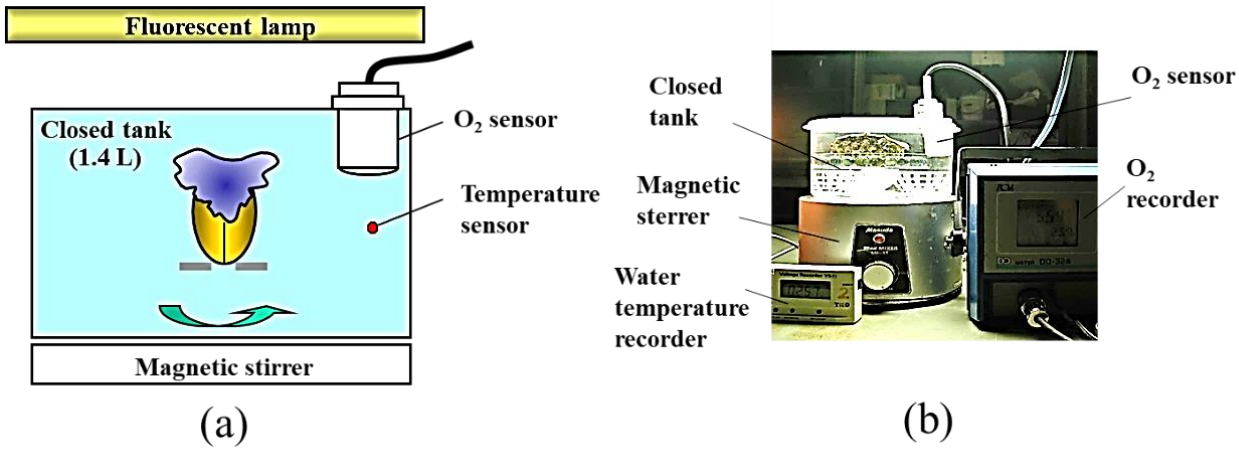

Figure 2. Outline (**a**) and photo (**b**) of the measurement system for O_2 exchange rates of *Tridacna crocea* clams under different dissolved oxygen concentrations, light intensities, and water temperatures.

The oxygen exchange rate was determined from the change in dissolved oxygen concentration over time in the sealed container and expressed as a value per dry mass excluding the clam shell. A dissolved oxygen meter (DO-32A, Toa DKK Co., Ltd., Tokyo, Japan) was used to measure the dissolved oxygen concentration.

Since the dissolved oxygen meter itself and the microalgae and bacteria present in seawater absorb oxygen, the oxygen exchange rate of the experimental system without clams, which was the background value, was first measured. The change in the dissolved oxygen concentration caused by factors other than the *T. crocea* clam was measured over time in the system without clams. The transparent plastic container without the *T. crocea* clam inside was sealed, and the changes in the dissolved oxygen concentration over time were measured as background values.

The *T. crocea* clam was subsequently placed in a transparent plastic container containing seawater with an initial dissolved oxygen concentration of approximately 4.5 mg L^{-1} and sealed. The oxygen exchange rate of the experimental system was calculated from the increase or decrease in dissolved oxygen concentration per unit time using the following equation:

The oxygen exchange rate of the clam = the oxygen exchange rate of the system − (the microorganism oxygen exchange rate + the oxygen meter exchange rate)

The mean water flow velocity was set at approximately 30 mm s^{-1}, and the water temperature was 28 ± 0.3 °C. The seawater used was stabilized at 400 µmol mol^{-1} atmospheric CO_2 in advance.

2.3. Effects of Light Intensity and Water Temperature on the Oxygen Exchange Rates of T. crocea Clams

Effects of light intensity and water temperature on oxygen exchange in *T. crocea* clams. The oxygen exchange rate was determined under 12 conditions, combining four levels of

photosynthetic photon flux density (PPFD) (0, 115, 215, and 415 μmol m^{-2} s^{-1}) and three levels of water temperature (26, 28, and 30 °C).

Four clams (52.3 ± 6.8 mm in shell length, 27.8 ± 3.9 mm in shell width, and 33.7 ± 3.4 mm in shell height) were individually tested. For each individual, the same measurements were repeated three times. The light intensity was adjusted to four levels in the PPFD: 0, 115, 215, and 415 μmol m^{-2} s^{-1}, and the water temperature was adjusted to three levels: 26, 28, and 30 °C. Using the experimental apparatus shown in Figure 2, the oxygen exchange rate of clams was measured under 12 conditions with a combination of four light intensities and three water temperatures. The gross photosynthetic rate was calculated by subtracting the OER at a PPFD of 0 μmol m^{-2} s^{-1} under each temperature condition. The expanded mantle area under each light intensity (hereafter referred to as the apparent mantle area) was also measured with the same image analysis method as mentioned in Section 2.3.

2.4. Effect of Daily Integrated PPFD on the Relative Growth Rates of T. crocea Clams

Three *T. crocea* clams were cultured for 28 days in each of the four areas with different light intensities using supplemental lighting and shade curtains above the culture tank (1000 L of seawater volume) at a depth of 10 cm in a natural light greenhouse. Two halogen lamps (250 W each) were installed 20 cm above the water surface as supplemental lighting sources, and the light intensity was adjusted according to the distance from the lamp. The experimental system is shown in Figure 3.

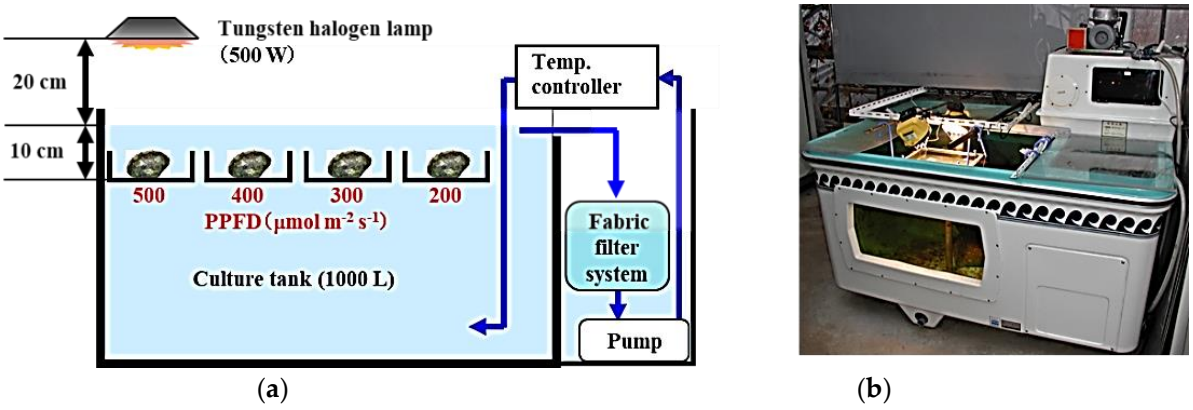

Figure 3. Outline (**a**) and photo (**b**) of the experimental culture system for evaluating relative growth rates of *Tridacna crocea* clams under different light intensities.

The light intensity was adjusted depending on the distance from the lamp. The PPFDs under supplemental light were 500, 400, 300, and 200 μmol m^{-2} s^{-1} without natural light. The water temperature was 28 °C, the salinity was 3.0–3.5%, and the duration of supplemental light was 12 h. The shell length, width, and height were measured every week to estimate the dry mass.

The relative growth rate (RGR) was calculated from the change in dry mass over time using the following equation:

$$RGR = (\ln W_2 - \ln W_1)/(t_2 - t_1)$$

where W_1 and W_2 are the dry masses of each clam at times t_1 and t_2, respectively. In this experiment, t_1 and t_2 were 0 and 28 days after starting the experimental culture, respectively.

2.5. Statistical Analysis

The Tukey-Kramer method was used after an analysis of variance for the significance test to evaluate the effect of PPFD on the apparent mantle areas of the clams as a dependent sample test using four individual samples and the effect of daily integrated PPFD on the

relative growth rates of clams as an independent sample test using four individual samples. The effects of PPFD and temperature and their interaction on the oxygen exchange rate and the gross photosynthetic rate of the clam were analyzed using a two-way analysis of variance as a dependent sample test using four individual samples. The presence or absence of significant differences was indicated at the $p < 0.05$ level.

3. Results

3.1. Relationships Between T. crocea Shell Length, Shell Width, and Shell Height and the Fresh Biomass, Dry Biomass, and Mantle Tissue Area of T. crocea Clams

The fresh mass (mg/clam) (TW) and dry mass (mg/clam) (TD) excluding shells and the mantle tissue area (mm^2/clam) (MS) were strongly correlated with the product (SP) of shell length (cm) × shell width (cm) × shell height (cm) (Figures 4 and 5). The equations expressing the relationships among these variables are shown below.

$$TW = 0.1161\ SP - 0.3300\ (R^2 = 0.998)$$
$$TD = 0.0132\ SP + 0.0036\ (R^2 = 0.925)$$
$$MS = 0.581\ SP + 0.312\ (R^2 = 0.965)$$

The TWs, TDs, and MSs of living clams were confirmed to be affected by the SP.

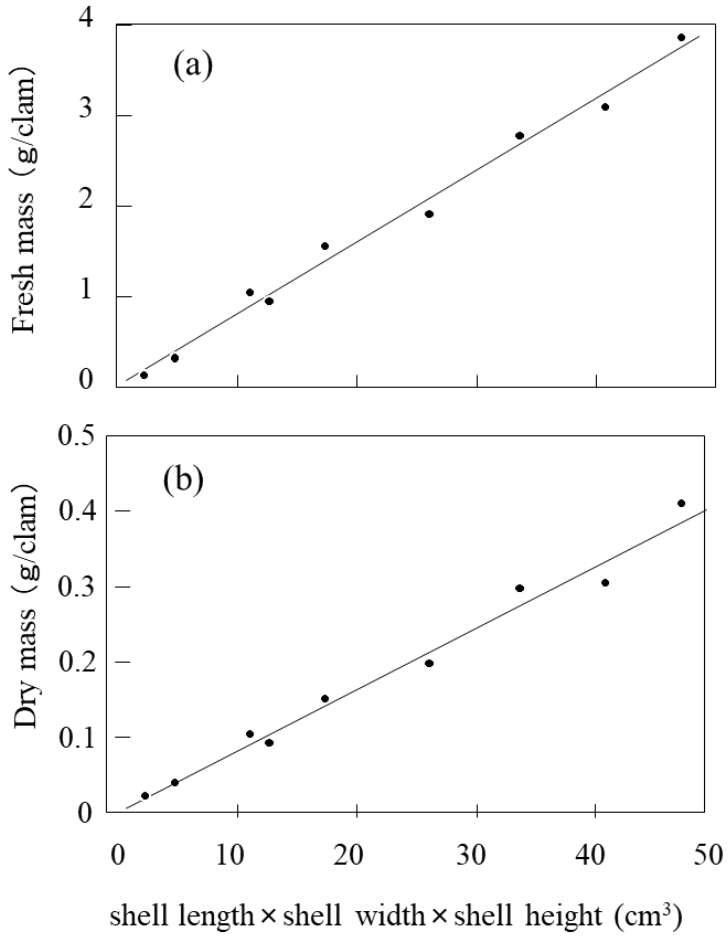

Figure 4. Relationships between shell length × shell width × shell height and the fresh (**a**) and dry (**b**) masses of *Tridacna crocea* cla.

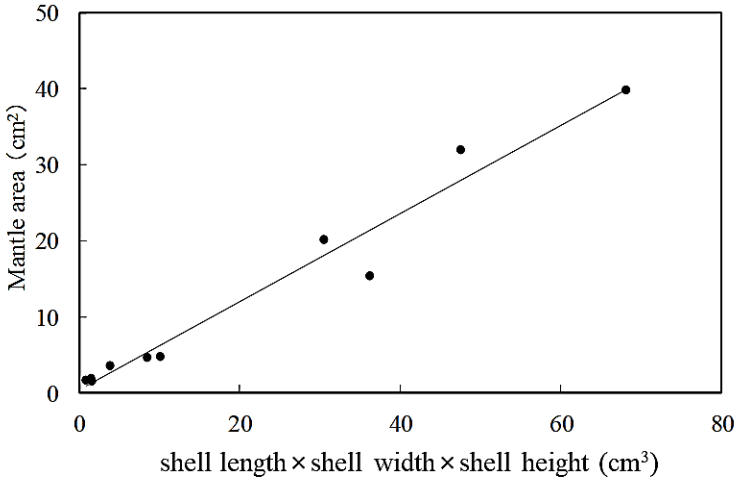

Figure 5. Relationships between shell length × shell width × shell height and the anatomically measured mantle tissue area of *Tridacna crocea* clams.

3.2. Effect of the Dissolved Oxygen Concentration on the Oxygen Exchange Rates of T. crocea Clams

The oxygen was consumed in the measurement system, excluding the clams, and the oxygen exchange rate was −0.185 $\mu gO_2\ s^{-1}$ as the background value. The oxygen exchange rate of the clams was calculated from the oxygen consumption rate of the whole system, including the clams, by subtracting the background value.

The oxygen concentration in the measurement system, including the clam samples, increased over time. The oxygen exchange rate of the clams at each dissolved oxygen concentration was calculated from the time-dependent change in the dissolved oxygen concentration in each range from 4.5 to 5.0, 5.0 to 5.5, 5.5 to 6.0, 6.0 to 6.5, 6.5 to 7.0, 7.0 to 7.7, and 7.5 to 8.0 mg L^{-1}.

The effect of the dissolved oxygen concentration on the oxygen exchange rate of the *T. crocea* clam as expressed per dry mass weight of the clam is shown in Figure 6. At dissolved oxygen concentrations ranging from 4.5 mg L^{-1} to 8 mg L^{-1}, the effect of the dissolved oxygen concentration on the oxygen exchange rate of the *T. crocea* clam was small. Although no significant difference was detected, the oxygen exchange rate tended to increase with increasing DO concentration from 4.5 $mgO_2\ L^{-1}$ to 6 $mgO_2\ L^{-1}$ and tended to decrease with increasing DO concentration above 6 $mgO_2\ L^{-1}$.

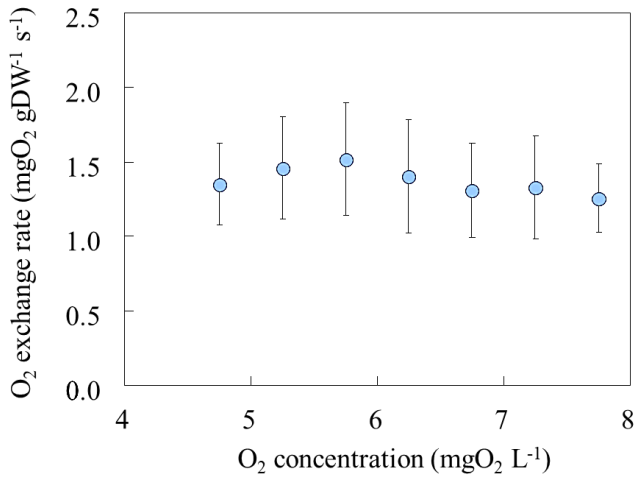

Figure 6. Effect of the dissolved oxygen concentration on the oxygen exchange rate in *Tridacna crocea* clams. The PPFD was 415 µmol $m^{-2}\ s^{-1}$, the water temperature was 28 °C, the water flow velocity was 30 mm s^{-1}, the pH was 8.0, and the salinity ranged from 3.0 to 3.5%. The vertical bars indicate standard errors (n = 3).

3.3. Effects of Light Intensity and Water Temperature on the Oxygen Exchange Rates of T. crocea Clams

A representative change in the dissolved oxygen concentration over time under different PPFD levels is shown in Figure 7. The dissolved oxygen concentration was reduced to approximately 4 mgO_2 L^{-1} for 30 min by the respiration of the clam in the dark. After the PPFD was set to 400 µmol m^{-2} s^{-1}, the dissolved oxygen concentration increased to approximately 9 mgO_2 L^{-1} by oxygen exchange in the clam.

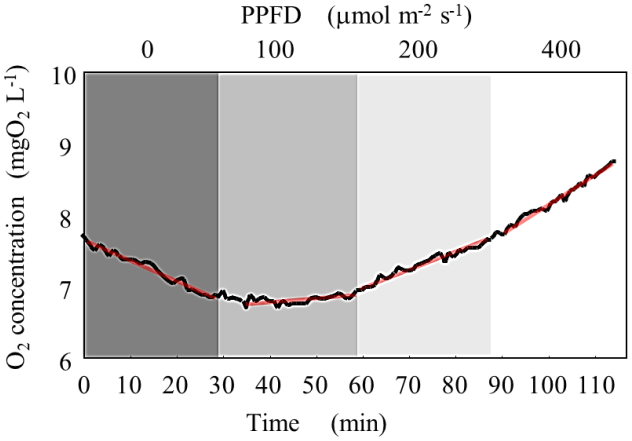

Figure 7. Changes in the dissolved oxygen concentration in a seawater container culturing a *Tridacna crocea* clam under different PPFDs. The red lines indicate the least squares approximation lines in the range where the trend is stable. The water temperature was 28 °C, the water flow velocity was 30 mm s^{-1}, the pH was 8.0, and the salinity ranged from 3.0 to 3.5%.

The oxygen exchange rate of the *T. crocea* clam increased with increasing PPFD (Figure 8), which is the net photosynthetic rate based on dry mass of the zooxanthellae and *T. crocea* clam system. At a PPFD of approximately 50 µmol m^{-2} s^{-1}, the oxygen exchange rate reached 0 at 28–30°. In this case, the net photosynthetic rate of the zooxanthellae and the respiration rate of the *T. crocea* clam became equal. At a PPFD of 100–400 µmol m^{-2} s^{-1}, the oxygen exchange rate of the *T. crocea* clams reached a maximum at 28 °C, which was approximately 1.8 and 1.4 times greater than those at 26 °C and 30 °C, respectively (Figure 8). Two-way analysis of variance did not detect any significant (p = 0.05) interaction effect between the PPFD and temperature.

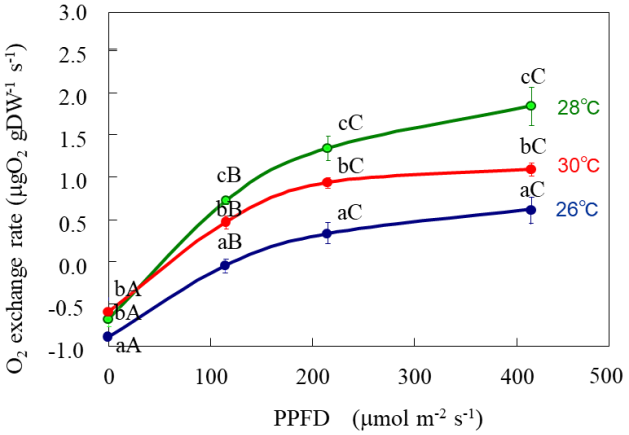

Figure 8. Effects of PPFD and water temperature on oxygen exchange rates based on the dry mass of *Tridacna crocea* clams. The water flow velocity was 30 mm s^{-1}, the dissolved oxygen concentration ranged from 6.5 to 7.5 mg L^{-1}, the pH was 8.0, and the salinity ranged from 3.0 to 3.5%. The vertical bars indicate standard errors (n = 4). Different lowercase and uppercase letters indicate significant differences (p = 0.05) among the temperatures at each PPFD and among the PPFDs at each temperature, respectively.

The gross photosynthetic rates per anatomically measured mantle tissue area of the zooxanthellae at 28 °C were approximately 1.7 and 1.4 times greater than those at 26 °C and 30 °C, respectively (Figure 9). The light saturation point at which the gross photosynthetic rate per mantle area reached a plateau was a PPFD of 400 μmol m^{-2} s^{-1} or higher (Figure 9). The apparent mantle areas of the *T. crocea* clam decreased with increasing PPFD (Figure 10). The value at a PPFD of 400 μmol m^{-2} s^{-1} was 0.75 times lower than that at 150 μmol m^{-2} s^{-1}.

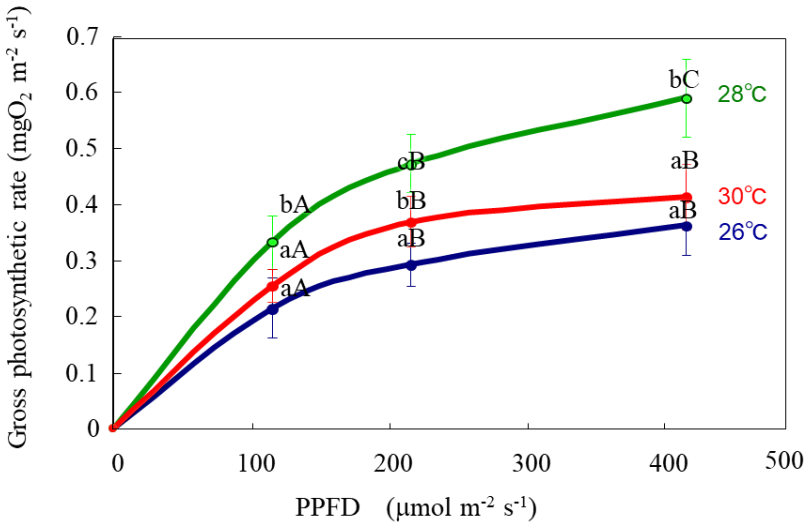

Figure 9. Effects of PPFD and water temperature on gross photosynthetic rates based on the anatomically measured mantle tissue area of *Tridacna crocea* clams. The water flow velocity was 30 mm s^{-1}, the dissolved oxygen concentration ranged from 6.5 to 7.5 mg L^{-1}, the pH was 8.0, and the salinity ranged from 3.0 to 3.5%. The vertical bars indicate standard errors (n = 4). Different lowercase and uppercase letters indicate significant differences (p = 0.05) among the temperatures at each PPFD and among the PPFDs at each temperature, respectively.

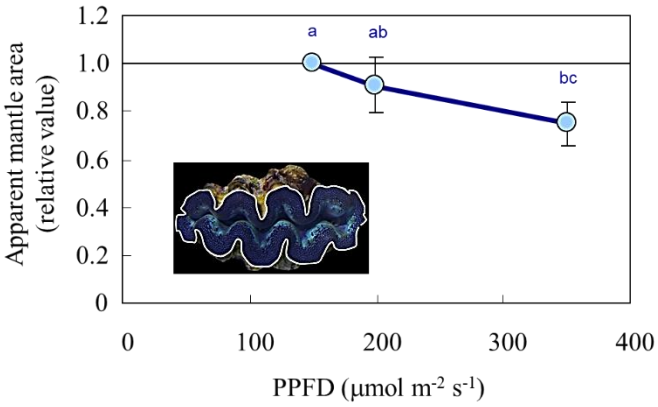

Figure 10. Effect of PPFD on the apparent mantle areas of *Tridacna crocea* clams. The apparent mantle area was measured using an image analysis. The water temperature was 28 °C, the water flow velocity was 30 mm s^{-1}, the dissolved oxygen concentration ranged from 6.5 to 7.5 mg L^{-1}, the pH was 8.0, and the salinity ranged from 3.0 to 3.5%. The vertical bars indicate standard errors (n = 4). Different letters indicate significant differences (p = 0.05).

Gross photosynthetic rates were converted from values based on the anatomically measured mantle tissue area to values based on the apparent mantle area (Figure 11). The oxygen exchange rates in Figure 11, which reflect the actual photosynthetic capacity of the mantle based on directly illuminated area, increased compared with the values at 28 °C in Figure 9, especially at higher PPFD levels. The light saturation point based on

the apparent mantle area was relatively further beyond the PPFD of 400 μmol m^{-2} s^{-1} (Figure 11) compared with that per anatomically measured mantle tissue area.

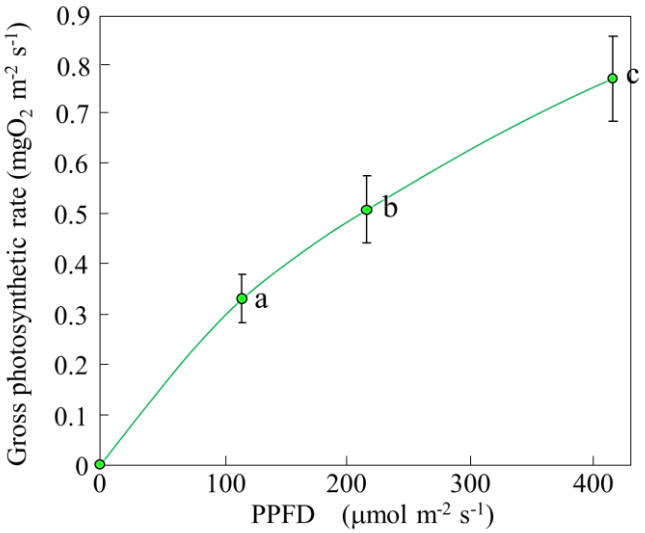

Figure 11. Effects of PPFD and water temperature on gross photosynthetic rates based on the apparent mantle area of *Tridacna crocea* clams. The water temperature was 28 °C, the water flow velocity was 30 mm s^{-1}, the dissolved oxygen concentration ranged from 6.5 to 7.5 mg L^{-1}, the pH was 8.0, and the salinity ranged from 3.0 to 3.5%. The vertical bars indicate standard errors (n = 4). Different letters indicate significant differences (p = 0.05).

3.4. Effect of Daily Integrated PPFD on the Growth of T. crocea Clams

The dry mass of the *T. crocea* clam increased slightly logarithmically with time (Figure 12). The effect of the daily integrated PPFD on the relative growth rates is shown in Figure 13. As the daily integrated PPFD increased from 9 mol m^{-2} d^{-1} to 18 mol m^{-2} d^{-1}, the relative growth rate increased almost linearly, but the increase in the relative growth rate tended to plateau above 18 mol m^{-2} d^{-1}.

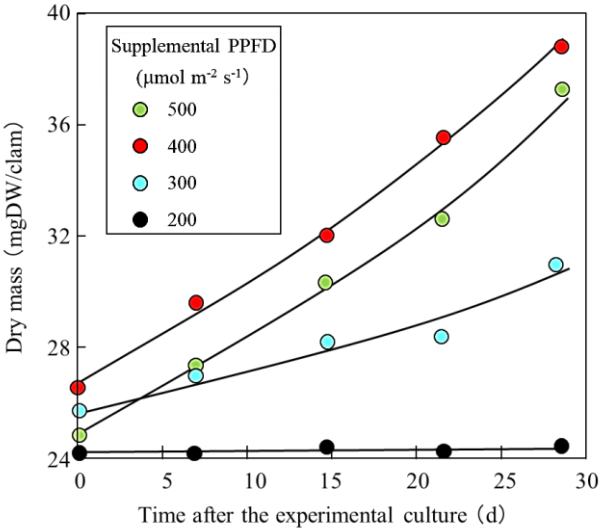

Figure 12. Time courses of dry masses of *Tridacna crocea* clams at different supplemental PPFDs. Representative sample values at each PPFD are shown. The water temperature was 28 °C, the water flow velocity was 30 mm s^{-1}, the dissolved oxygen concentration ranged from 6.5 to 7.5 mg L^{-1}, and the salinity ranged from 3.0 to 3.5%.

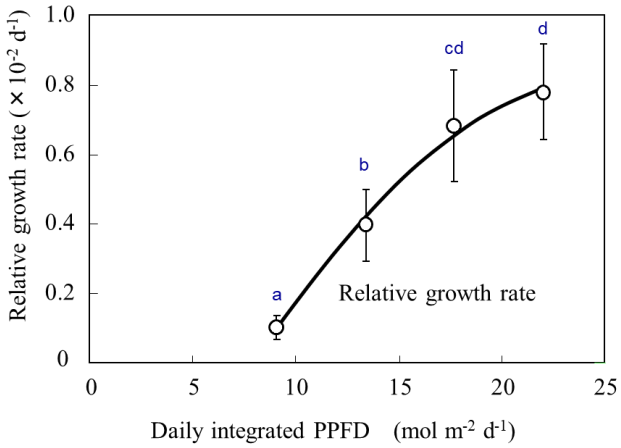

Figure 13. Effects of daily integrated PPFD on the relative growth rates of *Tridacna crocea* clams. The water temperature was 28 °C, the water flow velocity was 30 mm s^{-1}, the dissolved oxygen concentration ranged from 6.5 to 7.5 mg L^{-1}, and the salinity ranged from 3.0 to 3.5%. The vertical bars indicate standard errors (n = 3). Different letters indicate significant differences (p = 0.05).

4. Discussion

In this experiment, strong relationships existed between the fresh and dry masses and the product of shell length × shell width × shell height of the *T. crocea* clam (Figure 4). A similar relationship can also be observed in the estimation of biomass information from the sample size for other clam species. By investigating these relationships in advance, it may be possible to observe, for example, changes in the fresh and dry masses over time for the same individual using measurements of shell size parameters of living clams.

The oxygen release of the *T. crocea* clam is due to the photosynthesis of zooxanthellae, and the growth of the *T. crocea* clam is considered to be promoted by promoting the photosynthesis of zooxanthellae. The oxygen release rate, which is the positive oxygen exchange rate, is an index of photosynthetic activity, so a relatively high oxygen exchange rate indicates relatively high photosynthetic activity of symbiotic zooxanthellae, which in turn promotes the growth of clams. Since the oxygen exchange rate tended to be greatest at approximately 6 mgO_2 L^{-1} dissolved oxygen at a relatively high PPFD of 415 µmol m^{-2} s^{-1} (Figure 6), the clam growth rate seemed to reach a maximum at that dissolved oxygen concentration and to gradually decrease with increasing dissolved oxygen concentration above 6 mgO_2 L^{-1} at high PPFDs. This finding implies that the growth of *Tridacna* sp. clams might be suppressed at excessively high dissolved oxygen concentrations. Photorespiration in zooxanthellae isolated from *T. maxima* clams increased at elevated oxygen levels [19]. Low dissolved oxygen concentrations are often observed in coral reef waters [20]. The photosynthesis of *Tridacna* sp. that evolved in such an environment may have been adapted to relatively low oxygen concentrations. The occurrence of high oxygen concentrations observed in the coral–algal interface and water between coral branches [20] could negatively affect the photosynthesis and thus the survival of *Tridacna* sp. This finding also suggests the importance of controlling the oxygen concentration in the aquaculture of *Tridacna* sp.

The growth rate of the clams seemed to reach a maximum at a water temperature of approximately 28 °C because the net photosynthetic rate of the zooxanthellae was highest at 28 °C (Figure 8). The growth rate of the clams, however, seemed to decrease at 30 °C based on their photosynthetic behavior (Figures 8 and 9), because of the strong relationship between the photosynthetic capacity of the zooxanthellae and the nutrient supply dependence of *T. crocea* clams. Notably, high-temperature conditions can cause oxidative stress through repressed antioxidant ability, apoptosis activated by the unfolded protein response, and further the collapse of the symbiotic system, which threatens the growth and reproduction of *T. crocea* [13]. The decrease in the oxygen exchange rates (net photosynthetic rates) of the symbiotic system of *T. crocea* and zooxanthella at 30 °C in this study seems to support the phenomenon mentioned above. Compared with normal temperature,

exposure to elevated water temperature by 3 °C caused a significant decrease in the net photosynthetic rate, which was mainly due to the significantly increased respiration rate of *T. squamosa* [21]. Similar properties were reported for *T. gigas* and *T. derasa* [22]. Elevated temperatures reportedly cause nearly total mortality, and the highest temperature at which *T. squamosa* survived was 32.8 °C [23]. Many other animal species that live in symbiosis with zooxanthellae in coral reefs are thought to be sensitive to elevated seawater temperatures, similar to *Tridacna sp*. Therefore, frequent ocean warming events drive the loss of coral reef cover [24].

At a water temperature of 30 °C, the light saturation point decreased to approximately the PPFD of 300 μmol m^{-2} s^{-1}, and the maximum gross photosynthetic rate also decreased (Figure 9). It seems that the growth of clams living symbiotically with zooxanthellae is suppressed when excess high light intensity occurs at high temperatures, especially in shallow water at low tide.

The apparent mantle area decreased with increasing PPFD (Figure 10). This behavior may lead to an apparent reduction in size in bright sunlight without decreasing the photosynthetic rate (Figure 11), making them less visible to predators when viewed from above.

The RGR increased with increasing daily integrated PPFD, and a daily integrated PPFD of approximately 20 mol m^{-2} d^{-1} allowed the clam to grow to its maximum value (Figure 13). A light intensity of 15 klx was previously reported to be suitable for the growth of *T. crocea* clams with 45–90 mm shell lengths between 5 and 15 klx under a 12 h light/12 h dark light cycle with halogen lamps [1]. The light intensity of 15 klx corresponds to the daily integrated PPFD of 9.33 mol m^{-2} d^{-1}. The light intensity is mostly the minimum value in this study, and the relative growth rates linearly increased with increasing daily integrated PPFD from 9 to 18 m^{-2} d^{-1} in this study (Figure 13).

Environmental factors other than those in this study, such as salinity [25], the diurnal light cycle [26], light spectral characteristics [21,22], pH [15,27], the partial pressure of carbon dioxide [28,29], and water flow velocities [30], can affect clam growth. To improve environmental control technology for *T. crocea* clam culture, it is necessary to assess the combined effects of the environmental factors mentioned above in addition to those examined in this study.

5. Conclusions

Basic knowledge was obtained for the development of optimal environmental control technology for *T. crocea* clam culture. The optimum water temperature and dissolved oxygen concentration for photosynthesis in the *T. crocea* and zooxanthellae symbiotic system were 28 °C, 5–6 mgO_2 L^{-1} and 500 μmol m^{-2} d^{-1}, respectively. The optimum daily integrated PPFD for clam growth was 20 mol m^{-2} d^{-1}.

The number of clams of *Tridacna* sp. in the natural ecosystem can be increased by releasing juvenile clams that have been cultured until they reach an optimal size, which increases their chances of survival in the life cycle of *Tridacna* sp. Therefore, aquaculture not only prevents population declines due to fishery capture from the natural ecosystem but also contributes to a positive increase in population through the release of cultured juvenile clams.

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References

1. Liu, C.; Li, X.; Wu, C.; Wang, A.; Gu, Z. Effects of three light intensities on the survival, growth performance and biochemical composition of two size giant clams *Tridacna crocea* in the Southern China Sea. *Aquaculture* **2020**, *528*, 735548. [CrossRef]
2. Jameson, S.C. Early life history of the giant clams *Tridacna crocea* Lamarck, *Tridacna maxima* (Roding), and *Hippopus hippopus* (Linnaeus). *Pac Sci.* **1976**, *30*, 219–233.
3. Hirose, E.; Iwai, K.; Maruyama, T. Establishment of the photosymbiosis in the early ontogeny of three giant clams. *Mar. Biol.* **2006**, *148*, 551–558. [CrossRef]
4. Mies, M. Evolution, diversity, distribution and the endangered future of the giant clam–Symbiodiniaceae association. *Coral Reefs* **2019**, *38*, 1067–1084. [CrossRef]
5. Ishikura, M.; Adachi, K.; Maruyama, T. Zooxanthellae release glucose in the tissue of a giant clam, *Tridacna crocea*. *Mar. Biol.* **1999**, *133*, 665–673. [CrossRef]
6. Hean, R.L.; Cacho, O.J. A growth model for giant clams *Tridacna crocea* and *T. derasa*. *Ecol. Model.* **2003**, *163*, 87–100. [CrossRef]
7. Long, C.; Zhang, Y.; Li, Y.; Li, J.; Zhou, Z.; Qin, Y.; Li, X.; Ma, H.; Wei, J.; Zhou, Y.; et al. Effects of symbiodiniaceae phylotypes in clades A–E on progeny performance of two Giant clams (*Tridacna squamosa* and *T. crocea*) during early history life stages in the South China Sea. *Front. Mar. Sci.* **2021**, *8*, 633761. [CrossRef]
8. Fitt, W.K.; Fisher, C.R.; Trench, R.K. Contribution of the symbiotic dinoflagellate Symbiodinium microadriaticum to the nutrition, growth and survival of larval and juvenile tridacnid clams. *Aquaculture* **1986**, *55*, 5–22. [CrossRef]
9. Sayco, S.L.G.; Kurihara, H. Bleaching and recovery in the giant clam *Tridacna crocea* from the subtropical waters of Okinawa. *Coral Reefs* **2024**, *43*, 773–786. [CrossRef]
10. Sayco, S.L.G.; Pomares, A.A.; Cabaitan, P.C.; Kurihara, H. Reproductive consequences of thermal stress-induced bleaching in the giant clam *Tridacna crocea*. *Mar. Environ. Res.* **2024**, *193*, 106280. [CrossRef]
11. Dawson, R.F.; Philipson, P.W. The market for giant clams in Japan, Taiwan, Hong Kong and Singapore. In *The Marketing of Marine Products from the South Pacific*; Philipson, P.W., Ed.; Suva (Fiji) Institute of Pacific Studies: Suva, Fiji, 1989; pp. 90–123.
12. Bell, J.D. Restocking of giant clams: Progress, problems and potential. In *Stock Enhancement and Sea Ranching*; Howell, B.R., Moskness, E., Svasand, T., Eds.; Blackwell Science: Oxford, UK, 1999; pp. 437–452.
13. Zhou, Z.; Liu, Z.; Wang, L.; Luo, J.; Li, H. Oxidative stress, apoptosis activation and symbiosis disruption in giant clam *Tridacna crocea* under high temperature. *Fish Shellfish. Immunol.* **2019**, *84*, 451–457. [CrossRef] [PubMed]
14. Zhou, Z.; Li, J.; Ma, H.; Li, Y.; Qin, Y.; Wei, J.; Zhou, Y.; Zhang, Y.; Yu, Z. The evaluation of culture performance and mantle coloration of two boring giant clam (*Tridacna crocea*) strains. *Aquac. Rep.* **2021**, *20*, 100646. [CrossRef]
15. Syazili, A.; Niartiningsih, A.; Jompa, J. Effect of ocean acidification and temperature on growth, survival, and shell performance of fluted giant clams (*Tridacna squamosa*). In *IOP Conference Series: Earth and Environmental Science*; IOP Publishing: Bristol, UK, 2020; Volume 473, No. 1, p. 012141.
16. Moore, D. Farming giant clams in 2021: A great future for the 'Blue Economy'of tropical islands. In *Aquaculture: Ocean Blue Carbon Meets UN-SDGS*; Springer International Publishing: Berlin/Heidelberg, Germany, 2022; pp. 131–153.
17. Neo, M.L.; Lim, K.K.; Yang, S.; Soong, G.Y.; Masucci, G.D.; Biondi, P.; Wee, H.B.; Kise, H.; Reimer, J.D. Status of giant clam resources around Okinawa-jima Island, Ryukyu Archipelago, Japan. *Aquat. Conserv. Mar. Freshw. Ecosyst.* **2019**, *29*, 1002–1011. [CrossRef]
18. Armstrong, E.J.; Watson, S.A.; Stillman, J.H.; Calosi, P. Elevated temperature and carbon dioxide levels alter growth rates and shell composition in the fluted giant clam. *Tridacna squamosa. Sci. Rep.* **2022**, *12*, 11034. [CrossRef]
19. Downton, W.J.S.; Bishop, D.G.; Larkum, A.D.; Osmond, C.B. Oxygen inhibition of photosynthetic oxygen evolution in marine plants. *Funct. Plant Biol.* **1976**, *3*, 73–79. [CrossRef]
20. Nelson, H.R.; Altieri, A.H. Oxygen: The universal currency on coral reefs. *Coral Reefs* **2019**, *38*, 177–198. [CrossRef]
21. Elfwing, T.; Plantman, P.; Tedengren, M.; Wijnbladh, E. Responses to temperature, heavy metal and sediment stress by the giant clam *Tridacna squamosa*. *Mar. Freshw. Behav. Physiol.* **2001**, *34*, 239–248. [CrossRef]
22. Blidberg, E.; Elfwing, T.; Plantman, P.; Tedengren, M. Water temperature influences on physiological behaviour in three species of giant clams (*Tridacnidae*). In Proceedings of the Ninth International Coral Reef Symposium, Bali, Indonesia, 23–27 October 2000; Volume 1, pp. 561–565.
23. Eckman, W.; Vicentuan, K.; Todd, P.A. Effects of low light and high temperature on pediveligers of the fluted giant clam *Tridacna squamosa*. *Mar. Freshw. Behav. Physiol.* **2019**, *52*, 255–264. [CrossRef]
24. Alderdice, R.; Voolstra, C.R.; Lendo, C.I.N.; Boote, C.; Suggett, D.J.; Edmondson, J.; Goyen, S.; Haydon, T.; Camp, E.F. Loss of coral thermotolerance following year-long in situ nursery propagation with a consecutively high summer heat-load. *Coral Reefs* **2024**, *43*, 919–933. [CrossRef]
25. Militz, T.A.; Southgate, P.C. Salinity influences hatchery production of the giant clam *Tridacna crocea*. *Aquac. Res.* **2021**, *52*, 5105–5108. [CrossRef]
26. Yamaguchi, M. Conservation and cultivation of giant clams in the tropical Pacific. *Biol. Conserv.* **1977**, *11*, 13–20. [CrossRef]
27. Watson, S.A. Giant clams and rising CO_2: Light may ameliorate effects of ocean acidification on a solar-powered animal. *PLoS ONE* **2015**, *10*, e0128405. [CrossRef] [PubMed]

28. Brahmi, C.; Chapron, L.; Le Moullac, G.; Soyez, C.; Beliaeff, B.; Lazareth, C.E.; Vidal-Dupiol, J. Effects of elevated temperature and pCO_2 on the respiration, biomineralization and photophysiology of the giant clam Tridacna maxima. *Conserv. Physiol.* **2021**, *9*, coab041. [CrossRef] [PubMed]
29. Kurihara, H.; Shikota, T. Impact of increased seawater pCO_2 on the host and symbiotic algae of juvenile giant clam *Tridacna crocea*. *J. Coral Reef Stud.* **2018**, *20*, 19–28. [CrossRef]
30. Grizzle, R.E.; Langan, R.; Howell, W.H. Growth responses of suspension-feeding bivalve mollusks to changes in water flow: Differences between siphonate and nonsiphonate taxa. *J. Exp. Mar. Biol. Ecol.* **1992**, *162*, 213–228. [CrossRef]