Effect of ocean acidification and temperature on growth, survival, and shell performance of fluted giant clams
(Tridacna squamosa)

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Abstract. This study aims to determine the effect of ocean acidification and temperature on growth, survival, and shell performance of fluted giant clam (Tridacna squamosa). Juvenile fluted giant clam put into an aquarium which is given a combination of CO₂ pressure treatment (415, 1000 and 1800 ppm) and temperature (30, 32, and 34°C). Measuring the length, width and height of the shell perform in every two weeks for five times. CaCO₃ content and shell strength was test at the end of the study. The best growth of shell length, shell width, and shell height in the treatment of CO₂ concentrations of 415 ppm and temperatures of 30°C were 23.28 mm, 11.51 mm, and 0.69 mm respectively. Survival live also obtained in the treatment of CO₂ concentrations of 415 ppm and temperatures of 30°C were 100%. The strength of the shell and CaCO₃ content decreased in the treatment of CO₂ concentration and high temperatures. Higher concentration of CO₂ and increased temperature negatively affected the growth of length, width, survival of scales, reduced strength and the CaCO₃ content of shell.

1. Introduction
Ocean acidification and rising temperatures threat the sustainability of marine ecosystems such as coral reefs, seagrass beds, and mangrove ecosystems. Giving a negative impact on living organisms in all of these ecosystems including corals [1], molluscs, crustaceans, phytoplankton, zooplankton, fishes, crustaceans [2], affect the biological and physiological processes of organisms as shell formation and calcification [3], reproduction, development, metabolism, immune system, and apoptosis [4].

Mollusks form exoskeleton shell structures with certain benefits. The main function of the shell is as a defense against predators, providing protection from the influence of the external environment such as temperature, salinity, and pH. Although the formation of mollusk shells is basically biologically and genetically controlled [5], however it is also strongly influenced by environmental factors such as temperature, salinity and pH which can change mineral strength and composition. At present, many researchers have proven the negative effects of ocean acidification and temperature increases on mollusks such by inhibiting the growth of shells and decreasing rates of gastropod calcification, bivalves
[6,7], growth and survival rate, and metabolism rate [8]. Specifically for shellfish, there is very little research on the effects of rising temperatures and ocean acidification [9] which proved the effects of ocean acidification and increased temperatures can trigger shellfish mortality rates, affect shellfish growth and trigger zooxanthellae death.

Giant clams are iconic organisms that live in coral reef ecosystems, found in western Indo-Pacific waters. Clams have high economic value for Indo-Pacific coastal communities because they can be used as a source of highly nutritious food [10], popular aquarium organisms and their shells can be used as decoration, bowls and floor tiles. Highly exploitation such as uncontrolled capture and habitat destruction, and high market demand affects the deterioration in nature population. At present, all species of clams have been protected by the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) and are listed as endangered species at IUCN. In addition, both human captivity and environmental factors for example ocean acidification and temperature levels are mostly the common pressures of clam’s abundance. Clams are soft-bodied invertebrates, which live permanently somewhere, very vulnerable to environmental changes. This type of marine animal is considered a good model for climate change research because it is an ectothermic animal that has a limited ability to regulate body temperature. This study aims to find out how the interaction of the effects of increasing CO$_2$ and temperature on growth, survival rate, calcification and shell strength.

2. Methods
This research was conducted at the marine station hatchery of Hasanuddin University in Barrang Lompo Island, Makassar City, South Sulawesi, Indonesia in April - July 2018. Juvenil scale clams (T. squamosa) used were derived from spawning during September 2015, with an average length of 22.43 ± 0.31 mm. Juvenils are placed in an 40x30x20 cm aquarium filled with 40 liters of water and maintained for 2 weeks before treatment. This aquarium is equipped with a water circulation and aeration system that runs continuously.

Factorial pattern of ocean acidification and temperature increasing was used in a completely randomized design [9]. The treatments tested were carbon dioxide concentration factor in three different levels i.e. 415 ppm, 1000 ppm and 1800 ppm. The temperature factor consisted of 30°C, 32°C and 34°C with three replications. Measurement of water quality such as temperature, pH and salinity was measured everyday. Water samples were collected at the beginning of the study to measure the total acidity level which was then used to measure bicarbonate ions (HCO$_3^-$), carbonate ions (CO$_3^{2-}$), Ω aragonite dan Ω calcite using CO$_2$ Calc software for 1.2.0 version. Measurement of length, height and width shell is conducted every two weeks for 10 weeks by using a 0.01mm caliper. Survival rate was observed every day. After measurement, the clam shell was cleaned from attached parasites and cleaned the aquarium. At the end of study, clams were collected to test CaCO$_3$ content and shell strength using a free compressive strength test in accordance with SNI 03-1973-1990 [11].

2.1. Statistical analysis
Length and thickness of shell, calcium content and data were processed using SPSS 17 software. The variation of each treatment and analysis of variance (ANOVA) results are displayed in tabular and graphical form.

3. Results
Environmental and chemical parameters of carbonate results are shown on pH on CO$_2$ concentration of 415 ppm ranged from 8.06-8.00, while on 1000 ppm pH ranged from 7.73-7.69 and at 1800 ppm the pH value is 7.47-7.46. The pH and temperature values measured during study indicate.

Length shell growth of scale clams provide different results as shown on Fig 1. The highest growth is found on CO$_2$ concentration 415 ppm around 23.28±0.045 mm on 30°C. The growth decrease when giving injection on CO$_2$ 1000 µatm which around 22.43±0.02 mm on 34°C, and the lowest range is 22.38±0.03 mm on CO$_2$ concentration 1800 µatm on 34°C. Based on ANOVA, it can be seen that CO$_2$ and temperature and their interaction had significant effect (P <0.05) on the growth of scale lengths.
Tukey's further test results show a significant difference between each treatment temperature and CO$_2$ concentration. Growth rate of shell width has different ranges as present on Fig 1B. The highest width growth was obtained at CO$_2$ concentrations of 415 µatm around 11.51 mm on 30°C. Furthermore, the total growth decreases at 1000 µatm CO$_2$ injection which is 11.44 mm on 34°C and the lowest value is 11.43 mm at 1,000 CO$_2$ concentrations on 34°C.

ANOVA results show significantly different (P<0.05) on the scale width. The results of the tukey follow-up test showed that the treatment temperature of 30°C was significantly different from the treatment of CO$_2$ concentration 415 µm which was significantly different from the treatment of 1000 µm and 1800 µm. The same results are shown on both 32°C and 34°C as well as the treatment of CO$_2$ concentrations of 100 µm and 1800 µm. The content of CaCO$_3$ shells after research can be seen in Figure 3B. The highest CaCO$_3$ content obtained at a treatment temperature of 30°C and a concentration of 415 ppm CO$_2$ was 89.05 ± 0.22%, followed by a treatment temperature of 30°C and a concentration of 1000 ppm CO$_2$ from 88.07 ± 0.91%. The lowest CaCO$_3$ content was obtained at a treatment temperature of 34°C and a CO$_2$ concentration of 1800 ppm was 80.15 ± 0.49%. The results of the analysis of variance (Table 1) showed that there was a real effect of the treatment temperature and CO$_2$ concentration (P <0.05) but there was no interaction between the two treatments (P> 0.05). The results of the tukey follow-up test (Table 2) showed that there was a significant difference between each temperature treatment and the 415 ppm CO$_2$ concentration treatment was not different from the 1000 ppm CO$_2$ concentration treatment, but it was different from the 1800 ppm CO$_2$ concentration treatment. The treatment of 1800 ppm CO$_2$ concentration was not different from the treatment of CO$_2$ concentration of 1000 ppm, but it was different from the treatment of CO$_2$ concentration of 415 ppm.

![Figure 1](image-url)

**Figure 1.** (A) The scale length of clamshell (B) The scale width of clamshell and after given a combination of temperature treatment (°C) and CO$_2$ concentration for 10 weeks.
4. Discussion
This study shows that increasing CO₂ and sea temperatures tend to reduce growth in length, width, height of shells and survival of scales (*Tridacna squamosa*). At the end of the study, the length, width and height of the shells in the control treatment experienced the fastest growth compared to other treatments. Shellfish growth is strongly influenced by CO₂ concentration and ambient temperature. The survival of shellfish after 10 weeks in experimental conditions, shows all individuals in the treatment of CO₂ control and live temperature, shows that the cause of the decrease in survival occurs due to an increase in CO₂ or temperature, or a combination of both. Very low survival (<15%) occurs in the highest
CO₂ treatment at 34°C. This suggests that juvenile T. squamosa may be highly vulnerable to the higher CO₂ levels predicted at the end of this century, and may be more vulnerable to an increase in relevant CO₂ than an increase in temperature.

Shell clams are currently threatened by various local pressures such as overfishing, habitat destruction and environmental influences and are listed at IUCN as Threatened Species. This study shows that giant clams are also threatened by ocean acidification and rising temperatures. Shellfish growth is greatly influenced by environmental factors such as temperature, salinity, pH, zooxanthella and nutrients in seawater. The results of this study prove that the increase in CO₂ concentration in the ocean which triggers a decrease in sea water pH and an increase in temperature has been shown to inhibit the growth of the length, width and height of shellfish and reduce the survival rate by 87.3%. Talmage and Gobler (2010) [12] found that shellfish maintained at higher CO₂ concentrations has slow growth, shell structure damage, and low survival. A decrease of 0.5 units of pH can cause low survival in marine mollusks, as has been studied with Mercenaria mercenaria, Argopecten iradians and Crassostrea gigas [13], while decreasing pH 0.7 units can cause abnormal development and high mortality rates in C. gigas and Mytilus galloprovincialis.

Slow growth and low survival rate at high CO₂ concentrations and high temperatures are thought to be influenced by physiological conditions of clams, but it is not yet known how these physiological mechanisms affect the growth and survival of shellfish or other clams. Research conducted by [14] stated that T. squamosa had slow growth when maintained at CO₂ concentrations of 700 to 1400 μm CO₂. The metabolic rate, photosynthesis, and interactions with zooxanthellae, shell growth, calcification, other physiological influences, and ecological processes can influence the growth and survival of clams.

Clams are mollusks that have large size and are very dominant to inhabit the seabed, using shells to avoid predators, reducing environmental impacts such as waves, currents, hydrodynamic forces and the movement of large objects that can damage the shell. The strength of the shells is greatly influenced by the CaCO₃ content. In this study it was proven that increasing the temperature and acidification of the sea can damage or reduce the strength of the shell. The high CaCO₃ content in the shell is related to the strength of the shell in holding the applied pressure. Clamshell consists of three main layers, namely the prismatic layer and the nacreous layer. The prismatic layer consists of calcite and aragonite while the nacreous layer consists of aragonite [15]. Mollusk shell formation is controlled biologically and genetically [5], but it is also influenced by environmental factors such as temperature, salinity, and pH which can change the strength and composition of their minerals [16]. The data can be seen on the table 1.

**Table 1.** Summary of the results of further tests (Tukey) interactions between the effect of increasing CO₂ concentration and temperature on Fluted Giant Clams (T. squamosa).

| Variabel                  | Treatment | Temperature | CO₂ concentration |
|---------------------------|-----------|-------------|-------------------|
|                           |           | 30°C        | 32°C              | 34°C              | 415   | 1000  | 1800  |
| Shell length (mm)         |           |             |                   |                   |       |       |       |
|                           |           | 22.3978a    | 22.7178b          | 22.9671c          | 22.4504a | 22.6894b | 22.9439c |
| Shell width (mm)          |           | 11.43a      | 11.431a           | 11.4819b          | 11.4402a | 11.4472b | 11.4676b |
| Shell thickness(mm)       |           | 0.6794a     | 0.6864a           | 0.6873b           | 0.68a  | 0.6837b | 0.6894b |
| Survival rate (%)         |           | 33.3333a    | 57.7778b          | 75.5556c          | 35.5556a | 51.1111b | 80.00c  |
| CaCO₃ (%)                 |           | 80.6956a    | 85.7756b          | 88.3589c          | 83.94ab | 85.0911bc | 85.7989c |
| Compressive strength (Kg/Cm²) |         | 13.5556a    | 18.2222b          | 24.000c           | 17.1111a | 18.2222a | 20.4444b |
Increased shell calcification can be caused by mechanical and ecological problems, when the matrix and mineral ratios change, it will affect the strength of the shell. In general, increasing the change in matrix ratio will make the shell heavier but more brittle, the matrix is very important to be used to reduce the shell fracture or make the shell stronger against pressure. Clams that have fragile shells will be more easily devoured by predators, one of the functions of the shell is to protect the shell from predator attack [17]. report that predators can destroy pearl oyster shells, *Pinctada margaritifera* which have an optimal size range for prey, and this size range generally increases with increasing predator size. The higher CO$_2$ concentration and the increase in temperature negatively affect the growth of length, width, survival, weakening of the shell and lower content of CaCO$_3$ shells. Changes in mechanical function, shell mineral composition and physiological responses due to increased temperatures and ocean acidification are closely related to behavior and ecology that require further research.

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