Seaweed Plantlet Growth *Kappaphycus alvarezii* with different lengths of maintenance time

Author contributions

This work was carried out in collaboration between all authors. All authors read and approved the manuscript end.

Seaweed *Kappaphycus alvarezii* has a high economic value. This seaweed is included in the class *Rhodophyceae* or red algae which is known for its diverse nutritional content, high vitamin content, protective pigments, and sufficient mineral content, so that this seaweed is widely used in various industries, such as the food industry, health sector, and cosmetics industry. The problem often faced by cultivators is limited seeds, so tissue culture seeds are needed. Different seaweed harvest periods will affect the quality produced. From this statement there has been no related research regarding the different lengths of maintenance for seaweed plantlets resulting from tissue culture, especially in Gerupuk Bay. Therefore, it is important to carry out research regarding different lengths of maintenance, with the aim of finding out the optimal harvesting period for cultivators. The method used in this research was an experimental method with a completely randomized design. This treatment was carried out with 4 treatments and 3 repetitions, namely P1: 30 days, P2: 35 days, P3: 40 days and P4: 45 days. The four treatments were repeated 3 times to obtain 12 experimental units. The initial seed weight used in each treatment was 10 grams. The water quality parameter values obtained during the research are still considered optimal. The results of the research carried out can be concluded that the harvest age for seaweed *Kappaphycus alvar*ezii have a real influence on absolute growth, specific growth in seaweed. The best absolute growth was obtained in the P4 treatment with a 45 day harvest with a weight of 388 grams, as well as the best specific growth rate in the P4 treatment of 5.91%.

Key words: Seaweed, tissue culture, harvest period.

UNDER PEER REVIEW

1. INTRODUCTION

Seaweed of the species *Kappaphycus alvarezii* has high economic value. Based on FAO data (2020) in world seaweed production, Indonesia is recorded as a promoter with an average growth of 19.3% per year from 2007 to 2019 (Arthatiani *et al.*, 2021). This seaweed belongs to the class *Rhodophyceae* or red algae which is known for its diverse nutritional content, high vitamin content, protective pigments, and sufficient mineral content, so that this seaweed is widely used in various industries, such as the food industry, health sector, and cosmetics industry. This can influence the high demand for seaweed production. Increasing seaweed production requires continuous availability of seeds without being influenced by the season, this is an obstacle for seaweed cultivators. Repeated use of seeds produces poor quality seaweed seeds which is one of the challenges that farmers often face. In this case it is necessary to use tissue culture seeds. Tissue culture seeds are one of the methods used to produce quality seeds and seedlings (Cokrowati *et al.*, 2018). Seed propagation through tissue culture has several advantages, namely that it has the same plant characteristics as the parent (uniform) and seedlings can be propagated in large quantities in a short time.

Different seaweed harvest periods will affect the quality produced. According to research results (Djami *et al.*, 2024), seaweed growth *Kappaphycus alvarezii* Tissue culture seeds provide more significant results than selected seeds. In particular, the absolute growth of selected seeds aged 25 days with seaweed seeds from tissue culture seems to be more significant. From this statement, there has been no research related to the optimal length of maintenance for seaweed plantlets resulting from tissue culture, especially in Grupuk Bay, so research into the length of maintenance for plantlets *Kappaphycus alvarezii* at different times this needs to be done. Research on the length of maintenance time for seaweed plantlets *Kappahycus alvarezii* The aim is to find out the optimal time to be harvested in Gerupuk Bay.

2. MATERIALS AND METHODS

2.1 Research Methods

This research activity was carried out for 45 days and will be carried out in Teluk Gerupuk, Sengkol Village, Pujut District, Central Lombok Regency, West Nusa Tenggara. This research was carried out on July 1-August 16. Percentage tissue slice data collection was carried out at the Fish Production and Reproduction Laboratory, Aquaculture Study Program, Faculty of Agriculture, Mataram University. Before this research activity took place, it was carried out

Tying seaweed to the prepared ris rope. The method used in this research was an experimental method with different lengths of maintenance for 45 days. The experimental design used was a completely randomized design (CRD) consisting of 4 treatments with 3 replications. So that twelve experimental units were obtained, the treatments given in this study were 30 days, 35 days, 40 days and 45 days. The tools used in this research are as follows: prepared ropes where the seaweed is tied, raffia ropes to tie each clump of seaweed, floating rafts where seaweed is planted that has been tied to the ropes, a knife to cut the ropes and raffia, a boat as means of transportation to the middle of the sea, buoys to assist researchers in carrying out sampling, markers to provide markings for each treatment.

Comment [SA1]: Does the 45 day research period include preparation, analysis and discussion?

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UNDER PEER REVIEW

2.2 Research Procedures

In this research, the method for planting seaweed seeds used was a floating raft consisting of rope and bamboo. The size of the floating raft used is 2×4 m. Then the raft is tied using ris ropes to bamboo which has been shaped into a square with 12 ris ropes, and each ris rope contains 7 clumps of plantlets, then tied with a ribbon knot and a little loosely, the distance used between seaweed and other seaweed is 25 cm, this is within the SNI provisions. Then a 6 mm ris rope is stretched. This stretched rope is used as an anchor for the floating raft, the purpose of which is to keep the raft from being carried away by the current.

Seaweed seeds used are seaweed types *K. alvarezii* The brown color is the result of tissue culture. The seeds used in this research were obtained at BPBL Gerupuk, then the length of maintenance was adjusted to the treatment given, namely 30 days, 35 days, 40 days and 45 days. Then the seeds were weighed with the initial seed weight being 10 grams each. Next, the seaweed seeds are tied to ris ropes. The ropes used as clump ropes are raffia ropes and the like with seaweed clumps 25 cm apart. The length of the ris rope used is 3 m.

The seaweed to be planted is weighed first, each clump weighing 10 grams, then tied to the rope that has been prepared. To facilitate the planting process, seaweed is planted in the morning with light levels that are not too high. In seaweed maintenance activities, growth control is carried out *K. alvarezii* against moss that sticks to rafts, ropes or other types of seaweed which can cause damage *K. alvarezii* planted, the aim is to avoid other plants and dirt that can interfere with the growth of seaweed.

Observation of growth in seaweed *K. alvarezii* This research was carried out from the beginning to the end of maintenance, the aim was to determine the growth and absolute weight of seaweed. Before the seaweed is tied, it is first weighed to determine the initial weight of the seaweed, and at harvest time it will also be weighed at each treatment, namely 30, 35, 40, 45 days, to determine the final weight of the seaweed. Each treatment will be labeled to make it easier for researchers to know the treatment and research replications, then water quality measurements and weighing will be carried out every 9 days until harvest on day 45.

The seaweed harvesting process is carried out after 45 days of maintenance. Harvesting is done by lifting the floating raft and taking all the seeds that have been planted, then weighing the total seaweed per rope. After that, each clump is broken and transferred to another rope to increase the number of seeds, and then tied back to the floating raft used.

2.3 Data Collection

The parameters measured in this research include two parameters, namely main parameters and supporting parameters. The main parameters consist of absolute weight of

UNDER PEER REVIEW

seaweed, specific growth rate and tissue slices.

Meanwhile, the supporting parameters in this research consist of water quality parameters (physical and chemical) such as temperature, *dissolved oxygen*, pH, and salinity. Absolute weight measurements use the formula Wm = Wt -Wo, LPS measurements use the formula (ln Lt – ln L0)/t x 100%.

2.4 Data Analysis

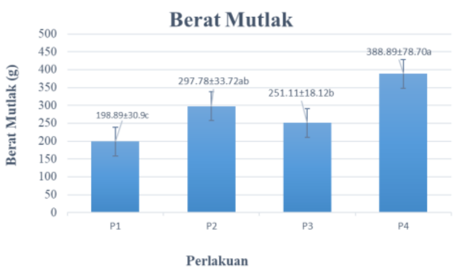
The statistics of this study include specific and absolute weight gain. After that, quantitative analysis was carried out on the weight data collected for this research, and the results were displayed in the form of tables and figures. Seaweed carrageenan content, tissue slices, and water quality were the characteristics examined. To determine whether there was a significant difference in the average weight of each treatment, the seaweed weight data was examined using analysis of variance (ANOVA) at a significance level of 5% with a 95% confidence interval. If the final results are significantly different, then further testing is carried out using the Duncan test. Then for the results of water quality parameters.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Absolute growth

Based on the results of the ANOVA test, it shows that rearing seaweed with different lengths of maintenance has a significant effect ($P < 0.05$) on the absolute weight of seaweed plantlets. *K. alvarezii*. So further tests were carried out using Duncan. The results of the absolute weight growth of seaweed are displayed in the form of a bar chart as follows:

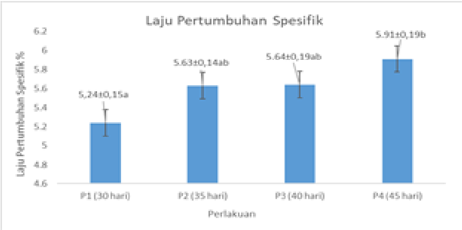


Ficture 1. Absolute weight growth of seaweed *K. alvarezii* with different maintenance times.

Duncan's test results showed that the absolute weight in treatment P4 had no significant effect on treatment P2, but had a significant effect on treatments P3 and P1. P1 had a real influence on treatments P2, P3, and P4 on the absolute weight growth of seaweed *K. alvarezii.*

3.1.2 Specific growth rate

The results of the anova test showed that there was a significant difference ($P < 0.05$) in the specific growth rate of seaweed, so a further test was carried out using Duncan. The results of the specific growth rate of seaweed are displayed in the form of a bar chart as follows:

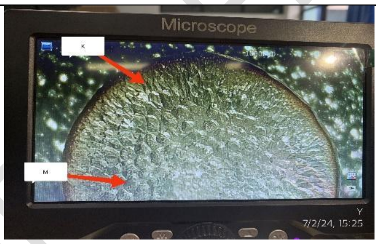
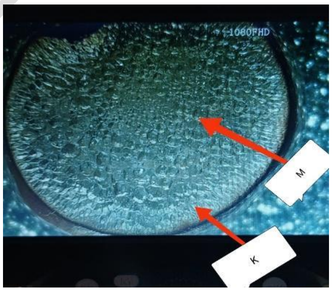


Ficture 2. Specific growth rate of K. Alvarezii seaweed with different lengths of cultivation

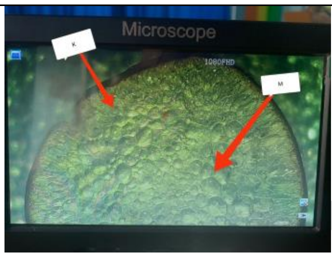
The Duncan Test results showed that treatment P1 had a real influence on each treatment. Treatments P2 and P3 did not have a real effect, but had a significant effect on treatments P1 and P4.

3.1.3 Network Slices

Observation results of seaweed tissue slices *K. alvarezii* at the beginning of maintenance, and for each treatment at the end of maintenance, namely 30 days, 35 days, 40 days and 45 days, the following data were obtained. The following image of network slices is presented in tabular form: Table. 1 Table of seaweed tissue slices *K. alvarezii* at the beginning of maintenance and at the end of maintenance.

No	Treatment	Cell Structure	Description
1	Early Seed (day 0)		K: Cortical (The surface of the talus, which is smaller and slightly oval). M: Medullary (large inner cells).
2.	P1 Day 30		K: Cortical (Cells at the edge, near the cells wall small). M:Medulary (Inner cells, large and round).

UNDER PEER REVIEW

3.	P2 Day 35		K: Cortical (Cells at the periphery, near the cell wall small). M:Medulary (Inner cells, at the large and round).
4.	P3 Day 40		K: Cortical (Cells at the periphery, near the cell wall small). M:Medulary (Inner cells, at the large and round).
5.	P4 Day 45		K: Cortical (Cortical sells at the edge, near sized cell walls small). M:Medulary (Cells located in section deep, and not too big solid, and irregular).

Picture 3. Network Slice Seaweed K. alvarezii

UNDER PEER REVIEW

3.1.4 Kualitas Air

3.1.4 Water Quality

Based on the results of the research, water quality observations were carried out, several parameters were measured, namely temperature, pH, salinity and DO. The temperature values obtained during the research ranged from 26 – 30 ^{0}C, then the pH results obtained ranged from 7.9-8.0. Then the salinity values obtained during the research ranged from 28-

35 ppt, and the DO (Dissolved Oxygen) measurement results obtained during the research ranged from 5.6 -8 mg/L. Below is a table for measuring water quality during seaweed maintenance in tabular form:

Table 2. Water Quality Parameters

No	Parameter	Measurement Results	Optimal Range
1.	Temperature	27-28,50C	26 – 32 SNI (2010)
2.	Salinity	31-32 ppt	28 – 34 SNI (2010)
3.	pH	7,1-7,6	7 - 8,5 SNI (2010)
4.	Disolved Oxygen	5,6-6,5 mg/L	>5 SNI (2010)

From the results of water quality measurements in the table above, both temperature, salinity, Ph, and DO (Dissolved Oxygen), can be said to be optimal for seaweed cultivation activities at this location.

4.2 Discussion

4.2.1 Absolute Weight

Based on the research results in (Figure 1), it was found that treatment (P4) with a harvest period of 45 days gave the highest absolute weight results in seaweed. *Kappaphycus alvarezii*, namely 388.67 (g). The second highest absolute weight treatment was (P2) which was 297.67 (g), then treatment (P3) gave a value of 251.11 (g), and the lowest value was obtained in treatment (P1) which gave a value of 198.89 (g), The 30 day harvest period produced the lowest absolute weight compared to other treatments.

The high absolute growth value in treatment P4, namely (45 days), is thought to be because harvesting at 45 days can be said to be optimal. It is said to be optimal because seaweed is able to absorb nutrients and elements in greater quantities, so it can produce a higher absolute weight than other treatments. This research is in line with Andi's research *et al.*, (2020) stated that the harvest age that has high productivity is the 45th day of harvest. After passing the 45 day harvest age, seaweed productivity tends to decrease. In Rofik's research *et al.*, (2021) stated that the harvest age of 45 days is the optimal harvest age for seaweed, so this statement is in accordance with this research so that the quality of the harvest age in treatment P4 (45 days) produces better seaweed compared to lower harvests.

Absolute growth in treatment P1 obtained a lower growth value than other treatments. The

UNDER PEER REVIEW

low weight in treatment P1 is thought to be due to the harvesting period being not optimal or the harvesting time being too short so that the nutrients and nutrients obtained are not optimal. This is in line with Rifaid's opinion *et al.*, (2023) stated

That the low yield of seaweed was caused by harvesting too quickly so that the nutrients had not been processed optimally, so that treatment P1 obtained the lowest value. Apart from that, environmental factors such as sunlight are also very important. The low absolute weight is also caused by the small amount of light received by seaweed, so that the photosynthesis process is not optimal. According to Guntur and Arami (2016), photosynthesis is a basic physiological process that is important for seaweed nutrition, where seaweed utilizes C02 for the photosynthesis process. The photosynthesis process occurs due to the optimal intensity of sunlight received by seaweed. So at different harvest ages you will certainly get different nutrients and nutrients.

Apart from that, there are other factors that cause the growth of seaweed to decrease, such as experiencing shedding due to strong current movements, causing seaweed to be close together and even cut or fall out. This is in line with Cokrowati's opinion *et al.*, (2018) stated that the talus between ties (between clumps) touch each other so that it can break the movement of water. This can minimize the occurrence of talus loss due to breaking by water movement.

4.2.2 Specific Growth Rate

Based on the results of the research in (figure 2) which was carried out for 45 days in Gerupuk Bay, the results of the specific growth rate of seaweed were obtained. *K. alvarezii* shows different averages in each treatment. In treatment P4, a value of 5.91% was obtained, giving the highest specific growth rate value, followed by treatment P3, a value of 5.64% was obtained, and in treatment P2, a value of 5.63% was obtained, then in treatment P1 it was 5.32%, which was the lowest value of the other treatments. .

The P4 treatment had the highest specific growth rate value with a value of 5.91% with an initial weight of 10 g. This is thought to be due to the 45th day of seaweed harvest *K. alvarezi* absorb nutrients in large quantities and have an optimal harvest period so as to obtain a high growth rate. In this study, the value obtained was 5.91%, this indicates that the growth rate value obtained in this study can be said to be optimal, because a good specific growth rate is considered to occur if the growth rate exceeds 3%. This is the same as Damayanti's research *et al.*, (2019) & Ikhsan *et al* (2022) stated that the growth rate of seaweed *K. alvarezii* Optimal growth is above 3% per day. This is in line with Rifaid's research *et al.*, (2023) stated that the high growth rate of seaweed is due to greater harvest time and nutrient availability, because the ideal age of seaweed is 45 days. A good specific growth rate is considered to occur if the growth rate exceeds 3%.

UNDER PEER REVIEW

The low specific growth in treatment P1 of 5.36% is thought to be due to a faster harvest period resulting in less than optimal growth. This is in line with Rifaid's research *et al.,* (2023) stated that the low value of the specific growth rate is due to the faster harvest age so that the talus formation process is not optimal which causes the growth value at P1 to be low. This is in line with Novandi's opinion *et al.,* (2022) stated that the low number of seaweed harvests in treatment P1 was due to the lack of nutrients such as (nutrients, carbon and phosphorus) to fulfill its growth. Chen *et al.,* (2016) stated that sunlight is one of the main factors for the growth of seaweed. If the light received is below the required level, then the energy produced through the photosynthesis process is unbalanced or not fulfilled, so that if one clump of seaweed is too clustered and too close together then the parts covered by other thallus will have difficulty penetrating the incoming sunlight. cannot carry out photosynthesis optimally.

There are several factors that can influence the growth of seaweed, such as the presence of nutrients and the intensity of sunlight. Nutrients and sunlight have a big influence on the growth of seaweed as an energy source for the photosynthesis process. This is in line with the Prime's opinion *et al.,* (2022) Nutrients or nutrients such as nitrogen are really needed by seaweed for its growth, because its function is to stimulate growth. Lack of nitrogen will inhibit the growth of seaweed because it is an element used in the photosynthesis process. Other factors are the planting distance and initial seed weight used at the start of maintenance. This is in line with Tiwa's opinion *et al.,* (2013) stated that the growth of seaweed is greatly influenced by the weight of the initial seeds used, where small initial seeds provide faster growth results because there is no competition between the thallus in obtaining nutrients, and in Ismariani's opinion *et al.,* (2015) stated that the farther away the seaweed seedlings are, the more nutrients the seaweed uses to grow.

4.2.3 Network Slices

Based on the results of observations of slices of seaweed thallus tissue *K. alvarezii*, at the beginning of maintenance, the cells on the outside close to the skin tissue were round and oval in shape, small in size and looked dense, while in the center of the thallus the cells appeared larger but not as dense as the cells near the skin tissue. These small cells are young cells that have just been formed. This is in line with the opinion of Darmawati, (2012) who states that the inside of the cortex contains cells.

polygon cells (polygonal-ovoid) that get smaller towards the edges. The cells outside this Identical to the tip (apical) cells of the talus, where it is stated that the apical part consists of assimilator cells or can be called cells that are actively growing. In Putri's research *et al.,* (2021) stated that the histology of seaweed tissue *K. alvarezii* Healthy ones have cells that are small, oval in shape, located on the surface of the thallus (cortical), and the cells get bigger in the middle of the thallus (medulla). This is also supported by Maulani's statement *et al.,*

UNDER PEER REVIEW

(2018) stated that seaweed tissue *K. alvarezii* with a healthy cell shape showing that the cell walls are tight and not loose. Observation of tissue slices in treatment P1 (harvest 30 days) showed that the cell arrangement was relatively the same as the cell arrangement at the beginning of cultivation, the cortex cells were arranged densely and regularly with a dense number of oval-shaped cells, as well as the medulla cells looked smooth and not too big in size. big. Then the results of observations at P2 (35 days), P3 (40 days), P4 (45 days) gave different results because the diameter of seaweed cells will increase with the maintenance time, this is in line with the opinion of Darmawati, (2012) stated that Seaweed cell diameter increases with increasing cultivation time.

The 40th and 45th harvest treatments showed that the medullary cells were larger and rounder in size, but less dense than the cortical cells. This is characterized by the longer the maintenance period, the greater the cell size or changes in the state of a number of cells to form organs that have different structures and functions. thallus growth shows fewer cortex cells and medullary cells appear to tend to be more prominent and rounded. According to Putri *et al.,* (2021) stated that the histology of healthy seaweed tissue is characterized by round/oval shaped cells in the cortex that are regular and not loose. Meanwhile, the medulla is thinner than the cortex cells and the cells are larger in size.

Seaweed tissue *K. alvarezii* shows that the seaweed cells are round and oval on the outside or after the thallus wall, then become larger in the middle of the thallus and are arranged irregularly. This was stated by Darmawati's research *et al.,* (2012) stated that the smaller cells that are elongated in shape with thick and dense cell walls in the talus can be called cortical. Then these cells decrease linearly and increasingly develop into medullary cells. These medullary cells are larger and rounder, but less dense compared to cortical cells. Seaweed cells increase as the length of cultivation increases. This is in line with Hayashi's opinion *et al.,* (2007) stated that thallus growth is an increase in cell size or a change in the state of a number of cells forming an organ

Organs that have different structures and functions. The purpose of this tissue section is to identify good cell tissue and seaweed cell tissue that is affected by disease. Good seaweed cells are characterized by small/oval and regular cortical cells, then as you move towards the center the cells become larger and more irregular. According to Putri *et al.,* (2021) stated that the histology of healthy seaweed tissue is characterized by round/oval shaped cells in the cortex that are regular and not loose. Meanwhile, the medulla is thinner than the cortex cells and the cells are larger in size. Meanwhile, cells affected by the disease are characterized by the presence of large empty spaces in the medulla of the seaweed tissue, this was stated by Yatin's research. *et al.,* (2023).

4.2.4 Water Quality Measurement

During research on the optimal length of maintenance for seaweed, water quality

UNDER PEER REVIEW

measurements were carried out. Water quality parameters are one of the most important factors for the success of cultivation activities. Seaweed cultivation activities can be successful if the physical and chemical parameters match the optimal needs for seaweed growth. Water physical parameters include temperature, salinity. Meanwhile chemical parameters include pH and DO.

The results of temperature measurements obtained during the seaweed maintenance period *K. alvarezii* during the research, namely 27.5-28.5 ^{0}C. The temperature obtained during the research can be said to be optimal and classified as suitable for seaweed cultivation. The measurement results are still considered good for seaweed maintenance, which is still within the range of the Indonesian National Standard (SNI 2010), which is around 26 – 32^{THE} C. This is in line with Khotijah's opinion *et al.,* (2020) The optimal temperature for seaweed growth ranges from 27-30 ^{0}C. According to Pauwah *et al.,* (2020) stated that temperature plays a very important role in the growth of seaweed *K. alvarezii* because it is related to the processes of photosynthesis, metabolism and respiration of seaweed, the speed of metabolism is due to increasing water temperature. Water temperatures that are too high can cause the talus to become pale and unhealthy, while low temperatures can cause seaweed growth to slow.

Salinity is a very important factor that needs to be considered in maintaining seaweed *K. alvarezii*. Salinity refers to the level of dissolved salts in seawater, and seaweed relies heavily on an optimal salinity balance for its growth. The results of salinity measurements in seaweed cultivation media at the time of the research ranged from 31-32 ppt, which is a good value to support seaweed growth. Meanwhile, according to Atmanisa *et al.,* (2020) stated that salinity between 28-34 ppt is good enough to support seaweed cultivation activities *K. alvarezii* while the optimal salinity value in grass cultivation activities sea, namely 33 ppt. This is in accordance with the Indonesian National Standard (SNI 2010) that the range of water salinity suitable for seaweed cultivation activities is 28-34 ppt. According to Yuliati *et al.,* (2023) that salinity plays a major role in thallus growth, color and morphogenic development, because it is directly related to osmoregulation that occurs in cells.

Dissolved Oxygen (DO) or what can be called dissolved oxygen is an important parameter to support the survival of seaweed. Dissolved oxygen is needed to support the metabolic and respiration processes of seaweed and other organisms around it. The DO values obtained during the study ranged from 5.6 – 6.5 mg/L. This is in line with Astriana's opinion *et al*., (2024) stated that the optimal DO value for seaweed growth is in the range of 4-5 – 8.9 mg/L. According to the Indonesian National Standard (SNI 2010), the optimal DO value range for waters is >5 mg/l. Seaweed uses dissolved oxygen for respiration. In respiration, cells need oxygen for glucose to become the energy needed by seaweed for the growth of Tarmizi *et al.,* (2022).

PH (degree of acidity) is an important factor in maintaining seaweed *K. alvarezii* because

UNDER PEER REVIEW

pH greatly affects water quality, the health of organisms, and the ability of seaweed to grow. The results of pH measurements during the research were found to range between 7.1-7.6. This is in line with Atmanisa's opinion *et al.*, (2020) stated that in seaweed cultivation activities, the pH that is good for seaweed growth is between 7-9. So the PH value obtained during the activities during this research is considered good for seaweed growth *K. alvarezii*. Meanwhile, the pH range according to the Indonesian National Standard (SNI 2010) is between 7.5 - 8.5, which is the optimal pH for maintaining seaweed. *K. alvarezii*.

5. Conclusion

The conclusion of this research is the harvest age of seaweed *Kappaphycus alvar*Different ezii have a real influence on absolute growth, specific growth in seaweed. The best absolute growth was obtained in the P4 treatment with a 45 day harvest with a weight of 388 grams, as well as the best specific growth rate in the P4 treatment of 5.91%.

UNDER PEER REVIEW

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