**Growth of Kappaphycus alvarezii Seaweed from Tissue Culture Cultivated with Different Initial Seed Weights**

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| Abstract-Seaweed, commonly called seaweed, is one of the abundant biological resources in Indonesian waters, covering around 8.6% of the total marine biota. The area of ​​seaweed habitat in Indonesia reaches 1.2 million hectares, making it the largest in the world. One type of seaweed that is commonly cultivated is Kappaphycus alvarezii. This study aims to analyze the effect of different initial seed weights on the growth of K. alvarezii seaweed from tissue culture. This study lasted for 45 days, carried out from May to June 2024, located in the waters of Gerupuk Hamlet, Gerupuk Village, Pujut District, Central Lombok Regency, West Nusa Tenggara. Data were analyzed using Analysis of Variance (ANOVA) with a 95% confidence level. If there is a significant effect (significantly different) from each treatment, further testing will be carried out using the Duncan test with the same confidence level. Meanwhile, water quality data and tissue sections were analyzed descriptively. The results of this study indicate that the use of different initial seed weights can increase the absolute weight of K. alvarezii tissue culture results ranging from 27.94g - 49.52g, the specific growth rate ranges from 0.39% - 3.14%. Carrageenan yield ranges from 0.37% - 0.54%. Seaweed production ranges from 307.2 - 998.2 g / m². The tissue structure of K. alvarezii seaweed with different seed weights obtained the results of P1 (25g) showing the results closest to the tissue structure before maintenance. The measurement of water quality during the study including salinity, pH, temperature, and phosphate was still ideal while light intensity and nitrate were not ideal for K. alvarezii seaweed cultivation. This study concludes that K. alvarezii seaweed cultivation with different initial seed weights can affect the specific growth rate but does not affect the absolute weight. |

Keywords: Initial weight, Cultivation, Kappaphycus alvarezii, Tissue culture, Gerupuk Bay.

**1. INTRODUCTION**

Seaweed, commonly called seaweed, is one of the abundant biological resources in Indonesian waters, covering around 8.6% of the total marine biota. The seaweed habitat area in Indonesia reaches 1.2 million hectares, making it the largest in the world (Suparmi & Sahri, 2009). One type of seaweed that is commonly cultivated is Kappaphycus alvarezii. The amount of seaweed production has increased by 2.25%, namely with a production volume of 28,332,249 tons (Ministry of Maritime Affairs and Fisheries, 2022).

 The increasing demand for seaweed requires farmers to be able to produce seaweed faster and with better quality, so that they can meet the increasing market demand. To meet the increasing market demand, seaweed seeds are needed that have superior criteria in terms of time, quality and quantity. One method that has been developed to obtain higher quality seaweed seeds is through tissue culture. This seaweed seed propagation technique is expected to produce quality seeds in large quantities in a relatively short time, without being affected by the seasonal cycle (Fadilah et al., 2016).

Tissue culture is a method for aseptically propagating plants involving cell or tissue parts of the plant. These cell or tissue parts have the ability to regenerate themselves, so that the resulting plants are similar to the parent plant (Jaelani et al., 2021). Seaweed obtained through tissue culture has a faster daily growth rate compared to conventionally cultivated seaweed. In addition to improving quality, tissue culture also plays a role in the provision of sustainable seaweed seeds (Makmur & Mulyaningrum, 2018).

 The method thatcommonly used by cultivators in seaweed cultivation is the floating raft method. In this method, seaweed is cultivated in the water column close to the surface, where the seaweed is tied to a rope with a predetermined distance, then the rope is tied to a bamboo raft (Calari et al., 2019). The floating raft method has been widely used and the planting distance used by cultivators is very good in the NTB area. However, it is not yet known the number of initial seeds that will be planted in each tie so that it can provide optimal growth in seaweed seeds from tissue culture.

The aim of this study was to analyze the effect of different initial seed weights on the growth of tissue cultured seaweed K. alvarezii.

**2. MATERIALS AND METHODS**

**2.1 Research Methods**

The research was conducted from May to June 2024 with a location in the waters of Gerupuk Hamlet, Gerupuk Village, Pujut District, Central Lombok Regency, West Nusa Tenggara. For data collection, tissue slices and carrageenan yield were carried outin the Fish Production and Reproduction Laboratory, Aquaculture Study Program, Faculty of Agriculture, University of Mataram. This study used an experimental method with a Completely Randomized Design (CRD), consisting of 12 experimental units, namely 4 treatments with 3 repetitions. The treatment tested was the difference in the initial weight of K. alvarezii seaweed seedlings from tissue culture, the treatment given in this study wasinitial seed weight 25g, initial seed weight 50g, initial seed weight 75g, initial seed weight 100g. The equipment used during this study included stationery, bamboo, boats, scissors, cameras, rulers, pH meters, refractometers, thermometers, scales, lux meters, nitrate kits, and phosphate kits. While the materials used were brown K. alvarezii seeds, bottles, sacks, sand, 3 mm polyethylene rope, 8 mm diameter polyethylene rope, and raffia rope.

**2.2 Research Procedures**

The floating raft used is made of bamboo with a raft length of 4 m and a width of 2 m. The raft is then tied with a ris rope (polyethylene rope) with a distance between each ris rope of 30 cm. The raft that has been made is then placed in the middle with an anchor attached so that the raft does not drift away with the current or waves.

 Seaweed seeds K. alvarezii were obtained from BPBL Lombok, Pujut District, Central Lombok Regency as much as ± 3kg. The seaweed seeds used have been cleaned from pests and dirt that stick to them. The seaweed seeds from tissue culture used were 2 months old after the planlet period. The characteristics of seaweed seeds K. alvarezii used as seeds are still young, fresh and clean and free from pests that inhibit the growth of seaweed K. alvarezii. The selected seaweed seeds were weighed with an initial seed weight of 25g, 50g, 75g & 100g then tied to a rope. Each rope contains 7 bundles of seaweed with a planting distance between ties of 25 cm. Planting of seaweed seeds is carried out in the morning when the weather is calm using the floating raft method.

Maintenance of K. alvarezii seaweed is carried out for 45 days with weighing every 9 days. Maintenance is carried out to control the seaweed so that it is free from pests and dirt that sticks to the seaweed and the ris rope, so as not to interfere with the photosynthesis process of the seaweed. The weighing process is carried out simultaneously with water quality measurements, namely salinity, temperature, light intensity, nitrate, phosphate and acidity (pH).

 Seaweed harvesting is done after 45 days of maintenance. Harvesting is done by releasing all the ris ropes and taking them to the beach to weigh the final weight of the seaweed. Weighing the weight of the seaweed is done by untying all the ties on each ris rope.

**2.3 Research Parameters**

**2.3.1Absolute Weight**

The absolute weight of K. alvarezii seaweed was obtained from measuring the average value from the beginning to the end for each treatment. Absolute growth was calculated using the formula proposed by Efendie (1997) inThe Cokrowati*et al*., (2020):

**G = Wt – W0**

 Information:

 G : Average absolute weight (g)

 Wt: Average weight at the end of the study (g)

 Wo: Average weight at the beginning of the study (g)

**2.3.2 Daily Growth Rate**

According to Dawes (1994) inThe Cokrowati *et al.,*(2018)Daily growth can be measured using the specific growth formula as follows:

**SG = (Ln Wt – Ln Wo)/tx 100%**

 Information:

 SG = Daily growth rate (%/day)

 Ln Wt = Average weight at the end of the study (g)

 Ln Wo = Average weight at the beginning of the study (g)

 t = Time (Maintenance Duration)

**2.3.3 Carrageenan Refining**

Carrageenan yield is the extraction result calculated based on the ratio between the weight of the carrageenan produced and the weight of the dried seaweed used in each treatment. The formula used to calculate the carrageenan yield refers to the FMC Corp formula (1997) in Failu et al., (2016):

**Carrageenan Percentage = Carrageenan weight x 100%**

 **Seaweed weight**

**2.3.4 Seaweed production**

Seaweed production can be calculated using the Faisal et al., (2023) formula in (Failu et al., 2016) as follows:

**Seaweed production = final biomass – initial biomass**

 **Maintenance area size**

**2.3.5 Network slice**

Observation of tissue slices was carried out at the beginning and end of the study after harvesting. Observation of tissue slices was carried out by slicing the thallus very thinly, then observing using a microscope.

**2.3.6 Water Quality**

Water quality checks are carried out every 9 days, with measurements that include temperature, salinity, pH, light intensity, nitrate, and phosphate. This water quality check uses tools such as salinity checks using a refractometer, temperature using a thermometer, pH using a pH meter and light intensity using a lux meter.

**2.4 Data Analysis**

Data were analyzed using Analysis of Variance (ANOVA) with a 95% confidence level. If there is a significant effect (significantly different) from each treatment, further testing will be carried out using the Duncan test with the same confidence level. Meanwhile, water quality data and tissue sections were analyzed descriptively.

**3. RESULTS AND DISCUSSION**

**3.1 Results**

**3.1.1 Absolute Weight**

 The results of the research that has been conducted show that the absolute weight of K. alvarezii seaweed maintained for 45 days with different initial seed weight treatments ranged from 22.89g - 92.56g. The highest absolute weight was found in the initial seed weight treatment of 75g (P3) of 92.56g. Then followed by the initial seed weight treatment of 25g (P1) of 81.44g, and the initial seed weight treatment of 50g (P2) of 79.44g. While the lowest absolute weight was found in the initial seed weight treatment of 100g (P4) of 22.89g.



 Fig 1- Effect of different initial seed weight treatments on the absolute weight growth of K. alvarezii

The results of the Anova statistical analysis showed that different initial seed weight treatments did not have a significantly different effect (P > 0.05) on the absolute weight growth of K. alvarezii seaweed.

**3.1.2 Specific Growth Rate**

 The results of the research that has been conducted show that the specific growth rate of K. alvarezii seaweed maintained for 45 days with different initial seed weight treatments ranges from 0.39 - 3.14% / day. The highest specific growth rate is in the initial seed weight treatment of 25g (P1) of 3.14% / day. Then followed by the initial seed weight treatment of 50g (P2) of 2.09% / day, the initial seed weight treatment of 75g (P3) of 1.69% / day. While the lowest specific growth rate is in the initial seed weight treatment of 100g (P4) of 0.39% / day.

 

Fig 2-Effects of different initial seed weights on the specific growth rate of K. alvarezii

 The results of the Anova statistical analysis showed that different initial seed weights had a significantly different effect (P < 0.05) on the specific growth rate of K. alvarezii. The results of Duncan's further test showed that the highest specific growth rate was found in the initial seed weight treatment of 25g (P1) and was significantly different from all other treatments.

**3.1.3 Carrageenan Reduction**

 The results of the research that has been conducted show that the average carrageenan yield of K. alvarezii seaweed maintained for 45 days with different initial seed weight treatments ranged from 0.37% - 0.54%. The highest carrageenan yield was found in the initial seed weight treatment of 75g (P3) at 0.54%. Then followed by the initial seed weight treatment of 25g (P1) at 0.51%, the initial seed weight treatment of 50g (P2) at 0.44%. While the lowest carrageenan yield was found in the initial seed weight treatment of 100g (P4) at 0.37%



Fig 3-Effects of different initial seed weights on the average carrageenan yield of K. alvarezii seaweed

**3.1.4 Seaweed Production**

 The results of the research that has been conducted show that the production of K. alvarezii seaweed maintained for 45 days with different initial seed weight treatments ranged from 307.2g/m² - 998.2g/m². The highest seaweed production was found in the initial seed weight treatment of 75g (P3) of 998.2 g/m². Then followed by the initial seed weight treatment of 50g (P2) of 822.5g/m², the initial seed weight treatment of 25g (P1) of 618.2g/m². While the lowest average carrageenan yield was found in the initial seed weight treatment of 100g (P4) of 307.2g/m²



Fig 4-Effects of different initial seed weights on the seaweed production

**3.1.5 Network Structure**

 The results of observations of the seaweed thallus tissue of K. alvarezii at the beginning and at the end of the study showed that all treatments still had cortical and medullary tissue with varying shapes and structures.

List 1- Seaweed thallus tissue of *K. alvarezii* under different treatments

|  |  |  |  |
| --- | --- | --- | --- |
| **No** | **Treatment** | **Cell Structure** | **Information** |
| 1 | Early Seeds |  | The cortex cells (K) and medulla cells (M) are still closely packed. |
| 2 |  P1(25 g) |  | The cortex cells (K) and medulla cells (M) are still closely packed. |
| 3 |  P2(50 g) |  | The cortex cells (K) and medulla cells (M) are irregular. |
| 4 |  P3(75 g) |  | The cortex cells (K) and medulla cells (M) are irregular. |
| 5 |  P4(100 g) |  | The cells of the cortex (K) and medulla (M) appear irregular. |

Information:

K = Cells that are round/oval in shape at the edge of the cell

M = Cells that are round/oval in shape in the middle of the cell.

**3.1.6 Water Quality**

 The results of water quality measurements during the study, including salinity, pH, temperature, and phosphate, were still classified as ideal, while light intensity and nitrate were classified as not ideal for cultivating K. alvarezii seaweed.

List 2- Outcome of different parameters

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| No | Parameter | Mark | Ideal | References |
| 1 | pH | 7.1- 7.6 | 7.5-8.6 | Nikhlani & Kusumaningum, 2021 |
| 2 | Salinity | 31 | 28–34 ppt | Syafikri et al., 2019 |
| 3 | Temperature | 28 – 30.1 | 26-31°C | Nikhlani & Kusumaningum 2021 |
| 4 | Light intensity | 4027 - 4103 | 4750 lux | Rohman et al., 2018 |
| 5 | Nitrate | 10 | 0.9 - 3.2 mg/l | Logo & Priyono, 2019 |
| 6 | Phosphate | <1 | 0.02-1mg/l | Susanto et al., 2021 |

**4.2 Discussion**

**4.2.1 Growth**

Growth is a form of change with increasing weight and length of an organism. Many factors affect the growth of K. alvarezii, such as internal factors and external factors. According toCokrowati et al., (2020)that the internal factors that influence the growth of K. alvarezii seaweed include the type, strain, part of the thallus and age of the thallus used as seeds, while the external factors that influence the growth of this seaweed are the physical and chemical environmental conditions of the waters.

 Overall, the results of this study indicate that the use of different initial seed weights in the cultivation of tissue cultured K. alvarezii seaweed does not affect the absolute weight. This indicates that the use of initial seed weights in the range of 25 - 100 g in this study is the optimum seed weight range used for seaweed cultivation. It is suspected that in the initial seed weight range, K. alvarezii seaweed can still obtain sufficient nutrients for its growth. According to Sakdiah (2009) in Novandi et al., (2022)that high density can cause seaweed seeds to have difficulty obtaining nutrients because of competition and it is difficult for them to grow. Marisca (2013) inGultom et al., (2019)also stated that optimal seaweed growth will be achieved when the seaweed gets enough nutrients from its environment. In addition, the use of initial seed weight in the range of 25 - 100 g in this study is still optimum for seaweed defense against wave impacts during its growth process. This is in accordance with Hartono's opinion (2015) inCokrowati et al., (2018)that the seeds used should be seeds weighing 50-150 g, have young and strong thallus, are easy to tie and are resistant to wave action.

 The use of initial seed weight in the range of 25 – 100 g in this study provided the absolute weight of seaweed*K. alvarezii*at the end of the maintenance period ranged between 22.89 - 92.56 g. This absolute weight range is still very low. Based on the research resultsNovandi et al., (2022)shows the average value obtained is 316.78 g. The results of the studyCalari et al., (2019)shows that the absolute growth obtained is 1632.5 g. The low absolute weight value obtained from this study is thought to be due to the high nitrate levels in Gerupuk waters which causes eutrophication in the waters so that seaweed growth is less than optimal. Eutrophication is a phenomenon where the level of water fertility is excessive, causing an explosion in the number of algae and phytoplankton (Hasibuan, 2016).Tarmizi et al, (2022), stated that if the nitrate levels in waters do not comply with what has been determined, it will cause seaweed growth to be less than optimal.

 Meanwhile, the use of different initial seed weights in seaweed cultivation*K. alvarezii*tissue culture results greatly affect its specific growth rate. Specific growth rate describes the ability of seaweed to grow specifically within a certain time span. The results of this study indicate that the best specific growth rate was found in the initial seed weight treatment of 25 g (P1). This is thought to be because the initial seed weight of 25 g is the lowest seed weight used in this study so that there is not too much competition for nutrients. According to Sakdiah (2009) inNovandi et al., (2022)that high density causes seaweed seeds to have difficulty in obtaining nutritional intake because of competition and it is difficult for them to grow. According toGultom et al., (2019)Maximum growth will be achieved when seaweed gets sufficient nutrients from the environment, whereas conversely, if the nutrients obtained from the environment are lacking, this will cause low and inhibited growth of seaweed.

The use of initial seed weight of 25 g in this study gave a specific growth rate of K. alvarezii seaweed at the end of the maintenance period of 3.14%/day. This specific growth rate value is still considered optimal for cultivation. Based on the results of the studyCalari et al., (2019)that the specific growth rate value obtained was 3.61%/day.Cokrowati et al., (2018)stated that the specific growth rate value obtained was 3.92%/day. This is in accordance with Erpin et al., (2013) inNovandi et al., (2022)which states that if seaweed cultivation experiences a 3% increase in growth rate, it is worth cultivating.

**4.2.2 Carrageenan yield**

Carrageenan is the result of primary metabolism of seaweed as a polysaccharide compound. Based on the results of the research that has been done, the yield value of carrageenan produced by K. alvarezii seaweed with different initial seed weight treatments ranges from 0.37% - 0.54%. The yield results obtained in this study are relatively low. This is because the yield of carrageenan produced in this study has not met the standard requirements for carrageenan yield set by the Indonesian Ministry of Trade, which is a minimum of 25% (Widyastuti, 2010). The low value of carrageenan yield obtained is due to the large amount of moss attached to the seaweed, so that the nutrient absorption process in the thallus is not optimal. It is known that nutrients are needed by seaweed for growth and carrageenan formation. The presence of moss attached to the seaweed thallus causes the absorption process to be hampered. This is in accordance with the opinion of Mudeng (2017) who stated that the presence of emphyte plants attached to the surface of the thallus will hamper the nutrient absorption process. According to Pakniany et al., (2023), the impact of emphyte attacks can be competitors or filters in obtaining dissolved nutrients and nutrients so that it can affect growth and partial or total loss of seaweed biomass.

**4.2.3 Seaweed Production**

Seaweed production is the total production generated during the maintenance period with a cultivation area. Based on the results of the research that has been conducted, the value of seaweed production produced ranges from 307.2 - 998.2 g / m². The value of seaweed production is greatly influenced by the specific growth rate. This is in accordance with the opinion of Failu et al., (2016) that the production of cultivated seaweed depends on the growth rate, production increases with the increasing growth rate. Seaweed production is also influenced by changes in weather which cause parasites to attach to the seaweed thallus. The attached parasites cause the nutrients obtained to decrease. According to Pakniany et al., (2023) the impact of emphyte attacks can be competitors or filters in obtaining dissolved nutrients and nutrients so that they can affect growth and partial or total loss of seaweed biomass. In addition, during the maintenance process, there are large waves and changes in the water to become cloudy, disrupting the growth of seaweed. The presence of high waves causes the ropes that bind the seaweed to become loose and come loose so that the amount of production produced decreases. This is in accordance with Sasmita (2019) who stated that fluctuating environmental changes cause pests and diseases to emerge which have an impact on the growth of seaweed, and high waves cause seaweed to break free from its bonds, thus affecting production capacity.

**4.2.4 Network Structure**

The best specific growth rate value in the initial seed weight treatment of 25 g (P1) is supported by the results of visual observations which show that the condition of the thallus tissue is still good and has similarities with the tissue structure of the initial seeds used before maintenance. The results of observations of the structure of the seaweed thallus tissue of K. alvarezii which are seen visually show that the seaweed cells look round and oval on the outside (cortex) and look very dense. While the inside (medulla) looks round and somewhat loose, not as dense as in the cortex. The cells on the outside (cortex) are young cells that have just formed. According to Darmawati (2014) in Yatin et al., (2023) the shape of seaweed tissue cells is round and rounded. The closer to the middle the cell size is, the larger and more irregular it is. While the cell wall is surrounded by small cells. According to Maulani et al., (2017) the seaweed tissue of K. alvarezii has a small, round, regular and dense cell shape in the epidermis. Dwiyanti & Muahiddah (2023) stated that normal cells can be seen from the walls not being damaged and the cortex and medulla appearing arranged and tight.

 Meanwhile, in the initial seed weight treatment of 50-100 g, the condition of the cortex and medulla cells was not tight and began to look irregular with each other, there were many empty spaces indicating abnormal cell tissue. According to Maulani et al., (2017), the condition of severe or abnormal thallus tissue is when the distance between the cortex and medulla cells is loose. This is because there is moss attached to the seaweed thallus which causes growth to slow down so that the cells become abnormal or develop.

**4.2.5 Water Quality Measurement**

Water quality is one of the most important parameters in cultivation activities. Water as a living medium for biota must have properties that are suitable for the life of the cultivated biota, because water quality can affect the growth of cultivated biota.

 In addition to being influenced by the weight factor, the growth of K. alvarezii seeds is also influenced by water quality, such as temperature. Temperature is an important component in seaweed growth, namely for the growth metabolism process. Unstable temperatures can disrupt metabolism which causes seaweed stress so that its growth is inhibited. The results of temperature measurements during the research activities ranged from 28-30.1 ° C, this shows that Gerupuk waters are still within the optimal range for the growth of K. alvarezii seaweed. According to SNI (2011) inMaulani et al., (2017)The optimal temperature for seaweed cultivation activities is between 26-31°C.

 The degree of acidity (pH) is one of the chemical factors that determine the growth of seaweed. The high or low degree of acidity of the water is influenced by the compounds contained in the water. The results of pH measurements during the study ranged from 7.1 to 7.6, this value is still considered good for the growth of K. alvarezii seaweed. This is in accordance withMaulani et al., (2017), which states that the range of water acidity levels suitable for seaweed cultivation activities is 7.5-8.6. According toSyafikri et al., (2019)The appropriate pH range for seaweed cultivation is 7.5–8.

 Salinity is the value of salt content and mineral ions dissolved in water. Salinity in each water has different values ​​depending on the density of the water mass and the solubility of the mineral ions contained therein.(Muqsith et al., 2022). The results of salinity measurements carried out during the study obtained a value of 31 ppt, this value is still considered good for the growth of K. alvarezii. This is in accordance withAtmanisa et al., (2020), stated that the appropriate salinity for K. alvarezii to grow well ranges from 28-34 ppt.

 The results of light intensity measurements during the study ranged from 4027 - 4103 lux, this level is still not suitable for the growth of K. alvarezii because light intensity affects the photosynthesis process so that it affects the growth of K. alvarezii seaweed. The low light intensity value is because the light intensity measurement was carried out in the morning. According toMuslimin & Sari, (2018), the high or low light intensity will always influence the productivity value of photosynthetic organisms.Rohman et al., (2018), the appropriate light intensity for seaweed growth is 4750 lux.

 Nitrate is one of the elements needed for the growth process of seaweed. The results of nitrate measurements during the study were 10 mg/l. Based on these results, the nitrate levels in the Gerupuk waters are classified as very high. According toTarmizi et al, (2022), if the nitrate levels in a body of water do not comply with what has been determined, it will cause seaweed growth to be less than optimal.Patricia et al., (2018)also stated that excess nitrate causes eutrophication to accelerate. The appropriate nitrate levels for seaweed growth are between 0.9 – 3.2 mg/lLogo et al., (2018).

 The results of the measurements that have been carried out show that the phosphate content in Gerupuk waters is <1 mg/l. Based on the data obtained, the nitrate content is still at a good level for seaweed growth. This is in accordance withSusanto (2021)states that the phosphate content suitable for seaweed cultivation ranges from 0.02 - 1 mg/l. The higher the phosphate content in a body of water, indicates that the body of water is classified as fertile. Sufficient phosphate concentration in a body of water will greatly determine the level of productivity in the aquatic ecosystem.

**5. Conclusion**

 Cultivation of K. alvarezii seaweed with different initial seed weights can affect the specific growth rate but does not affect the absolute weight. The initial seed weight treatment of 25 g (P1) is the best treatment because it can increase the specific growth rate by 3.14%/day supported by an absolute weight of 81.44 g, a carrageenan yield of 0.36% and a production of 618.2 g/m².

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

**Author's contribution**

This work was done collaboratively by all authors. All authors read and approved the manuscript.

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