Recovery of *Kappaphycus alvarezii* (Rhodophyta) thallus of due to damage by *Siganus* sp. bite and physical impacts

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Abstract. The intense attacks of *Siganus* sp. are among the problems of cultivating *Kappaphycus alvarezii*. The species graze seaweed and cause an important production decrease. Therefore, this study aims to compare the recovery rate of the *K. alvarezii* thallus, affected by fish bites and fractured by physical impacts. The study was conducted during the attack season of *Siganus* sp. on seaweed, namely from June to August, in the waters of Lakeba Beach, Bau-Bau City, Southeast Sulawesi Province, Indonesia. The method involved the storing of 20 thalli eaten by fish and 20 thalli broken by physical fractures in protected and unprotected areas. The parameters observed were thallus recovery, specific growth rate (SGR), and water quality. The results showed that the seaweed thallus recovery time in the protected area from fish bite marks and physical fractures was on average 11 and 13.7 days, respectively, while in the unprotected area it was 15 and 22.2 days, respectively. Furthermore, the SGR value for the thallus bitten by fish and the physical fracture in the protected area were 5.57 and 5.18% per day, respectively, while the SGR in unprotected areas were 4.20 and 4.56% per day, respectively. The specific growth rate of *K. alvarezii* seaweed reared in protected areas was significantly influenced by water quality parameters such as current velocity.

Key Words: fish, protected, recovery, seaweed, thallus.

Introduction. One of the critical problems that disturb seaweed farmers is the attack of fish pests (*Siganus* sp.) through intense grazing. These pests target some potential areas for seaweed cultivation in Indonesia, Thailand, the Philippines, Malaysia, and India (Kasim et al 2017). According to Kasim et al (2020), the attack reduces seaweed production by approximately 60% in one production cycle for areas with medium and high intensity. *Siganus* sp. are species of reef fish from the Siganidae family, including herbivorous fish with various diversity, spread in several Indonesian water bodies (Sitepu et al 2018; Kasim et al 2021). They feed on plants, including various types of algae (Selviani et al 2018). A previous report discovered that seaweed is the favorite food of *Siganus* sp. (Faisal et al 2013). The bite of *Siganus* sp. can cause fractures and injuries to the seaweed thallus, which will change according to the strength of the seaweed and environmental factors. Meanwhile, the main change that occurs in the seaweed's recovery is the growth of a new thallus (Kasim et al 2016), which is highly dependent on environmental conditions and genetic strength (Kasim et al 2019).

The growth of *Kappaphycus alvarezii* seaweed is influenced by environmental conditions, namely substrate type, light, nutrients, currents, etc., the seed quality, and the suitability of the technology (Hilda et al 2019). Thallus growth is an increase or a change in the biomass of several cells that form organs with different structures and functions, thereby increasing the number of cell masses (Hayashi et al 2007). When the main thallus starts to grow, it focuses more on increasing its diameter and length. The number of new cells is less at the time of growth than the old thallus. Subsequently, the diameter ceases to enlarge the cells and is more focused on multiplying new branches (Kasim et al 2017). The main thallus cells pass through division to form new cells in the new thallus (Kasim 2016). However, the original cells are not replaced or renewed because seaweed growth is more focused on widening branches (Rozaki et al 2013). Shoot formation occurs when a new thallus grows on the main thallus of the cultivated *K. alvarezii* seaweed seedlings. These new shoots resemble green spots that begin to form after an incision wound in the seedling has been covered with new cell tissue. The process of wound closure or healing by new tissue lasts for approximately 5-6 days (Rustam et al 2020). Changes in the growth of new shoots of *K. alvarezii* can occur in areas completely protected from *Siganus*, which can be overcome by a repellent (Kasim et al 2020). Protected seaweed will experience good growth compared to unprotected ones. The growth of the thallus can be repeatedly damaged by the bite of fish that eat seaweed. The purpose of this study was to compare the growth of new thallus from different types of damage, namely damage by fish bites and physical fractures.

Material and Method. This study was conducted from June to September 2022 in one of the largest seaweed cultivation areas in Lakeba coastal area, (50°48′78.2″N, 122°056″26.3″E), Southeast Sulawesi, Indonesia. The observations of water quality were carried out at the study location. The fish population increases from July to September every year, and the experiment was conducted at two different sites, namely an open area without any protection from attacks by *Siganus* sp. and a protected area, without the fish.

Seaweed sample collection and treatments. Seaweed samples were obtained from farmers around the cultivation area. The samples prepared were seedlings that had been kept together with *Siganus* sp. with thalli bitten by fish. Physical damage or physical fracture is obtained from the collision of the thallus, occurring during the binding of the thallus by seaweed farmers. This often happens when planting seaweed, the thallus being cut off due to physical impacts. We collected 20 thalli cut off by physical impacts as the research sample. Seaweed seedlings were cut using a knife and weighed to obtain the initial weight of 20 g. 20 thalli, 10 cut off and 10 with fish bite marks were tied to the maintenance media in each treatment.

Observations were made every 2 days to determine the recovery of the thallus and every 7 days to weigh the seaweed for determining its growth. The number of days to obtain samples of fish bites and physical fractures were 30 days and the period for observation of thallus recovery was 40 days. The study consisted of 2 treatments.

The specific growth rate (SGR) was calculated based on the formula used by Luhan & Sollesta (2010):

$$SGR = (\ln Wt - \ln Wo)/t \times 100\%$$

Where: SGR - specific growth rate (% days); lnWt - ln weight at the end of the study (g); lnWo - Ln weight at the start of the study (g); t - number of observation days.

Water quality parameter measurements. Environmental parameters were determined. Measurements were performed *in situ*, simultaneously with the seaweed collection. Measurement of salinity was carried out using a hand-refractometer. Temperature (°C) was determined using a thermometer. Brightness was measured using a Secchi disk, and depth was determined using a string meter scale and weights. Current velocity was determined using a current meter. Nitrate samples were analyzed using the Brucine method, while the phosphate samples were analyzed using the spectrophotometer method.

Statistical analysis. To determine the differences in the growth of seaweed in both rearing treatments, samples were tested to see the correlation between environmental factors and thallus recovery time after fish bites and by physical fracture, performed using statistical software (SPSS v. 24) with a 95% confidence level. The correlation was determined by the Pearson correlation coefficient (SPSS v. 24). To analyze the correlation between SGR thallus in protected and unprotected areas, Pearson's correlation coefficient analysis was carried out with a 95% confidence interval ($p<0.05$).

Results and Discussion

Seaweed thallus recovery time comparison. Seaweed thallus recovery time in protected areas has a slightly different range, from 9 to 13 days for thallus with cuts due to fish bites by *Siganus* sp. and from 12 to 17 days for the cuts by physical friction, as presented in Figure 1.

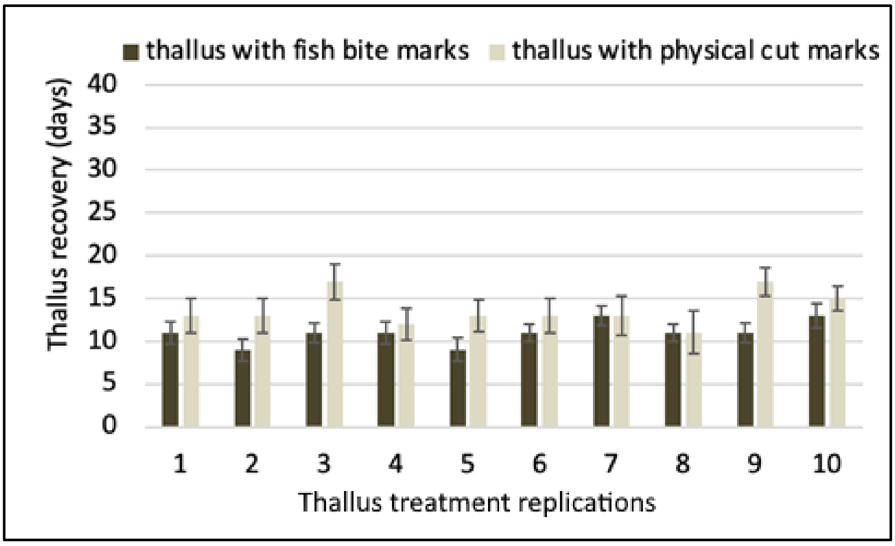


Figure 1. Histogram of recovery time for thallus cut by the bite of *Siganus* sp. and physical cuts in protected areas.

Although there was no significant difference, the recovery time for the thallus growing into new shoots in unprotected areas was lower. The time needed to recover for thallus eaten by fish is in the range of 13-17 days, while for thallus with fractures due to physical impact it is 19-28 days (Figure 2).

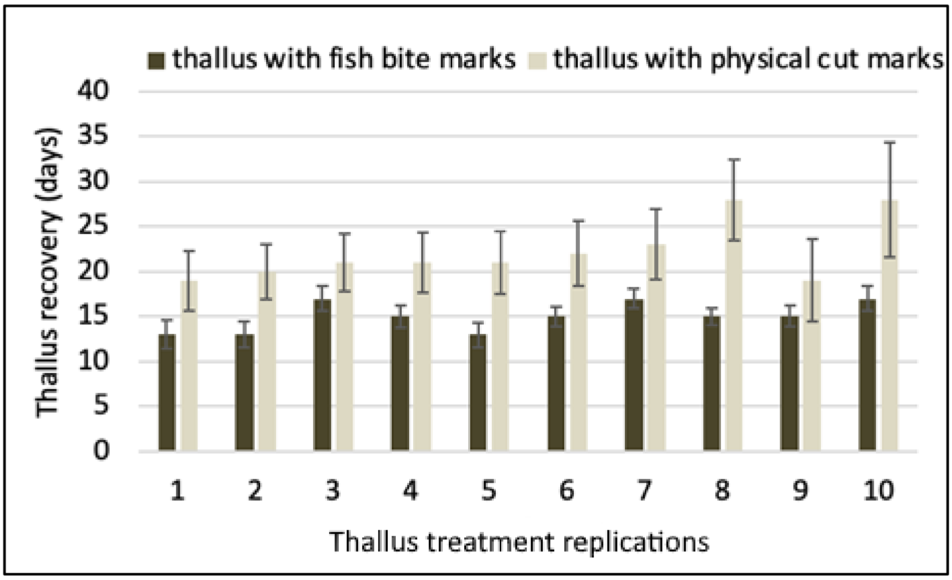


Figure 2. Histogram of recovery time for thallus cut after being bitten by *Siganus* sp. and physical cuts in unprotected areas.

The time required for recovery and growth into a new thallus for the seaweed bitten by fish and with physical impacts in protected and unprotected areas is significant different from the average recovery time, namely 11, 13.7, 15, and 22.2 days, respectively, as presented in Figure 3.

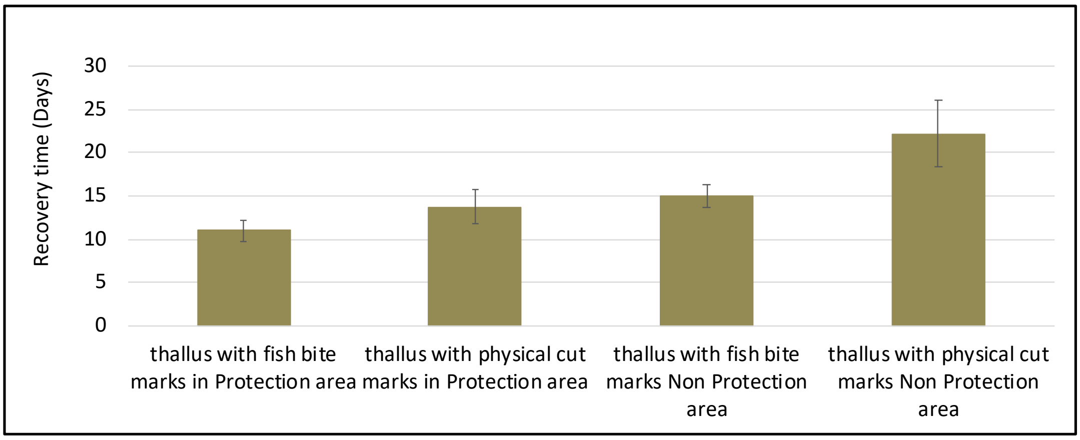


Figure 3. Histogram of the average recovery time (days) for the thallus cut by the bite of *Siganus* sp. and physical cuts in protected and unprotected areas.

Based on observations, it was discovered that the recovery rate of *K. alvarezii* thallus cultivated in protected and unprotected areas was 11 and 15 days, respectively. This was specifically for damage caused by bite marks of *Siganus* sp., while thallus with physical cuts needed 13.7 and 22.2 days, respectively. For the thallus bitten by fish, the recovery begins with reduced mucus in the fish bite marks; the explants are brownish yellow with a small bulge in the middle, which becomes more visible and resembles the original thallus. According to Mulyaningrum et al (2012), the thallus is formed in the middle (medulla) and the cortical layer. It is dark brown and consists of filaments from cell division, from the response to surface injury. Mizuta et al (2002) stated that the recovery of the thallus is influenced by the availability of nutrients, phytohormones possessed by the seaweed, and water quality. However, under certain conditions, thallus damage does not recover but becomes more susceptible to infectious diseases, including ice-ice disease (Tolanamy et al 2017). It was discovered that the antimicrobial effect can occur against bacteria isolated from the seaweed attacked by ice-ice disease. *K. alvarezii* also produces antimicrobials for protection against the disease (Tokan et al 2015). The process of growth and preparation of seaweed cells is very dependent on the intensity of sunlight to carry out photosynthesis. In this process, the seaweed cells can absorb nutrients to stimulate development through cell division activities and the formation of new shoots (Julizar et al 2018). Anatomically, the thallus consists of 3 layers of cells from the outside to inside, namely epidermal, cortex, and medulla layers. It was reported that the epidermis consists of one or 2 layers of the smallest-sized cells. The size of the epidermis and cortical cells is given by two layers of cells. When there is growth of new thallus in plant cells, there is an increase inward. This increased number of cells are known as medullary cells, which are located in the middle, or deepest part of the thallus (Pramesti et al 2016). Cells regenerate in each explant until they form a complete thallus (Fadel et al 2013). The process of algae growth also occurs because of the active role of phytohormones, namely organic substances that are needed in small amounts to ensure the continuity of a physiological process. These phytohormones are substances that support the development process, often called growth hormones, and include auxins and cytokinins (Mulyaningrum et al 2012). In the development of acids, the auxin group functions in cell enlargement, where the growth regulators stimulate proton pumps to bind H+ ions into the cell. This is to ensure that the cell cytoplasm becomes more acidic, thereby causing polysaccharide bonds in the cell wall (Redjeki et al 2013). Auxin also urges cell growth to support the diameter enlargement of the thallus (Basmal 2009) and influences processes such as cell development, elongation, cell wall acidification, and thallus formation (Gaspar et al 1996). Physiologically, cytokinins originate from plants, control cell division and shoot growth, playing a role in cell development (Tarakhovskaya et al 2007). Natural cytokinins are produced by actively developing tissues, especially at the base cells of the thallus. The formation of shoots is characterized by the compaction of the filament, which regenerates to form shoots (Basmal 2009). *K. alvarezii* explants produce crystalline and pigmented thallus filaments (Munoz et al 2006). The formation of the thallus begins with pigmentation or a change in color on the explant surface area (cortical). Subsequently, filaments are created on the explants and grow into shoots (Rorrer & Cheney 2004). Good water quality parameters are important for the development and formation of thallus, and for thallus morphogenetic growth in seaweeds in general. For example, salinity is directly related to cell osmoregulation (Marsoedi et al 2011).

The results showed that the emergence of the new thallus in surface scars only appeared after 11-15 days, being influenced by the cultivation media. In a covered area, the thallus is protected from herbivorous fish pests and other problems. Meanwhile, in unprotected areas, the thallus is not protected from pests, which may cause repeated damages its thallus. It was reported that shoots begin to appear at the age of 9-15 days after cultivation (Redjeki et al 2013) and *K. alvarezii* will experience the emergence of new thallus after 15-16 days of rearing (Munoz et al 2006; Hayashi et al 2007). The thallus begins to develop after 2 weeks of rearing, starting with the presence of a small spot in the middle of the thallus explant (Raihani et al 2016), indicated by the presence of fresh greenish brown spots around the surface. These spots are new shoots produced through vegetative growth (Parenrengi & Amini 1994; Raihani et al 2016) and a fresh thallus may emerge during a 10-day maintenance period (Kumar et al 2007). The difference in the color on the surface of *K. alvarezii* is caused by the variation in the chlorophyll response to the received light. When a new thallus grows, it will be more obvious if there is damage due to physical impacts (Rustam et al 2020). The newly formed cells along the surface will gradually thicken, and the outer layer will divide to create a cortical network for wound healing and regeneration. On the 10th day of maintenance, the thallus is dark brown, and there is a green color in the middle of the surface (Redjeki et al 2014).

Specific growth rate (SGR). SGR for thallus damaged by fish bite and physical impacts in protected and unprotected areas have slightly different values. Based on the results, SGR for thallus that recovered after damage from physical impact and fish bites in protected areas were 5.57 and 5.18% per day, respectively. In unprotected areas, the SGR of the thallus that recovered after physical impacts and fish bites was 4.2 and 4.56% per day, respectively, as presented in Figure 4.

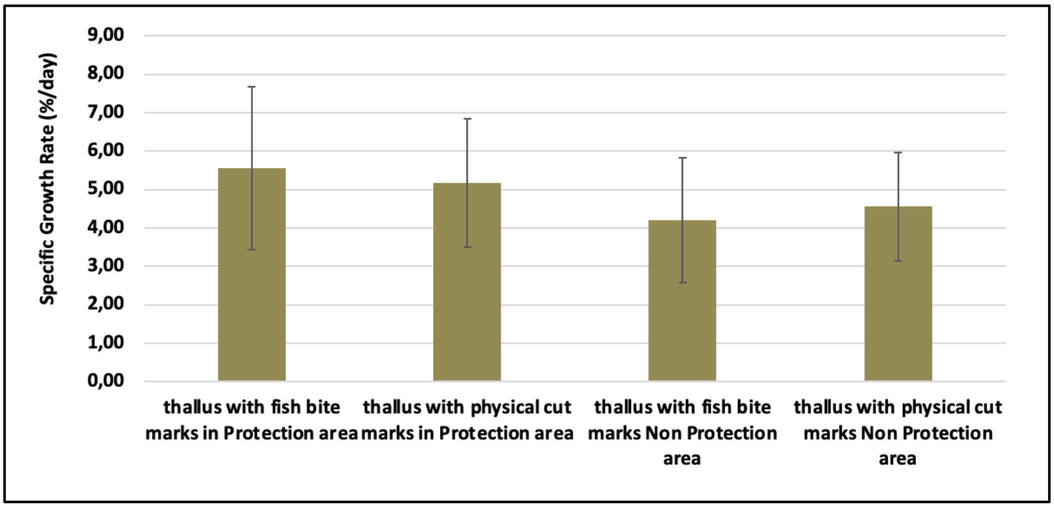


Figure 4. The specific growth rate of thallus with fish bites and physical cuts in protected and unprotected areas.

The growth and health of the thallus significantly determine the carrageenan content of seaweed (Patadjai et al 2019). Therefore, cultivation media that can protect seaweed from attacks by herbivorous fish is very important for *K. alvarezii* cultivation (Kasim et al 2019). Based on this study, it was discovered that the protected area had a better effect on the SGR of *K. alvarezii*. The protected area obtained an average SGR value in the sixth week of 3.13% per day, and the area without protection had a SGR of 2.95% per day. In the area protected from herbivorous fish pests, the thallus can properly carry out photosynthesis and growth.

The observations of *K. alvarezii* during the cultivation period showed that the highest growth rate was in the first week, but it began to decline the following week. This is partly because of the plant development and fertility phases. Furthermore, the decrease in SGR is due to competition in the acquisition of nutrients, along with the increasing age of seaweed. During the phase of vegetative growth, plants develop mature tissue cells for continuous growth (Nursyahran & Reskiati 2013). The decrease in the SGR is also due to the rapid saturation of cell division. Seaweed has passed through the adaptation process after a rapid development phase, which decreased the ability of cell development, causing slow growth (Yusnaini et al 2000). A low specific growth rate is the result of low thallus growth (Mutalib & Adi 2018). This shows that longer maintenance delays the growth rate of the cells in seaweed. Thus, development slows because they have reached the adult stage (Julizar et al 2018). A previous report also stated older seaweed will develop slower than younger seaweed (Kasim & Asnani 2012).

Table 1

Morphological characteristics of thallus recovered after damage from fish and physical cuts

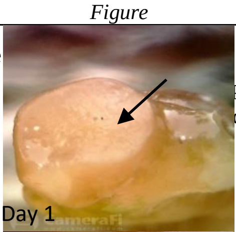
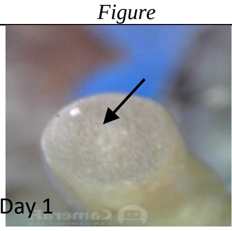
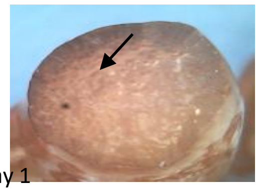
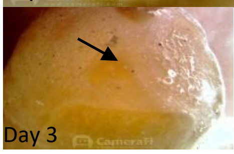
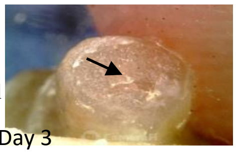
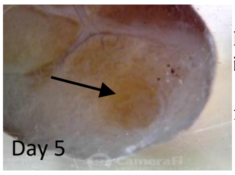
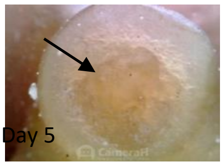
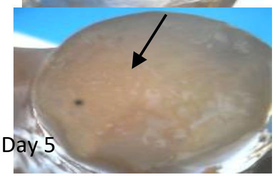
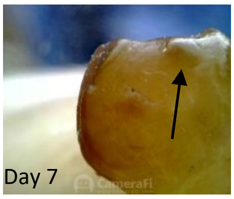
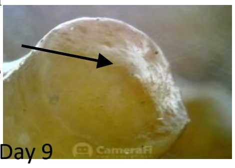
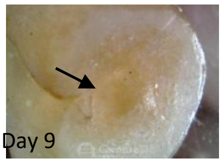
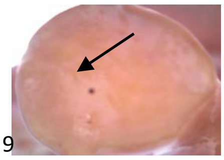
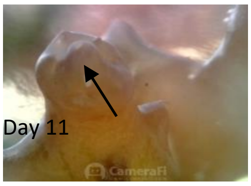
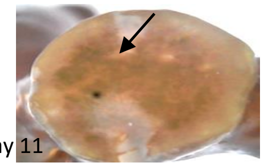
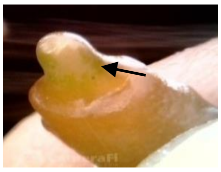
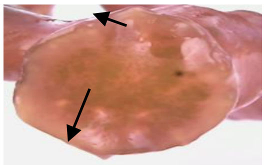
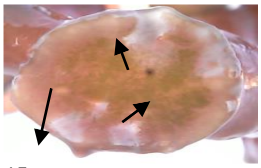
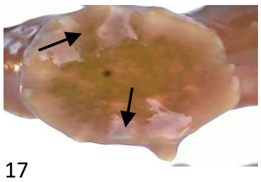
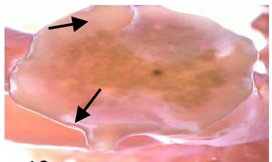
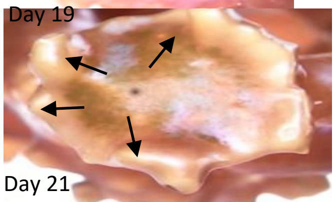
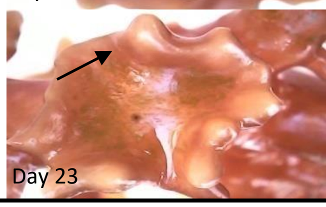
Note	*Figure*	*Note*	*Figure*	*Note*	*Figure*
Damage to the thallus due to fish bites, the thallus looks clear and secretes mucus.		Pieces of thallus due to fish bites are clear and secrete mucus.		The surface of the fractured thallus is clear	
The tip of the thallus begins to look brown in color.		Mucus begins to decrease in the hallus wound and looks dry		The surface of the thallus is slimy, and the side of the hallus begins to thicken	
The brown membrane on the side of the thallus is getting bigger.		The thallus of the fish bite begins to have a brown membrane in the middle.		The surface of the thallus is brownish yellow on the entire surface	
The thallus damage has improved, color starting to brown, and yellow. In the middle, there is a small bulge.		The thallus begins to heal and the white membrane already looks brownish yellow.		The surface of the thallus begins to turn light brown	
The protrusion began to become more visible and the wound on the side of the talus began to recover completely.		Middle part of the seaweed thallus that starts to brown and looks like a small bulge.		Thallus surface has begun to be completely covered and is light brown in color	

Table 1

Morphological characteristics of thallus recovered after damage from fish and physical cuts (continuation)

Note	*Figure*	*Note*	*Figure*	*Note*	*Figure*
The pieces of thallus that have been bitten by fish in the thallus are completely healed and a new thallus appears.	 Day 11	A bulge in the middle of the thallus begins to form.	 Day 11	Thallus surface is brown and in the middle of the thallus there is a green color	Day 11 
		The bulge on the side of the thallus is more visible.		Thallus surface begins to appear small lumps on the sides of the thallus	
		The bulge is more visible, and a new thallus appears along with the healing of the fish bite wound.	Day 13  Day 15	Thallus surface begins to have a lump on the side of the thallus	Day 13  Day 15
				Thallus surface surface begins to appear increasingly clear bulges	 Day 17
				Bumps on thallus surface are starting to clearly see new shoots	 Day 19
				The lump on the side of the thallus is getting more visible and clearer	 Day 21
				New thallus begins to grow on the sides of the thallus surface	 Day 23

Seaweed growth requires nutrients to create new tissue through the formation of shoots for a continuous lifespan. In brown seaweed, fucoxanthin is produced, which has a role in protecting the plant from environmental stress (Hitoe & Shimoda 2017). The growth rate of *K. alvarezii* in this study can be included in an excellent category. A SGR good for seaweed ranges from 2.03 to 2.36% (Hernanto et al 2015; Ariyati et al 2016). A profitable growth rate is above 3% (Julizar et al 2018). Patadjai et al (2019) obtained SGR values between 5.86 and 6.43%.

Seaweed growth can slow down due to unsupportive area conditions in certain months, this problem being often experienced by farmers (Paranrengi et al 2008). Under these circumstances, the seaweed is usually stunted and exposed to pests or diseases (Arisandi et al 2011). Seaweed cultivation in Madagascar is also affected by epiphytic outbreaks between November-December and, to a lesser extent, in March and October (Ateweberhan et al 2015). Each seaweed cultivation center is expected to have a time calendar that can be used to determine when to start planting and the species suitable for cultivation (Pong-Masak & Tjaronge 2009). The growth of *K. alvarezii* is related to cell formation and division in the thallus (Zainuddin et al 2018).

Environmental parameters. An overview of the existing environmental conditions at the research site compared to other areas is presented in Table 2.

Table 2

Water quality parameters during the study and the comparison with those of a different area

Water parameters	*Measurement unit*	*Range*	*Other values*	*Area*	*References*
pH	ppm	7.6-7.9	6.5-8.5	North Minahasa Regency, Indonesia	Mudeng et al (2015)
Salinity	ppt	28-32	28-34	Konawe Coast area, SouthEast Sulawesi, Indonesia	Julizar et al (2018)
Temperature	ºC	28-29	25-30	Lakeba Coast Area, Souteast Sulawesi, Indinesia	Kasim et al (2018)
Current speed	m s^{-1}	0.11-0.16	0.14-0.18	North Minahasa Regency, Indonesia	Mudeng et al (2015)
Water Transparancy	%	90-100	80-100	Konawe Coast area, SouthEast Sulawesi, Indonesia	Julizar et al (2018)
Depth	m	4-7	6-8	Konawe Coast area, SouthEast Sulawesi, Indonesia	Julizar et al (2018)
Nitrate	mg L^{-1}	0.102-0.116	0.01-0.07	Nambung Coast. NTB, Indonesia	Cokrowati et al (2018)
Phosphate	mg L^{-1}	0.0102-0.004	0.0021-0.03	Lontar Village, Tirtayasa District, Serang Regency	Saifullah et al (2015)

The specific growth rates for fish bite thallus in protected and unprotected areas were positively correlated with current strength. Based on Table 3, chemical factors such as phosphate are related to thallus growth, in the case where seaweeds were cut off due to physical impacts.

Table 3
Pearson analysis for correlation between specific growth rate and environmental factors

Specific growth rate	*Salinity*	*Temperature*	*pH*	*Current velocity*	*Nitrate*	*Phosphate*
Thallus with fish bite marks in protected areas	0.437	-0.72	0.309	-0.785	0.590	0.702
Thallus with fish bite marks in non-protected areas	0.369	-0.726	0.520	-0.83*	0.778	0.706
Thallus with physical cut marks in protected areas	0.509	0.592	-0.98*	0.989	0.288	0.323
Thallus with physical cut marks in non-protected areas	0.491	0.551	-0.97	0.985	0.325	0.263

Note: * - correlation is significant (2-tailed) ($p<0.05$).

Good and appropriate water quality is needed for the success of seaweed cultivation (Julizar et al 2018). During the study, the water quality was in a condition suitable for seaweed cultivation and the process was influenced by some environmental factors. This is in line with a previous report, where the growth parameters were affected by seasonal conditions and the topography of the water area (Nursyahran & Reskiati 2013). *K. alvarezii* tolerates changes in the environment (Cokrowati et al 2018).

Temperature showed values ranging from 28 to 30°C. This value is within the appropriate content for seaweed cultivation and is suitable for supporting the development of seaweed. The results of the observations showed a tendency of increase in temperature from the first day to the 40 days, which is relatively normal, the increase being low. Temperature directly affects the life of seaweed, especially photosynthesis, metabolic processes, and the reproductive cycle (Pong-Masak & Sarira 2008). Therefore, a suitable temperature value for cultivating *K. alvarezii* is between 20-28°C.

In this study, the values of salinity measurements ranged from 32 to 34 ppt. This value is good for *K. alvarezii* growth, because high salinity affects development and disease resistance (Arjuni et al 2015). Seaweeds experience slow growth when the salinity is very low (less than 15 ppt) or very high (more than 35 ppt) (Choi et al 2010). Seaweed requires a salinity of 32-35 ppt for optimal growth, a lower value being susceptible to cause ice-ice disease (Tisera & Naguit 2009). Other authors note a larger interval for seaweed optimal growth, between 25-33 ppt (Guo et al 2014).

The values of current velocity ranged from 0.11 to 0.18 m s^{-1}, which is good for cultivating *K. alvarezii*. It was reported that currents affect the absorption of nutrients from the environment (Rejeki et al 2012) and are very useful in supplying and increasing the diffusion of nutrients into plant tissues (Pong-Masak et al 2010). Too strong currents will disturb and endanger seaweeds. A good current velocity for seaweeds ranges from 0.2 to 0.4 m s^{-1}.

In the current study, the content of nitrate varied from 0.04 to 0.2 mg L^{-1}. This indicated that the waters can support seaweed development (Mudeng et al 2015). The ideal nitrate level for seaweed is between 0.0057-0.00185 g L^{-1} (Utojo et al 2007). Generally, water conditions with high nitrate concentrations are influenced by land activities that produce organic waste (Rujiman et al 2013). Furthermore, the phosphate content obtained was between 0.0020-0.0040 mg L^{-1}, while the values obtained in the surrounding areas ranged from 0.0007-0.0040 mg L^{-1} (Julizar et al 2018), values sufficiently good for seaweed growth. The phosphate range of 0.0021-0.050 is good for seaweed growth in several other areas in Southeast Sulawesi, Indonesia (Arfah et al 2015). According to Radulovich et al (2015), the optimal range of phosphate for seaweed growth is 0.05-1 mg L^{-1}.

Conclusions. Seaweed thallus recovery time in protected areas has a slightly different to fish bites by *Siganus* sp. and the cut by physical friction.

The time required for recovery and growth into a new thallus for the ones eaten by fish and physical impact on protected and unprotected areas is different from the average recovery time. Recovery rate of *K. alvarezii* seaweed thallus cultivated in protected was better than unprotected.

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