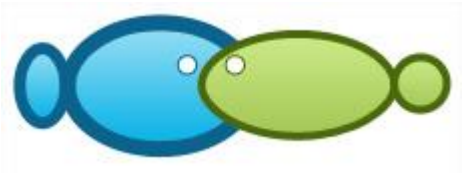

Eucheuma cottonii soaking time in a solution of atonic growth stimulants and its effects on the growth rate of thallus in vitro

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Abstract. The current research addresses the effect of immersion of *Eucheuma cottonii* in atonic growth stimulant (ZPT) solution. The study aimed to determine the effect of the dose and duration of immersion in atonic ZPT solution on the length and weight growth rates and on the survival of *E. cottonii* in vitro. The research had been conducted at the Seaweed Tissue Culture Laboratory, Research Center of Brackishwater Aquaculture, Takalar for 28 days. The method used was an experiment with a factorial RAL design, combining 2 factors: (A) the immersion duration (with several levels: 0, 6, 12, 18 and 24 hours), and (B) the dose of atonic ZPT (with several levels: 5, 10 and 15 ppm). The data observed included the rate of explant weight gain, the rate of increase in explant length and the survival rate of *E. cottonii* explants. Data were analyzed using a one-way ANOVA and continued with the Least Significant Difference (LSD) test, in order to identify a significant difference of the means. The highest weight growth rate (RGRW) in the solution at 5 ppm was found after 12 and 18 hours of immersion.
Key Words: seaweed, atonic ZPT dose, immersion, growth rate.

Introduction. Seaweed is a source of foreign exchange, a source of income for the coastal communities and one of the most popular marine commodities in world trade, due to its extensive use in everyday life, as a source of food, medicine and industrial raw materials (Indriani & Sumiarsih 1991). *Eucheuma cottonii* was originally obtained from the waters of Sabah (Malaysia) and the Sulu Islands (Philippines). Then it was developed to various countries as a cultivated plant. The locations for seaweed cultivation in Indonesia include Lombok, Sumba, Southeast Sulawesi, South Sulawesi, Central Sulawesi, Lampung, the Thousand Islands and the waters of Pelabuhan Ratu (Syamsuar 2007). The limited quality of seeds supply is one of the obstacles in the development of seaweed cultivation. One way to support the availability of seeds at any time is by using the seaweed propagation method by means of tissue culture. The thallus culture technique was performed by cutting the thallus explants from 0.5 to 2 cm, then culturing them in vitro until new thallus branches were formed (Titlyanov et al 2006). Nutrients contained in the maintenance medium were one of the factors that influenced the growth of seaweed. Insufficient nutrients can be supplied using fertilizers. Fertilization can be carried out indirectly, namely by soaking seaweed in a fertilizer solution before the maintenance process. Sufficient nutrients are used by seaweed for growth, through photosynthesis. Fertilizer application can be conducted by soaking seaweed prior to maintenance (Yuliyana et al 2013; Lideman et al 2014), however, fertilizer application must be done at an appropriate dose for the seaweed growth. The present study used growth stimulant (ZPT), which is more environmentally friendly, affordable to the community and contains the nutrients needed by seaweed. Based on this description, the study aimed to determine the effect of dosage on the duration of soaking *E. cottonii* in atonic ZPT solution against in vitro weight and length growth rates.

Material and Method

Research time and location. The present research was conducted during May-July 2019, at the Laboratory of Seaweed Tissue Culture of BBAP Takalar, Mappakalompo Village, Galesong Selatan District, Takalar Regency.

Tools and materials. The tools used in this research were: tissue culture room, lux meter, aeration equipment, aquarium, multiple chamber, autoclave, thermometer, analytical scale, aerator, aerator hose, razor blade, tweezers, sponge, basket, hand towel, dipper, petri dish, cleaning brush, erlenmeyer, measuring pipette, rubber band, vacuum pump, camera and stationery. The materials used in this study were: *E. cottonii*, sterile sea water, fresh water, ZPT atonik, 1% betadine, and sunlight soap.

Research design. The research was an experimental study using a completely randomized design (CRD) consisting of fifteen treatments and three replications. This study consisted of two factors with a fixed model (fixed factor 1, fixed factor 2). The treatments included treatment A (0 hours/control), treatment B (6 hours), treatment C (12 hours), treatment D (18 hours) and treatment E (24 hours), each one using three different doses: 5, 10 and 15 ppm. The research procedure began with the preparation of the container, acclimatization, sterilization, immersion of the explants, maintenance of the explants and data collection.

Data analysis. The variables observed in the present study were the relative growth rate of weight (RGRW), relative growth rate of length (RGRL) and survival rate (SR). Data retrieval and growth measurement of explants were carried out once a week using an analytical scale with 4 digits to measure the weight and using a ruler to measure the length.

The weight growth rate was calculated using the formula (Efendie 1979):

$$RGRW = [(\ln W1 - \ln Wo) / t] \times 100$$

Where:
RGRW - relative growth rate of weight (% day^{-1});
Wo- initial weight;
W1 - final weight;
t - the last weighing age.

For the calculation of the length growth rate, the following formula was used (Efendie 1979):

$$RGRL = [(\ln L1 - \ln Lo) / t] \times 100$$

Where:
RGRL - relative growth rate of length (% day^{-1});
Lo - initial length;
L1 - final length;
t - the last weighing age.

The explant survival was calculated using the formula according to Fadel (2013) as follows:

$$SR (\%) = (Nt / No) \times 100$$

Where:
SR - explant's survival rate (survival);
Nt - number of explants alive on day t;
No - number of explants at the start of maintenance.

The data analysis in the present study was carried out by describing the data obtained in the form of tables and graphs, in order to determine the ratio of weight and length of the explants, which showed the differences in the growth rate for different durations of immersion of the *E. cottonii* in atonic ZPT. The data entered were RGRW, RGRL and SR of *E. cottonii* explants. In order to determine the effect of each treatment, the analysis of variance (ANOVA) was used. ANOVA is a statistical analysis method that belongs to the inference statistics branch (Gaspertz 1991). If there was a difference, a further LSD (Least Real Difference) test was carried out.

Results

Explant weight growth rate. The effect of immersion of *E. cottonii* in atonik ZPT on the explant weight growth rate parameters are presented below.

ZPT Atonic immersion at a dose of 5 ppm. Following the research conducted for 28 days at the Takalar tissue culture center, weight data were obtained by weighing and calculating explants every week for 28 days, tested using the variance test or ANOVA to determine the effect of immersion of *E. cottonii* in atonik ZPT on the explant weight growth rate parameters (Figure 1).

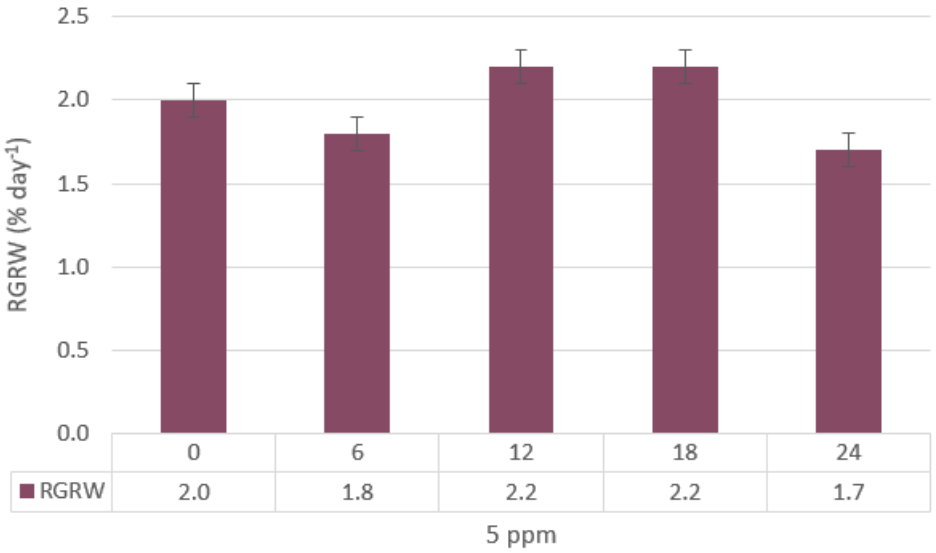

Figure 1. Relative weight growth rate of *Eucheuma cottonii* explants.

Based on Figure 1, it can be seen that the maintenance of *E. cottonii* explants by immersing into ZPT Atonic solution at 5 ppm showed that in terms of relative weight growth rate, the 18 hours and 12 hours treatments experienced the best growth, compared to other treatments, with a value of 2.2% day^{-1} after 12 and 18 hours of immersion. The lowest growth was at a dose of 5 ppm, for 24 hours of treatment with a value of 1.7% day^{-1}. The results of the analysis of variance showed that the treatment by immersion in the growth regulator solution (ZPT) at a 5 ppm dose had no significant effect on the relative weight growth rate of *E. cottonii* seaweed explants ($P>0.05$; significance value in the ANOVA table of 0.959).

ZPT atonic immersion at a dose of 10 ppm. The relative weight growth rate of *E. cottonii* explants immersed in 10 ppm ZPT dose is presented in Figure 2.

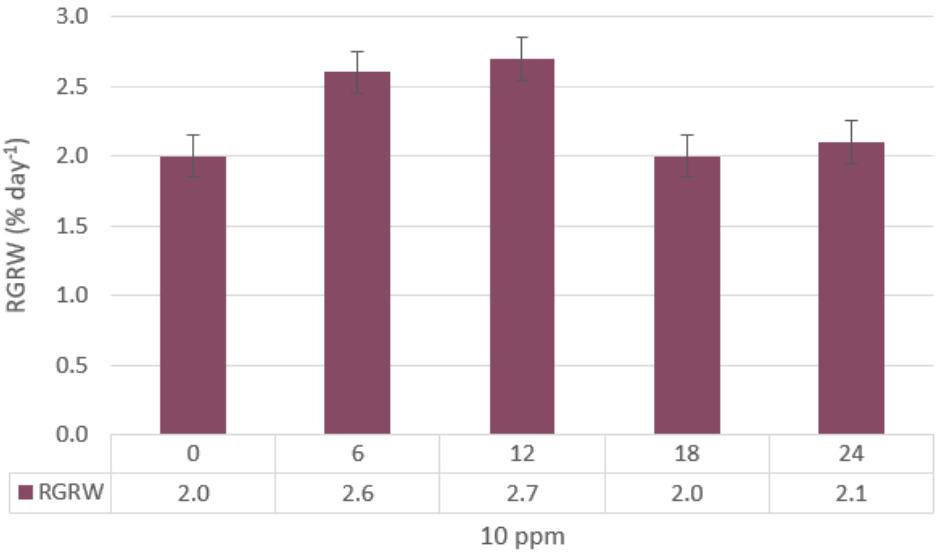

Figure 2. Relative weight growth rate of *Eucheuma cottonii* explants.

Based on Figure 2, it can be seen that the 24 hours immersion treatment of *E. cottonii* explants into ZPT atonic solution at a concentration of 10 ppm generates the highest growth rate, namely 2.7% day^{-1}, followed by the 6 hour immersion treatment (2.6% day^{-1}), the 24 hour treatment (2.1% day^{-1}) and the lowest percentage was observed in the treatments for 0 hours and 18 hours (2.0% day^{-1}). The results of the analysis of variance (ANOVA) showed that soaking the thallus of seaweed in atonic ZPT solution at an immersion dose of 10 ppm did not have a significant effect on the growth in terms of relative weight (relative length rate) of the thallus seaweed (P>0.05; significance value in the ANOVA table of 0.087).

ZPT atonic immersion at a dose of 15 ppm. The relative weight growth rate of *E. cottonii* explants immersed in 15 ppm ZPT dose is presented in Figure 3.

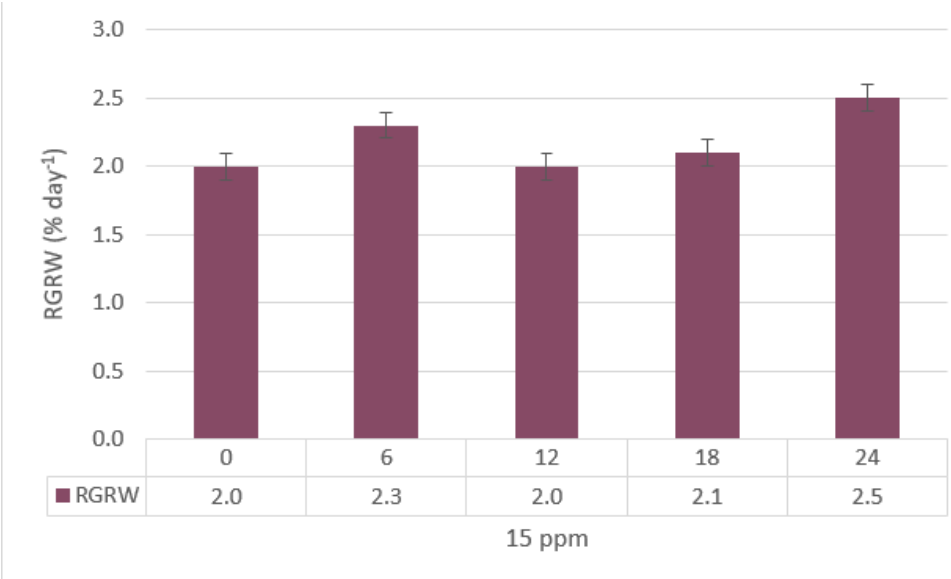

Figure 3. Relative weight growth rate of *Eucheuma cottonii* explants.

Based on Figure 3, it can be seen that the maintenance of *E. cottonii* explants by immersion into a 15 ppm ZPT atonik solution showed that the highest relative weight growth rate was observed in the treatment for 24 hours of immersion (2.5% day^{-1}), followed by 6 hours of treatment (2.3% day^{-1}), then by 18 hours of treatment (2.1% day^{-1}),

while the lowest relative weight growth rate was observed in the 12 hours and 0 hours treatments (2.0% day^{-1}). The results of analysis of variance (ANOVA) showed that the treatment of 15 ppm immersion dose in the atonic growth regulator (ZPT) solution had no significant effect on the relative weight growth rate of *E. cottonii* seaweed explants (significance value in the ANOVA table of 0.122).

The rate of increase in length of the explant. The initial length of the explants used in this study was 2 cm. This data was used as a benchmark for further measurements. Length observations were made for 28 days using a ruler. Then the research data were tested with the variance test (ANOVA) to determine whether the immersion treatment of the *E. cottonii* explants had a significant effect on the increase in explant length, up to 28 days of immersion in the atonic ZPT fertilizer.

ZPT atonic immersion at a dose of 5 ppm. The results of the length increase rate of ZPT atonic immersion explants at a dose of 5 ppm are presented in Figure 4.

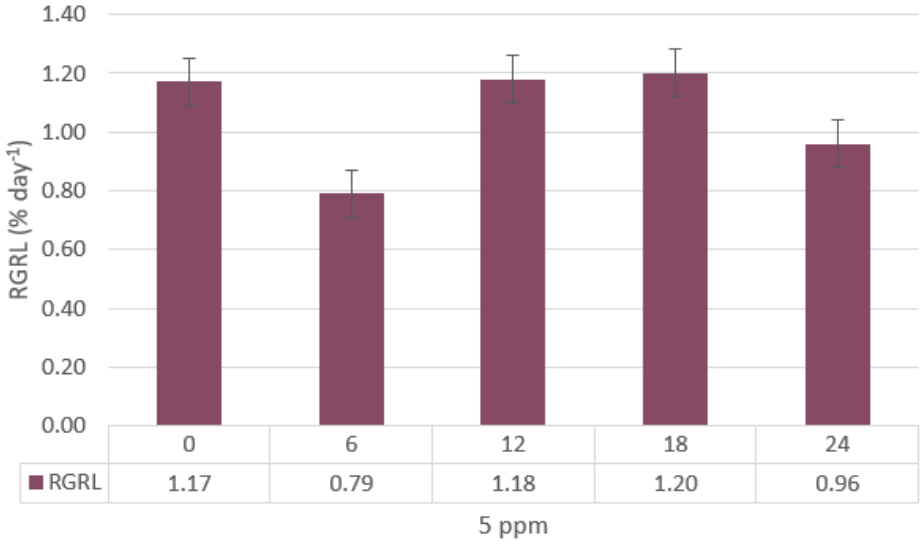

Figure 4. Relative length growth rate of *Eucheuma cottonii* explants.

Based on Figure 4, it can be seen that the maintenance of *E. cottonii* explants by immersion into a 5 ppm ZPT atonik solution showed the highest growth rate after 18 hours of treatment (1.20% day^{-1}), followed by the 12 hours treatment (1.18% day^{-1}), then by the 0 hours treatment (1.17% day^{-1}) and by the 24 hours treatment (0.96% day^{-1}), and the lowest was obtained in the 6 hours treatment (0.79% day^{-1}). The results of the analysis of variance (ANOVA) showed that the immersion into the 5 ppm atonic growth regulator (ZPT) solution significantly affected the relative length increase rate of *E. cottonii* seaweed explants (based on the significance value of 0.00, in the ANOVA table). The LSD test results showed that the value of the relative length growth rate for an immersion dose of 5 ppm at 0 hours immersion was significantly different from the 6 hours immersion. Soaking for 6 hours was significantly different of an immersion for 0, 12 and 18 hours. The 12 hours immersion was significantly different of the 6 and 24 hour immersion. The 18 hours immersion was significantly different of the 6 and 24 hours immersion. The 24 hours immersion was significantly different of the 12 and 18 hours immersion.

ZPT atonic immersion at a dose of 10 ppm. The results of the length increase rate of ZPT atonic immersion explants at a dose of 10 ppm are presented in Figure 5.

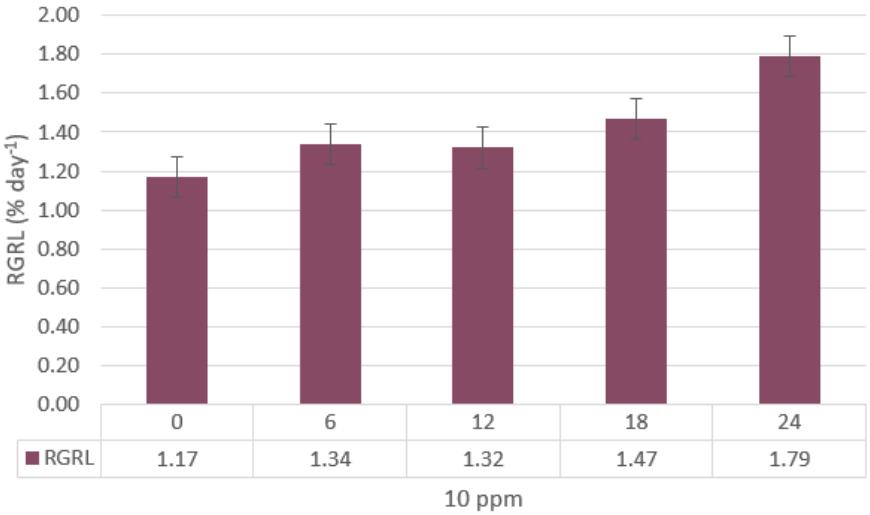

Figure 5. Relative length growth rate of *Eucheuma cottonii* explants.

Based on Figure 5, it can be seen that the maintenance of *E. cottonii* explants by immersion into a 10 ppm ZPT atonik solution showed the highest growth rate after 24 hours (1.79% day^{-1}), then after 18 hours (1.47% day^{-1}), while the control treatment had the lowest growth value (1.17% day^{-1}). The results of the variance test (ANOVA) showed that the different immersion durations in the atonic ZPT solution had a significant effect on the increase in relative length (the ANOVA significance value was 0.01). The LSD test results showed that the relative length growth rate for the 10 ppm immersion dose at 0 hours of treatment was significantly different from the treatments for 18 and 24 hours. Soaking for 6 hours was significantly different from the immersion for 24 hours. The treatment for 12 hours is significantly different from the immersion for 24 hours. The 18 hours immersion was significantly different from the treatments for 0 and 24 hours. The immersion for 24 hours was significantly different from the treatments for 0, 6, 12, and 18 hours.

ZPT atonic immersion at a dose of 15 ppm. The results of the length increase rate of ZPT atonic immersion explants at a dose of 15 ppm are presented in Figure 6.

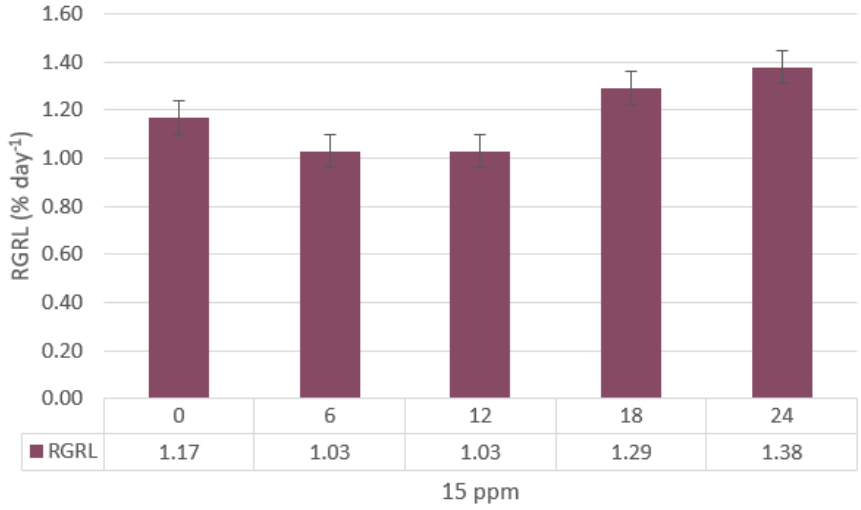

Figure 6. Relative length growth rate of *Eucheuma cottonii* explants.

Based on Figure 6, it can be seen that the maintenance of *E. cottonii* explants by immersion into a 15 ppm ZPT atonik solution showed the highest growth rate at 24 hours of treatment (1.38% day^{-1}), then at 18 hours of treatment (1.29% day^{-1}) and for 0 hours

of treatment (1.17% day^{-1}), and the lowest data value was observed after 12 and 6 hours of treatment (1.03% day^{-1}).

The results of the variance test (ANOVA) showed that the different immersion times in an atonic ZPT solution had a significant effect on the relative length increase (the significance value of ANOVA was 0.02). The results of the LSD test showed that the relative length growth rate for 6 hours of immersion at the dose of 10 ppm was significantly different from the 24 hours of immersion. An immersion for 12 hours was significantly different from the treatment for 24 hours, which in turn was significantly different from the immersion for 6 and 12 hours.

Survival rate (SR). The survival rates for the 5, 10 and 15 ppm dose are presented in Figure 7, Figure 8 and Figure 9.

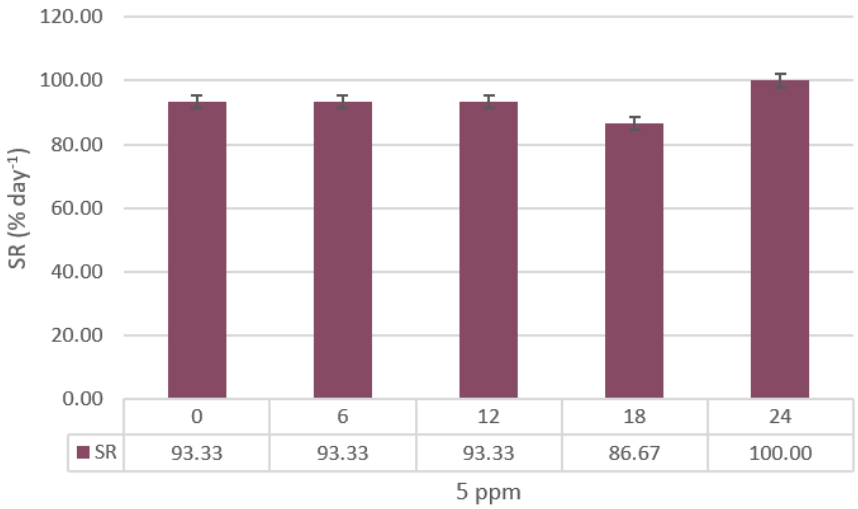

Figure 7. Average percentage of *Eucheuma cottonii* explant survival rate at an immersion dose of 5 ppm, for 28 days.

As it can be observed in Figure 7, the survival count for an immersion dose of 5 ppm, after 0 hours of treatment (control), was of 14 explants, after 6 hours of treatment it was of 14 explants, after 12 hours it was of 14 explants, after 18 hours of treatment it was of 13 explants and after 24 hours of treatment it was of 15 explants. The highest survival rate, with a value of 100%, was observed in the treatment E and the lowest survival rate was observed for 18 hours of treatment, with a value of 86.7%.

The survival rates for the 10 ppm dose are presented in Figure 8.

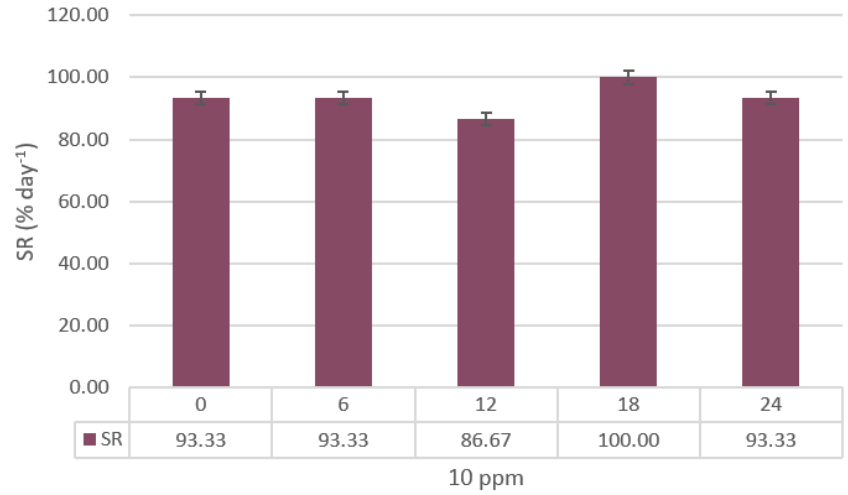

Figure 8. Average percentage of *Eucheuma cottonii* explant survival rate at a soaking dose of 10 ppm, for 28 days.

The survival count after the immersion at a dose of 10 ppm was of 14 explants after a treatment duration of 0 hours, 14 explants after 6 hours, 13 explants after 12 hours, 15 explants after 18 hours and 14 explants after 24 hours. The survival rate percentages for a dose of 10 ppm, were as follows: the highest survival rate (100%) was observed after 18 hours of treatment, while the lowest (86.67%) was observed after 12 hours of treatment, while in the control samples (without treatment) a survival rate of 93.33% was observed.

The survival rates for the 15 ppm dose are presented in Figure 9.

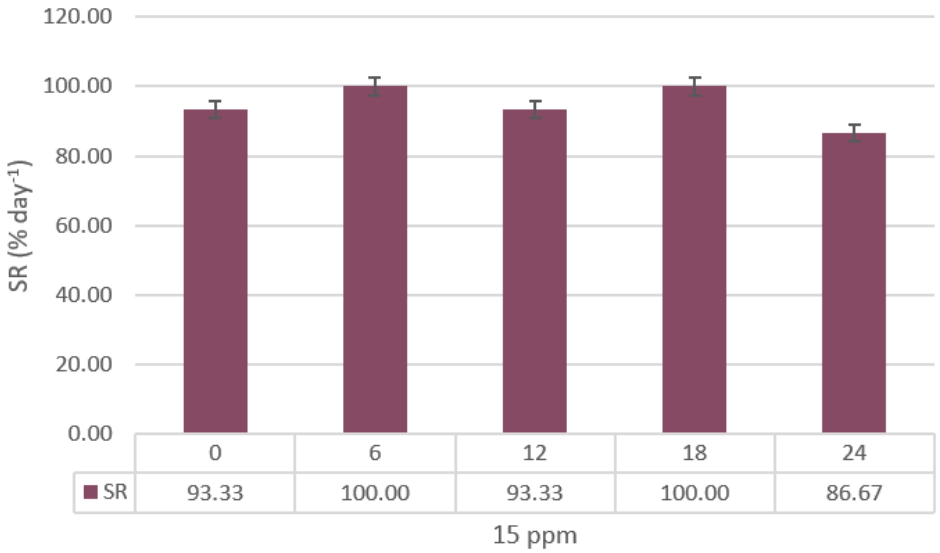

Figure 9. Average percentage of *Eucheuma cottonii* explant survival rates at a soaking dose of 15 ppm, for 28 days.

The survival count after the immersion at a dose of 15 ppm was of 14 explants after a treatment duration of 0 hours, 15 explants after 6 hours, 14 explants after 12 hours, 15 explants after 18 hours and 13 explants after 24 hours. Survival rates in treatments for 6 and 18 hours were the highest (100%), while the lowest rates were observed in the treatment E (86.67%) and in the control sample (93.33%).

Discussion

Weight growth of E. cottonii explants. Seaweed requires nutrients for growth and survival. According to Yuliana (2013) and Nursyam (2013), explant cell regeneration can form a complete thallus only if the explants live in a media that contains sufficient nutrients. The nutrients contained in liquid organic fertilizers, such as: C-organic, N, P_2O_5, K_2O, CaO, Mg, SO_4, trace elements (Cu; B; Mo; Mn; Zn and Co), 17 amino acids and 3 growth hormones (cytokinin, auxin and gibberelin), have functions that support the growth of *E. cottonii*. Elements like S, Ca, Na, K, Mg, Fe, Cu, Zn, N, C, O, and Mn are important for the algae growth (Kawaroe et al 2012).

In this study, the highest relative weight gain rates were: 2.2% day^{-1} after 18 hours of immersion at a dose of 5 ppm, 2.7% day^{-1} after 12 hours of immersion at a dose of 10 ppm and 2.5% day^{-1} after 24 hours of immersion at a dose of15 ppm. Thallus with a high growth value is likely due to an optimal absorption of nutrients from growth stimulating substances (ZPT). Silea & Masitha (2006) argued that the growth hormones (cytokinin, auxin and gibberelin) can accelerate the growth of new shoots in seaweed. In this study, a decrease in the average weight of explants occurred on day 21, presumably due to the reduction of the nutrients in the growth stimulants.

Length growth of E. cottonii explants. The initial length of the thallus used in the study was 2 cm, but the thallus length growth in the last week of the study, at the immersion dose of 5 ppm, decreased in the 6-hour immersion treatment, because the

thallus experienced death (marked by a white color), probably due to the variability of the plants ability to absorb the nutrients. In this study, the length growth of the thallus was very significant compared to the weight growth of the thallus, because the ZPT was designed to stimulate the growth of roots and shoots in plants. The thallus growth is also influenced by the its form. The number of thallus that grew at low density had a larger shape than the number of thallus that grew at high density, but had a relatively bigger size due to the process of nutrient competition. The same result was found by Yuliyana et al (2013), contrarily to the land plants, seaweed does not have roots to absorb nutrients, so the availability of nutrients around the thallus will greatly affect its growth. Lack of nutrients will usually cause stunted growth in seaweed. In *Caulerpha lentillifera*, the low growth is thought to be due to insufficient nutrient availability, the addition of fertilizers being expected to remediate this issue (Yuliyana et al 2013). Adding fertilizers or other growth stimulants to the seaweed living media provide important nutrients for the growth of seaweed (Wahidah 2011).

Water quality. The growth rate of explants weight and length during the 28 days of the study could not be separated from various external factors, including temperature, salinity, and light. Although many factors influence the growth of seaweed, such as currents, turbidity, depth and the substrate, this research is carried out at the laboratory scale, in vitro, so that the most influential factors are the temperature, salinity, light, nutrients and water quality. The temperature of the maintenance medium during the study was in the optimal range (24-26°C) for the growth of *E. cottonii*. Irawati et al (2016) cultivated *E. cottonii* at temperatures ranging from 23-26°C. Arisandi (2011) stated that *Kappaphycus alvarezii* marine algae explants cultured in vitro at a low temperature (20°C) and at a high temperature (40°C) can still live and match the growth patterns of *K. alvarezii* thallus cultured at the optimal temperature range (25 to 30°C). This is presumably because *K. alvarezii* has a fairly good ability to adapt to these temperature conditions, up to a certain time limit. Salinity measurements carried out during the maintenance period indicated 30 ppt, a still optimal value for the development of *E. cottonii* seaweed. Marisca (2013) stated that seaweed can grow well in waters with a salinity of 30-37 ppt.

The light intensity obtained during the study was around 300 lux. *E. cottonii* was still able to grow within this range of light intensity. This is in line with the research of Nurfebriani et al (2015), which states that *C. lentillifera* can grow at a light intensity higher than 300 lux. Several studies show that *Eucheuma serra* requires a temperature of 24-28°C for growing in vitro (Lideman et al 2011), while *Kappaphycus* sp. (Sumba strain) requires a temperature of 22-23°C and a sunlight intensity between 122-167 µmol photons m^{-2} s^{-1} (Lideman et al 2014).

Conclusions. Nutrient needs can be provided through the provision of liquid fertilizers. Besides that, the growth of seaweed is also very dependent on the conditions of air quality parameters such as temperature, salinity, light, currents, turbidity, air depth, and the bottom water substrate. Of the three treatments of ZPT immersion doses in this study, the highest relative weight gain rate was 10 ppt, namely 2.7% day^{-1} after 12 hours of immersion and the highest relative length growth rate was 10 ppm immersion dose for 24 hours of immersion with a value of 1.79% day^{-1}.

Conflict of interests. The authors declare no conflict of interest.

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