Performances of reproductive phases and carrageenan concentration of Kappaphycus cottonii (Weber Bosse) Doty in a non-cultivated environment (study case area: Likupang Minahasa coastal area)

by Rully Tuiyo

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Abstract. *Kappaphycus cottonii* is a red seaweed species from the Rhodophyta phylum, which can be developed for cultivation considering the large variety of species spread in tropical areas. The low natural performance of *K. cottonii* is considered one of several factors causing the species unfamiliarity with cultivation. This study aims to provide a relationship pattern between the emergence of *K. cottonii* reproductive phase and environmental conditions also the percentage of dry weight carrageenan in non-cultivated areas. Red seaweed *K. cottonii* samples, which grow naturally in Likupang Minahasa coastal area, were collected monthly for one year. Nitrate and rainfall positively correlate with individual algae's reproductive phase appearance, while temperature and salinity negatively correlate. The results also showed that *K. cottonii* was only found in the gametophyte and carposporophyte phases. Neither female gametophyte nor tetrasporophyte phases were not found. The number of individuals and carrageenan content measured from gametophyte phase samples outnumbered the carposporophyte. However, further study with various environmental parameters needs to be conducted to provide comprehension data related to reproductive phase, carrageenan content, and the natural ecosystem in which *K. cottonii* live and grow.

Key Words: Eucheuma, *Kappaphycus alvarezii*, red algae, Rhodophyta.

Introduction. Rhodophyta, commonly named red seaweed or red macroalgae, is well known widely and officially has various species. *Kappaphycus* and *Eucheuma* are two red seaweeds genera that belong to the phylum, which are familiar to be cultivated (Zuccarello et al 2006; Hung & Trinh 2020) in Asia regions. Both genera are commonly developed and universally processed as food materials (Agusman et al 2014; Anam et al 2020), medicine, and cosmetic products (Kasmiati et al 2021).

For more than five decades, seaweed cultivation has been applied in Indonesia. *Kappaphycus* and *Eucheuma* are currently becoming the two largest species produced globally (Kambey et al 2020). The identification and naming of *Kappaphycus cottonii*, *Eucheuma cottonii*, and *Kappaphycus alvarezii* as red seaweed species in several studies seems inconsistent and often interchangeable. It is due to the development of scientific studies successfully revealing many new things such as morphology, anatomy, histology, habitat, and including the content of carrageenan types.

E. cottonii (Ili Balqis et al 2017; Diharmi et al 2019; Sari et al 2019; Sari et al 2020) contains kappa-carrageenan (κ-carrageenan) therefore occasionally it is reported the similar species as *K. cottonii* that is unfortunately currently accepted by public with the trade name "cottonii" (Meinita et al 2012; Wijayanto et al 2019). In fact, Dumilag et al (2018) revealed that *K. cottonii* in the Philippines has the richest quantity of haplotypes for any *Kappaphycus* species. The phylogenetic trees showed that a lineage of *K. cottonii* exclusively separated from *K. alvarezii*, *K. inermis*, *K. malesianus*, and *K. striatus*. Tan et al (2014) also reported that *K. cottonii* and *K. alvarezii* are two different and separated species. Additionally, *Kappaphycus* and *Eucheuma* were subtle and confusing to be distinguished due to their morphological plasticity, lack of adequate characters for speciation purpose, and their common commercial name (Zuccarello et al 2006). However, *E. cottonii* was later taxonomically synonymous with *K. alvarezii* (Bakar et al 2017; Diharmi et al 2019; Dolorosa et al 2019).

Red algae certainly have an essential ecological role in ecosystem sustainability. Therefore, cultivation practices continue to be promoted to meet national and international market demands without threatening the availability and sustainability of the variety of species available in nature. The attainment of cultivation is primarily determined by managing environmental conditions that support algae's growth and survival.

It is different from other types of red algae that are heavily cultivated (Eggertsen et al 2020; Hendri et al 2020; Ndobe et al 2020; Pires et al 2021) and equipped with sufficient scientific information regarding the feasibility of waters supporting the algae survival (Kalhoro et al 2017; Serdiati & Widiastuti 2012; Khasanah et al 2016; Kumar et al 2020) as well as other relevant data (Farah et al 2020; Bouanati et al 2020; Thien et al 2020), *K. cottonii* species still lacks scientific data. In fact, in Indonesia and several tropical and sub-tropical countries, *K. cottonii* species potential is entirely prospective. This study provides data on the pattern of the relationship between the emergence of the reproductive phase, environmental conditions and also the percentage of carrageenan in non-cultivated areas.

Material and Method

Sampling area and parameters. The red algae *K. cottonii* growing naturally in Likupang coastal area, Minahasa District, North Sulawesi, Indonesia (Figure 1) was sampled monthly for one year from August 1998 until July 1999. For each specimen, its reproductive phase and carrageenan content were determined. Water temperature, salinity, and dissolved nitrates were also measured in the area along with the algae sampling. Meanwhile, the rainfall data was obtained from the Meteorology, Climatology, and Geophysical Agency report of the North Sulawesi Province (BMKG 1998; BMKG 1999).

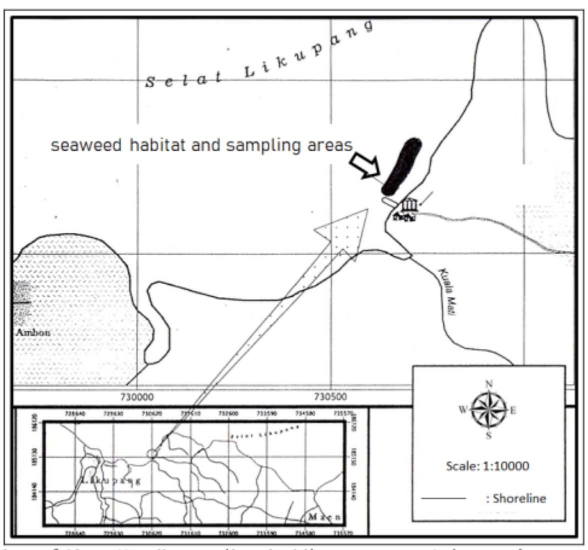


Figure 1. The location of *K. cottonii* sampling in Likupang coastal area (generated with ArcGIS).

Algae sampling technique. Algae sampling was carried out during the lowest tide using the line transect method (Krebs 1989). Two transects measuring 200×5 meters, separated by 10 meters, were placed parallel to the coastline ±25 meters from the shoreline. Individual *K. cottonii* algae at a depth of ±50 cm above sea level in the transect were collected, counted, then their growth phase and carrageenan content were determined.

Reproduction stage determination. Determining an individual's life phase was figured out by cutting transversely and longitudinally numerous protuberances or nodules on each collected sample's thallus's surface. The individual was classified as a gametophyte phase when only either male or female spores appear. If a mature cystocarp was found, then the individual was categorized as a carposporophyte phase. If tetraspores were found in the tetrasporangia, it was categorized in the tatrasporophyte phase.

Carrageenan content. Carrageenan was extracted using a water-extraction method modified from (Winarno 1990). Algae flour (A gram) was extracted with hot water as much as 300 ml (1:60), at a temperature of 85-95°C for 4 hours in an alkaline condition (pH 8-9). The extract was filtered with a chiffon layer. The filtrate obtained was then concentrated to ±150 ml through a heating process. The residuum was added with 200 ml of 95% alcohol solution then soaked overnight for carrageenan precipitating purpose. The formed residue then was refiltered with a sheer fabric. Finally, the precipitate was dried in an oven at 60°C for 8 hours. Dry carrageenan was then weighed (B gram). The proportion of carrageenan was calculated as follows:

$$\text{Carrageenan percentage (\%)} = \frac{\text{B (gram)}}{\text{A (gram)}} \times 100\%$$

Data analysis. All data was descriptively presented. Correlation analysis was applied to determine the relationship correlation level between measured environmental parameters and individuals presence in a particular reproductive phase. The significant differences of carrageenan content between gametophyte and carposporophyte reproductive phases were determined using the statistical t-test.

Results

Gametophyte and carposporophyte life stages. Morphologically, the male *Kappaphycus cottonii* in a gametophyte phase was characterized by a deformed thallus, irregular branching, white, green, brown in colors, and slightly open in the middle of the elongated or rounded groove (Figure 2a). The grooves' longitudinal section found spermatangia on the thallus cortex (Figure 2b).

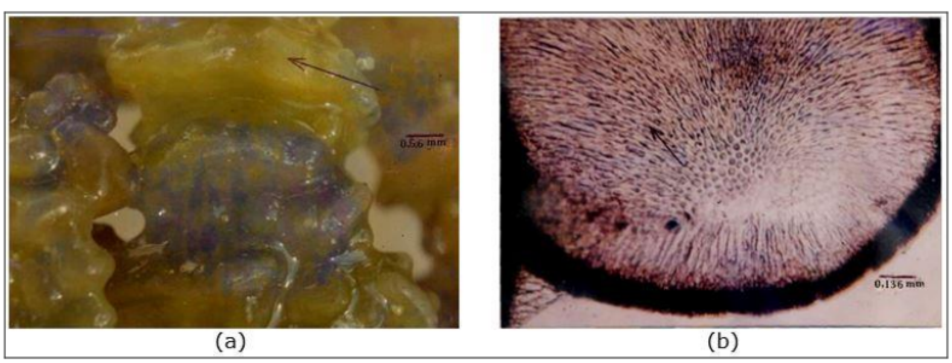
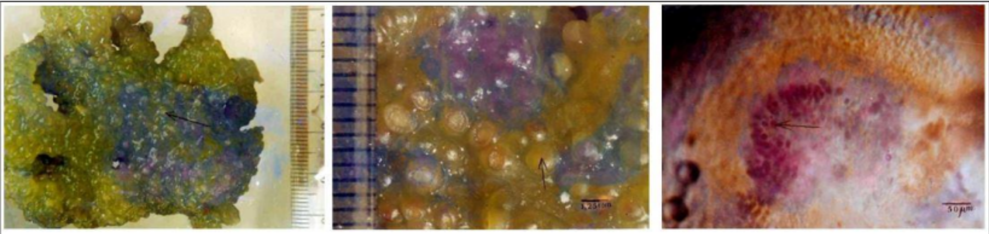


Figure 2. (a) Grooves (b) cross-section of spermatangia in the male gametophyte sample

In a carposporphyte *K. cottonii* phase, yellow or red colors observed on the groove tip's longitudinal sections indicate a cystocarp. It was located on and below the thallus surface (Figure 3a). The male and female gametes united to form a carposporophyte structure covered with pericarp. The carposporophyte and pericarp established a cystocarp organ characterized by bulges finding on and under the thallus surface. The spores were released through the tip of the mature cystocarp (Figure 3b). Carpospores (Figure 3c) were also observed in longitudinal sections of the cystocarp protrusions on and under the thallus cortex.



(a) (b) (c)

Figure 4. (a) cystocarp, (b) tip of mature cystocarp and (c) carpospore in the *K. cottonii* carposporophyte sample.

Temperature and Kappaphycus cottonii performance. Water temperature in the natural habitat of *K. cottonii* for the one-year observation ranged from 18 to 35°C. The fluctuation of temperature with the number of *K. cottonii* shows a contradictory pattern. The increasing of water temperature was in line with a decrease of *K. cottonii* numbers. In both phases, the least number of seaweeds was found in July, with the maximum water temperature of 35 °C (Figure 4). The correlation between male gametophyte and carposporophyte individual numbers with water temperature was -0.8 and -0.3, respectively.

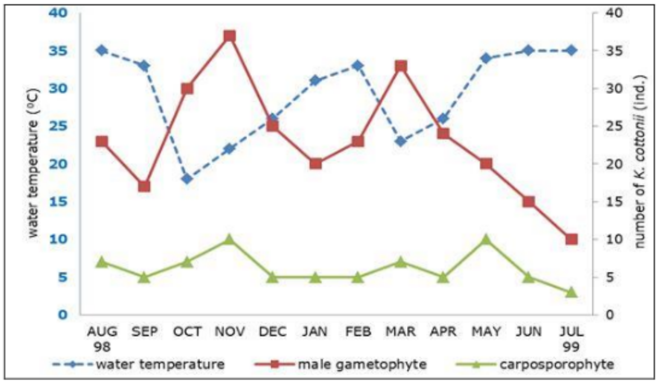


Figure 4. A relationship pattern between temperature and the number of *K. cottonii* in nature.

Salinity and Kappaphycus cottonii performance. The salinity of the water during the observation ranged from 18 to 35‰. The trend of water salinity and the number of individuals found in nature is also reversed. The rise in salinity does not follow by an increase in *K. cottonii* individuals' number. In October, the number of *K. cottonii* was relatively high in the lowest salinity environment. Conversely, in July, when the water salinity was highest, the *K. cottonii* performed in the lowest number (Figure 5). The correlation between male gametophyte and carposporophyte individuals with water salinity was -0.9 and -0.4, respectively.

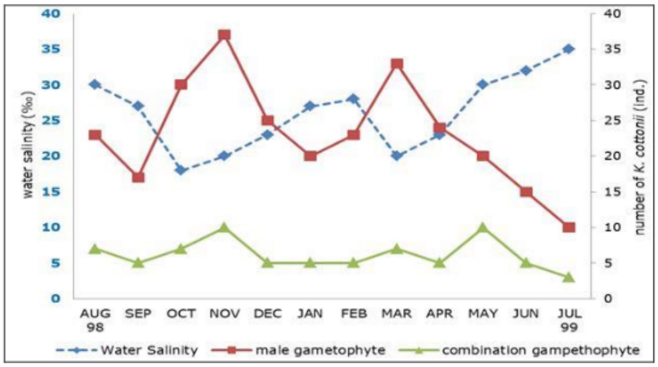


Figure 5. A relationship trend between water salinity and the number of *K. cottonii* in nature.

Rainfall and Kappaphycus cottonii performance. The pattern of fluctuation in rainfall throughout the year is in line with changes in the individual performance of *K. cottonii* (Figure 6). The higher the rainfall, the greater the number of *K. cottonii* individuals. Equally, the decrease in rainfall indicates a decline in the number of *K. cottonii*. The correlation between individual male gametophyte and carposporophyte with rainfall was 0.76 and 0.57, correspondingly.

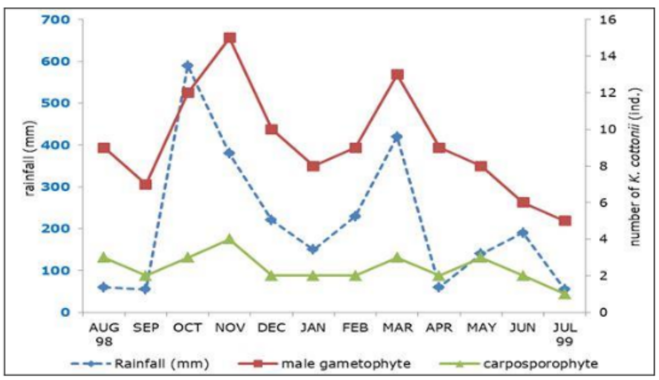


Figure 6. A relationship pattern between rainfall and the number of *K. cottonii* in nature.

Nitrate and Kappaphycus cottonii performance. Naturally, the trend of changes in nitrate content in the waters is followed by K. cottonii individuals' appearance pattern. However, the increased nitrate concentration in June indicated a decrease in the number of *K. cottonii* (Figure 7). The range of water salinity of natural habitat in this study ranged from 0.06 to 0.69 ppm. The correlation level between individual male gametophyte and carposporophyte with water temperature was 0.79 and 0.60, consecutively.

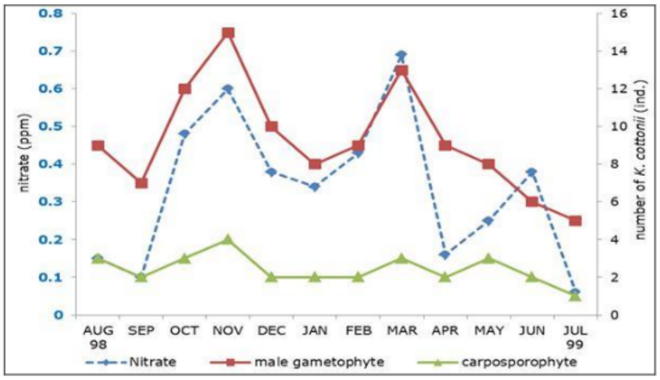


Figure 7. A relationship trend between nitrate content and the number of *K. cottonii* in nature.

Carrageenan concentration. The average carrageenan content of male gametophyte and carposporophyte of *K. cottonii* during the study were 2.64 grams (52 % dry weight) and 2.70 grams (54% dry weight), correspondingly. The percentage of male gametophyte outweighed the concentration of carposporophyte in September, December, January, March, June, and July. Meanwhile, the carrageenan content of *K. cottonii* carposporophyte was higher than that of gametophyte in August, October, November, February, April, and May (Figure 8). However, the statistical t-test did not show a significant difference (p-value > 0.05).

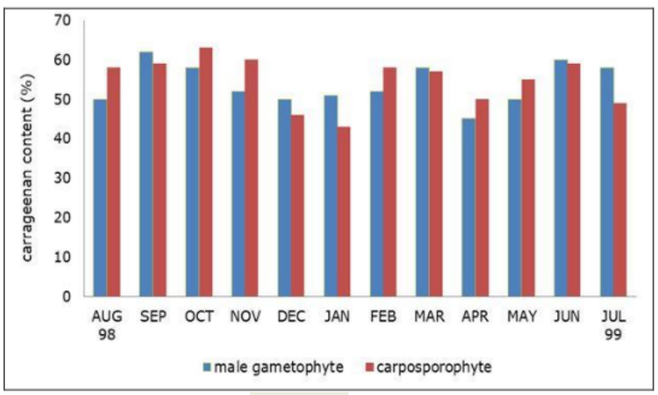


Figure 8. Comparison of carrageenan content of *K. cottonii* between male gametophyte and carposporophyte phases in nature.

Discussion. According to Doty (1988), *K.cottonii* thalli are compressed to flattened above the basal segment; prostate, irregular in form, or with linear segments sometimes in heads, or decumbent, or displacement from the initial position, with irregularly appearing protrusions or branches. A similar anatomical description also has been reported by Trono (1992) that the thalli of *K. cottonii* form large, crust-like clumps firmly attached to solid substrates by hapters arising from the thallus's undersurface. It may also consist of compressed irregular branches, attached by undefined haptera forming slightly amorphous fronds. A cross section of the thallus displays a medulla consisting of big round cells without a central nucleus of rhizoidial cells.

The environmental factors determining the survival of *K. cottonii* in nature observed in this research were water temperature, salinity, rainfall, and nitrate. This research's data cannot represent the existing condition of *K. cottonii* in Likupang Minahasa waters at present, as the character and condition of the waters must have changed over time. However, this study's results still can provide an overview of the correlation between each of four environmental factors and *K. cottonii* reproductive phases and its content. This information might be considered in further studies regarding the level of suitability and identification of the cultivation area's physical and chemical conditions. Data on this species is still minimal even though *Kappaphycus* is more diversified with a larger number of genotypes, strains, and unique species to Southeast Asia (Tan et al 2013).

Water temperature is one of the critical factors in adaptable life processes, development, reproduction, and seaweed distribution in waters. The optimum temperature for seaweed growth varies by species and geographic location. Algae in Arctic region grows optimally in ranging temperature of 0–10^{o}C. For algae in cold temperate regions, the optimal temperature ranges from 20 to 15^{o}C, while in warm temperate areas, it is ranging between 10 and 20^{o}C, and for tropical algae ranges from 15 to 30^{o}C (Bird et al 1986 in Aris et al 2020). Extreme temperature fluctuations can cause ice-ice disease attacks (Msuya & Porter 2014). Water temperature is closely related to the season in an area. Furthermore, the season is likewise influential water salinity.

Each algae species has a specific salinity tolerance for living and growing optimally. Variation in salinity is a factor that influences seaweed growth (Periyasamy et al 2014. Salinity and water temperature, and general season have been associated with response to seaweed resistance to disease. In dry season conditions, cultured seaweeds are susceptible to epiphytes. Vairappan (2006) informed that an epiphytic filamentous red algae burst linked with water temperature and salinity extreme alteration during the season. In the dry season, nitrates, salinity, and brightness contribute to seaweed production (Aris et al 2020). Therefore, seaweed production is higher in the rainy season than in the dry season. Hayashi et al (2017) also reported that carrageenophytes production in tropical and sub-tropical regions depends on the weather to facilitate increasing productivity and resilience of carrageenophyte seedlings against environmental instability.

Nitrogen is a significant element that often becomes a limiting factor for the growth of aquatic organisms. Nutrient deficiency stunted the marine algae growth. Hence, adding nutrients through the fertilization process is vital for the practice of seaweed cultivation. Tuwo et al (2020) informed that 0.9-3.5 ppm nitrate concentration is proper for *K. alvarezii* farming. Cahyani et al (2020) revealed that nitrate concentrations in *K. alvarezii* nursery cultivation's experimental results using tissue-cultured seedlings ranged from 0.41 to 0.61 mg/L. Meanwhile, a slightly similar concentration also was shown in this *K. cottonii* environment's nitrate ranging from 0.06 to 0.69 mg/L. Indeed, nitrate in waters cannot be used as a single factor for judging optimal water quality parameters for *K. cottonii* and other algal species' survival.

The benefits of carrageenan are comprehensively and widely known in the food, cosmetic, and pharmaceutical industries (Necas & Bartosikova 2013). Nonetheless, statistics on the carrageenan content of *K. cottonii* species are rarely published. Based on *K. cottonii* samples' measurement results in this study, the carrageenan content of male gametophyte *K. cottonii* species was 50-60%, while the carposporophyte was 42-62% dry weight. Therefore, it is suspected that there is a link between the carrageenan content performances of *K. cottonii* and the reproduction phase of individual red algae gametophytes. Talau (1998) reported that the carrageenan content in *K. alvarezii*, *K. cottonii*, and *E. spinosum* ranged from 46-48.6%, 53.2-56.7%, 40.6-43.3% dry weight, correspondingly. Naturally, it is a must to consider interactions with other environmental factors. Besides, there is no specific pattern showed by the carrageenan percentage average of a particular reproductive phase monthly during a year observation. It can be seen from the results that the carrageenan content of *K. cottonii* in the gametophyte phase was high in September, March, May, and June and low in August, October, November, December, April, and July. Meanwhile, its concentration of the carposporophyte phase was higher in September, October, February, May, and June compared to August, November, December, January, March, April, and July.

Conclusions. This research has revealed that in a non-cultivated natural condition, nitrate and rainfall positively correlate with individual algae's appearance, while temperature and salinity have a negative correlation. The results also showed that *K. cottonii* was only found in the gametophyte and carposporophyte phases. Neither female gametophyte nor tetrasporophyte phases were not found. The number of individuals and carrageenan content in the outnumbered gametophyte phase of the carposporophyte phase.

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

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