

A model experiment on discoloration and recovery of *Pyropia suborbiculata*

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Abstract

Low nutrient levels have been shown to cause deterioration in the quality of *Pyropia*, resulting in discoloration and detachment of the frond. In this study, we investigated the critical concentration of nitrogen that causes discoloration of *P. suborbiculata*, the effective period, and the appropriate nutrient concentration for the recovery of the discolored thalli. To calculate the critical nutrient concentration that causes the discoloration of the thalli, a culture experiment was conducted at 5 DIN concentrations (30, 50, 70, 90, 110 $\mu\text{g L}^{-1}$) for 14 days. To test recovery of discolored thalli, a culture experiment was conducted at 4 $\text{NH}_4\text{-N}$ concentrations (10, 20, 40, 60 mg L^{-1}) for 96 hours. After 8 days culture, discoloration of thalli occurred at nutrient concentrations between 70 and 90 $\mu\text{g L}^{-1}$ of dissolved inorganic nitrogen. When exposure to low nutrient levels lasted for less than 10 days, discoloured thalli recovered their colour within 72 hours when supplied with $\text{NH}_4\text{-N}$ at 20 ~ 40 mg L^{-1} . In this study, we revealed that the discoloration of *Pyropia* caused by low nitrogen concentrations on seaweed farms can be restored by supplying nitrogen at an appropriate concentration.

Introduction

Pyropia / *Porphyra* are one of the most commercially valuable macroalgae worldwide, with an annual production of approximately 3 million metric tons in 2020 and an economic value of 2.8 billion USD (FAO 2023). Korea produces 17.6% of the global *Pyropia* production, accounting for 18.0% of the global value (FAO 2023). While the growth rate of global seaweed production was 6.2% yr^{-1} (Duarte et al. 2022) between 2000 and 2018, the growth rate of Korean *Pyropia* farming has been significantly higher at 9.3% yr^{-1} .

Pyropia has been cultivated for several hundred years in Korea and has become one of the most successful industries in Korea, Japan and China (Mumford and Miura 1988; Pereira and Yarish 2008; Kim et al. 2017; Hwang and Park 2020). *Pyropia* vary their growth patterns in relation to various biological, physiological, ecological and environmental factors. Recently, abnormal weather conditions around culture farms have caused significant changes in seawater temperature, light, pH, salinity and nutrient concentrations (Kim et al. 2019; Kakinuma et al. 2017; Sasuga et al. 2018), which have reduced the productivity of *Pyropia* industry. If stable *Pyropia* production cannot be achieved by adapting to changing environmental conditions, the industrial foundation of the seaweed farming industry will inevitably be shaken.

Reclamation in the coastal area, particularly the blocking of sources of freshwater inflow to the marine environment, has reduced the amount of organic matter and inorganic nutrients available to seaweed farms. Nutrient depletion has been linked to discoloration of seaweed, turning fronds to a yellowish-white color rather than the normal deep red and can also cause fronds to detach from culture ropes (Amano and Noda 1987; Sakaguchi et al. 2003; Nishikawa and Yamaguchi 2006). Discolored seaweed has a very low value and therefore results in a significant loss of income for seaweed farmers.

In the early 2000s, the investigations into *Pyropia* discoloration in Japan found that blooms of diatoms in winter, reduced river inflows, and reduced nutrients due to the movement of high-salinity and low-nutrient water masses (Hori et al. 2008; Ishii et al. 2008; Tanda and Harada 2012) could all lead to discoloration. In Korea, discoloration appears to be caused by reduced levels of inorganic nutrients, and in particular, nitrogen deficiency was reported as the main cause (Kang and Koh 1977; Lee et al. 2018).

Pyropia suborbiculata (Kjellman) J.E. Sutherland, H.G. Choi, M.S. Hwang & W.A. Nelson is distributed in Korea, Japan, China, Taiwan and the Philippines (Sutherland et al. 2011). It is widely distributed throughout the south coast, west coast and Jeju Island in Korea (Hwang et al. 2013) and is used for aquaculture in some parts of the Jeollnamdo region. The characteristic of this species is that it is a cultivar that can be harvested much later than *P. dentata*, which is preferred in the domestic market in Korea.

Therefore, to reduce the potential for future catastrophic losses of *Pyropia* production in Korea, it is important to understand the nutrient conditions leading to discoloration and the potential to restore the color to *P. suborbiculata* by providing additional nutrients. This paper contributes to quality improvement and stable production by identifying the critical conditions that result in the discoloration and subsequent recovery of *P. suborbiculata*.

Materials And Methods

Algal sampling

Thalli of *P. suborbiculata* were collected from a commercial seaweed farm at Hwagori, Doripo, Muangun, Jeollanamdo (35°08'15" N, 126°20'58" E) on the southwestern coast of Korea in January 2022. The environmental conditions at the time of sample collection of the *Pyropia* farm were: seawater temperature 9.8°C, flow rate 0.5m sec⁻¹, turbidity 8 ppm, pH 7.85, salinity 33.5 psu, DIN 0.252 mg L⁻¹ and PO₄-P 0.027 mg L⁻¹. These conditions were appropriate for the normal growth of *Pyropia*. The thalli had been cultured at the farm for 15 days and randomly selected from the culture nets. They were visually free from macroscopic attached organisms. After collection, the thalli were transported to the laboratory in an insulated box under humid conditions. Transport took approximately 1 hour. Thalli with an average diameter of 3 cm were selected in the laboratory, and washed with sterile, filtered seawater. All experiments included 3 replicate flasks (500 mL, Pyrex) each containing 20 individuals. Artificial seawater (ASW, Tropic Marine®; Dr. Biener Aquarientchnik, Germany) with no inorganic nitrate or phosphate enrichment (detection levels were 0.5 and 0.2 µmol, respectively) was the base media used in the experiment. Thalli were kept in aerated ASW for 3 days at a light irradiance of 100 µmol m⁻² s⁻¹. before the discoloration experiments To prevent or delay spore formation in blades, the light level was reduced to 80 µmol m⁻² s⁻¹, during the discoloration experiments.

Discoloration induction

To calculate critical nutrient concentration that causes the discoloration of the thalli, the culture experiment tested 5 DIN concentrations (30, 50, 70, 90, 110 $\mu\text{g L}^{-1}$) for 14 days. Each DIN concentration was prepared in the ratio of $\text{NO}_2\text{-N}$ (10%), $\text{NO}_3\text{-N}$ (40%), $\text{NH}_4\text{-N}$ (50%). $\text{PO}_4\text{-P}$ was added at 30 $\mu\text{g L}^{-1}$ to each experiment. These nutrient concentrations in these experiments are typical concentrations found in coastal *Pyropia* seaweed farms (Korea Marine Environment Monitoring Network). The algae were placed in aerated 500 mL flasks with 350 mL of incubation medium during the experiments. Culture conditions were 8°C, 80 $\mu\text{mol m}^{-2} \text{s}^{-1}$, 10:14h L:D and aeration 240 mL min^{-1} . The temperature was controlled using a multi thermo incubator (EYELA Model MTI-202B, Japan, Mean $\pm$ 0.1°C). To check the change in nutrient concentration, 10 mL of culture medium was analyzed every 24 hours. The culture medium was changed each day. Three replicated flasks were used for each nutrient medium concentration. The color of the thalli, photosynthetic pigment, photosynthetic yield, and cell structure were analyzed at 48 hours intervals during the culture period. The experimental period for the discoloration induction was set to the normal production cycle of *Pyropia*.

Recovery induction

To test the recovery of discolored thalli, *Pyropia* samples on the 6, 8, 10, 12 and 14 days after discoloration were collected for the color recovery experiment. The experiment was conducted at 4 $\text{NH}_4\text{-N}$ concentrations (10, 20, 40, 60 mg L^{-1}) for 96 hours. $\text{PO}_4\text{-P}$ was added at 30 $\mu\text{g L}^{-1}$ to each experimental group. Culture conditions were 8°C, 80 $\mu\text{mol m}^{-2} \text{s}^{-1}$, 10:14h L:D and 240 mL min^{-1} of aeration. The color of the thalli, photosynthetic pigment, photosynthetic yield, and cell structure were analyzed at 48 hours intervals during the culture period. The experimental period for the color recovery was set to the normal production cycle of *Pyropia*.

Determination of discoloration and/or recovery

-Color

In order to find out the color change of thalli, the chromaticity of the plants was measured using a colorimeter (CR-1000, Minolta, Japan) and the reading from thalli were compared to analyze the chromaticity difference of seaweed. For measurement of chromaticity, the colorimeter was used after standardization or calibration each time. The thalli were spread out one by one and measured more than 5 times. A standard calibration plate used at this time had an L value of 96.95, a value - 0.03, and b value 1.42. Chromaticity was expressed as an L^* value indicating brightness, an a^* value indicating green from red, and a b^* value indicating blue from yellow. The color of the thalli was measured at 48 hour intervals in the discoloration experiments and at 24 hour intervals in the recovery experiments.

-Photosynthetic pigment

The pigments analyzed in thalli were chlorophyll a (Chl. a), phycoerythrin (PE), and phycocyanin (PC), which are the main pigments of photosynthesis. Chl. a was extracted by placing the dried seaweed in 80% acetone solution, grinding in a mortar, and then extracted by repeated centrifugation at 15,000 $\times$ g for 20

minutes. The extracted solution was standardized to 100 mL, and then the absorbance was measured at 663 nm using a spectrophotometer (U-1100 Spectrophotometer, Hitachi, Japan). Chl. a was calculated using the method of Meeks (1974) as 'Chl. a ($\mu\text{g/mL}$) measured at = $E_{663\text{ nm}} \times 11.9$ '. PE and PC were extracted by placing the dried seaweed in distilled water, grinding them in a mortar, and centrifugation at $35,000 \times g$ for 30 minutes. After the extracted solution was standardized to 100 mL, absorbance was measured at 565 nm and 615 nm, respectively, using a spectrophotometer. The contents of PE and PC were calculated according to Fujiwara (1955) and Hattori and Fujita (1959), 'PE ($\mu\text{g/mL}$) = $E_{565\text{ nm}} \times 124.3 - E_{615\text{ nm}} \times 62.4$, PC ($\mu\text{g/mL}$) = $E_{615\text{ nm}} \times 171.2 - E_{565\text{ nm}} \times 2.25$ ' was calculated. The photosynthetic pigments in the thalli were analyzed at the start of the discoloration experiment and after 14 days of culture.

-Photosynthetic yield

We estimated the tolerance of *P. suborbiculata* to DIN concentration by measuring the chlorophyll fluorescence of the thalli using a portable modulated fluorometer (Mini-PAM, Walz, Germany). According to the DIN concentration groups leaf clips (DLC-8, Walz, Germany) were used. Measurements were made on 3 replicate dishes containing 20 fronds for each DIN concentrations. The ratio of variable (Fv) to maximal fluorescence (Fm), Fv/Fm, after a 5-minute dark acclimation period was defined as the optimal quantum yield (Schreiber et al. 1986), and was measured after 14 days of exposure to different DIN treatments.

-Vacuole to cell area

An optical microscope (Carl Zeiss, Germany) was used to observe and photograph the cell structure of fronds after 14 days of exposure to different DIN treatments.

Statistical analyses

A paired t-test and 95% probability level was used to determine whether there was a statistically significant difference in the correlation between nutrient concentrations and discoloration, recovery, and time. Before statistical analyses, the percentage data for discoloration and recovery of the thalli was normalized by arcsine transformation (Parker 1979).

Results

Discoloration induction

The redness (a) and yellowness (b) values did not change during the culture period at a DIN concentration of $90\text{ }\mu\text{g L}^{-1}$ or higher. However, the a value decreased and the b value tended to increase with culture period (Fig. 1). Thalli cultured at some DIN concentrations for 14 days began to show discoloration (Fig. 2). In $90\text{ }\mu\text{g L}^{-1}$ or higher DIN concentration, no color change of the thallus was observed during the culture period. Discoloration began to appear on day-8 and -10 of culture at

concentrations of 50–70 $\mu\text{g L}^{-1}$ and day-6 of culture at a concentration of 30 $\mu\text{g L}^{-1}$ (Fig. 2). Therefore, the discoloration of thalli occurred when the dissolved inorganic nitrogen was exposed to 70 $\mu\text{g L}^{-1}$ or less for more than 10 days.

Recovery induction

When the thallus was exposed to 10 ~ 40 mg L^{-1} of $\text{NH}_4\text{-N}$ concentration within 10 days of discoloration occurring both the coordinates of redness a value and yellowness b value showed positive values. However, in the thallus treated more than 12 days after discoloration, the a value was less than 0. The b value tended to increase over culture time (Fig. 3). Treatment of discolored thallus at 10 mg L^{-1} of $\text{NH}_4\text{-N}$ concentration for 96 hours recovered the color of the thallus. In contrast, at 20 ~ 40 mg L^{-1} of $\text{NH}_4\text{-N}$ concentration, it took 72 hours. Therefore, considering the economic and industrial efficiency of nutrients supply to restore the color of *Pyropia* thalli, treatment for 72 hours at more than 20 mg L^{-1} of $\text{NH}_4\text{-N}$ concentration enabled the thallus to recover its color (Fig. 4).

Determination of discoloration and recovery of the thallus

-Photosynthetic pigment

After 14 days of culture under each DIN concentrations analysis of the thallus showed that Chl. a, PE and PC at 70 $\mu\text{g L}^{-1}$ or less were all lower than the control, but there was no significant difference from the control at 90 $\mu\text{g L}^{-1}$ or more (Fig. 5).

-Photosynthetic yield

The photosynthetic yield was significantly lower than that of the control in the concentration condition of 70 $\mu\text{g L}^{-1}$ DIN or less after 14 days of culture; however, there was no significant difference from the control in the concentration of 90 $\mu\text{g L}^{-1}$ DIN or more (Fig. 6).

-Vacuole to cell area

The ratio of the area occupied by the vacuole in the cell was the highest in the 30 $\mu\text{g L}^{-1}$ DIN concentration condition, and the ratio of the area of the vacuole gradually decreased as the nutrient concentration increased (Fig. 7). There was no significant difference from the control in the concentration of 90 $\mu\text{g L}^{-1}$ DIN or more.

Discussion

Since 2010, there has been a continuous increase in the number of seaweed farms in Korea. At the same time, the price of seaweed fell due to overproduction, disease, quality deterioration, and seaweed discoloration related to nutrient deficiency in seawater (NFRDI 2015). Understanding the causes of

seaweed discoloration will enable the industry to prepare countermeasures that address at least one factor that preserves the value of the industry (Kim et al. 2020).

The main elements that are found within seaweed are C, H, O, N, P, K, Ca, and Mg. Although N and P are present in large quantities in seawater (Lobban and Harrison 2000), they are also in high demand by seaweeds. If not present in sufficient quantities, they can limit the growth and proliferation of algae. Nitrogen is the element most frequently limiting the growth of seaweeds (Topinka and Robbins 1976; DeBoer and Ryther 1977) and phytoplankton (Ryther and Dunstan 1971).

The requirement for N is very high, and when *Pyropia*, *Undaria* and *Saccharina* farming occurs at high densities N depletion can occur. The flux of nutrients also affects the dissolved nutrient levels required for growth. For example, 100 $\mu\text{g L}^{-1}$ of N, 12 $\mu\text{g L}^{-1}$ of P or more are required for *Pyropia* farms with slow flow rates, and 70 $\mu\text{g L}^{-1}$ of N, 9 $\mu\text{g L}^{-1}$ of P or more are required with faster flow rates. However, if farms are overcrowded or the arrangement of farm facilities hinders the flow of seawater, then any shortage of nutrients will be exacerbated.

Nutrients enter coastal waters predominantly from the land through rivers and streams, or nutrients accumulated in the sediment of the coastal seabed are resuspended during storm events. Generally, productive seaweed farms are sited in large estuaries or shallow coastal areas with large tidal flats. However, in recent years, the amount of nutrients flowing into seaweed farms in coastal areas has been greatly reduced due to the expansion of sewage treatment facilities on land and the prevention of the inflow of pollutants from agricultural and livestock production into rivers. The nutrient deficiency in *Pyropia* farms is exacerbated by the increasing demand for nutrients as coastal seaweed farming increases.

Although seaweed growth rates are primarily driven by water temperature, they are also moderated by light intensity, day length and nutrient availability. The optimal seawater temperature for *Pyropia* growth ranges between 5 ~ 8°C, and the lower limit for growth is known to be 4°C. The nitrogen requirement for normal growth of *Pyropia* is 5.5 ~ 7.0% of the dry weight of seaweed, and the minimum and absolute requirements are 4 ~ 5% and 1.2 ~ 1.3%, respectively. Inorganic nitrogen in seawater is absorbed by *Pyropia* thalli and is used for the synthesis of various amino acids or nitrogen-containing compounds, such as pigment proteins (Chl. a, PE, PC, etc.) unique to *Pyropia*. Although seaweeds can absorb both ammonium salt and nitrate, they generally absorb ammonia nitrogen more quickly than nitrate (Kang and Ko 1977). Immediate enhancement of photosynthesis in *Gracilaria* spp. in response to ammonium enrichment has been reported by Nelson (1985). He suggested that these macrophytes can effectively exploit brief pulses of ammonium fertilizer. Nelson's (1985) work suggests that artificial fertilizers could be used to offset the limitations of low-nutrient conditions in *Pyropia* farms. Discoloration of *Pyropia* can be addressed by applying fertilizer or moving the seaweed to a farm in an area with higher nutrient levels. However, since moving a large-scale *Pyropia* farm is practically difficult, nutrient depletion may be more easily addressed by applying fertilizer.

The quality of *Pyropia*, is judged by both its taste and colour. The taste of *Pyropia* is determined by free amino acids, while pigment proteins determine the color of seaweed. Seaweed with high amino acid content and high pigment protein content tastes good and has a good color, and consequently is considered to be high quality. Both amino acid content and pigment content are heavily influenced by the N concentrations at the farm site. Generally, high-quality dried *Pyropia* has a photosynthetic pigment content of Chl. a of 600~700 mg, carotenoid 150~200 mg, PE 2,000~3,500 mg, PC 1,500~2,000 mg, (per 100 dry weight) and trace amounts of APC (allophycocyanin). On the other hand, low-quality of dried *Pyropia* has a photosynthetic pigment content per 100 dry weight of 190 mg of Chl. a, 80 mg of carotenoid, 380 mg of PE, and 440 mg of PC (Amano and Noda 1987).

This study found that the pigment content of farmed *P. suborbiculata* decreased markedly during the production cycle. As the farming season progressed, Chl. a, PE and PC values decreased, reflecting a decrease in the photosynthetic performance of the plants. The decrease in the pigment content of thallus can be attributed to factors such as irradiance and wavelength, water temperature, amount of carbon dioxide, pH, type and concentration of dissolved nutrients, nutritional status of the thallus itself, and amount of pigment. That is, all of these factors must be in an appropriate state for *Pyropia* to have a healthy pigment content. If any of these factors are lacking, the pigment content of *Pyropia* will be reduced no matter how well the other factors are satisfied.

In this study, when the DIN concentration in seawater was less than $70 \mu\text{g L}^{-1}$ for more than 8 days, the algal fronds became discolored, and when the DIN concentration was over $90 \mu\text{g L}^{-1}$, the algal fronds were not discolored. The critical DIN concentration of discoloration was found to be $80 \mu\text{g L}^{-1}$. In addition, algal fronds recovered their colour within 7 days of discoloration, when additional N was added. When discoloration lasted more than 8 days, the algal fronds did not recover their color (Fig. 8). The increasing period of exposure to low nutrients led to an increase in the degree of hypertrophy of intracellular vacuoles, the granulation of plastids, and the enlargement of the space between cells, making it impossible for the algal to recover thier color.

Amano and Noda (1987) reported that the discolored thalli had a relatively high sugar content and low crude protein or amino acid content, resulting in reduced disease resistance. This study did not consider the potential for reduced disease resistance at low nutrient levels, but this could be the focus of future work.

Because N is the primary nutrient resource that limits seaweed growth (Hurd et al. 2014; Roleda and Hurd 2019), variations in seaweed growth rates should parallel variations in N supply. Nitrogen-uptake capacity (V_{max}) is a direct function of the surface-area: volume (SA : V) ratio of the thallus (Lobban and Harrison 2000), whereas the nitrogen-storage capacity varies approximately inversely with the SA : V ratio (Duke et al. 1989). Thus, a seaweed's functional form (Littler and Littler 1980) may determine its ability to buffer nutrient variability (Norton 1991). In this study, seasonal growth species such as *Pyropia* often are annuals that feature rapid nutrient uptake and rapid growth potential, have high SA : V ratios, low nutrient-storage capabilities, and typically occur in high nutrient environments.

In case of the discolored thalli in Fig. 2, the L and b values tended to increase and the a values tended to decrease. In other words, when the nutrients are insufficient, the algal thalli show a discoloration in which the red is lowered and the yellow is increased. As the change of color and photosynthetic pigment content, the photosynthetic pigments of Chl. a, PE, and PC contents all tended to be significantly lowered. These results showed that the change in the photosynthetic pigment content of the thalli is directly related to the concentration of nutrients.

As shown in Fig. 3, thalli provided with additional nutrients within 10 days of discoloration, the thalli recovered, photosynthetic pigment content increased, and Fv/Fm values increased. These results suggest that when the degree of physiological damage to the algal thalli caused by nutrient deficiency is at a level that can be restored by nutrient supply, the thalli has the potential to recover to its normal state. The Fv/Fm values of normal thalli were 0.8 ~ 0.9, but for thalli cultured at DIN 30 ~ 70 L^{-1} concentration, they were 0.3 ~ 0.5, which is 38~56% lower than that of the control (Fig. 5). This is closely related to reduced photosynthetic pigment synthesis and metabolism when the thalli are exposed to low nutrient concentrations.

Recent experiments have shown that nitrogen most frequently limits the growth of seaweeds (Topinka and Robbins 1976), and phosphorus is the second most limiting nutrient (Lapointe et al. 1992). The concentration of a nutrient gives some indication as to whether or not the nutrient is limiting, but the nutrient's supply rate or flux is also important in determining the magnitude or degree of limitation. For example, if the concentration of a nutrient is low, but the supply rate is greater than the uptake rate by the algae, then the algae will not be nutrient-limited. Farmed *Pyropia* is harvested at intervals of 15 days under normal circumstances.

Amano and Noda (1987) found that when $\text{NO}_2\text{-N}$, $\text{NO}_3\text{-N}$, $\text{NH}_4\text{-N}$, urea, and amino acids were provided for the purpose of restoring the color of *Pyropia* thalli, the best result occurred when $\text{NH}_4\text{-N}$ was used. In this study, when $\text{NH}_4\text{-N}$ was provided at a concentration of 10~40 mg L^{-1} to discolored thalli, it was found to be very effective in restoring the color of algae. At a concentration of 20 mg L^{-1} or more, the color of the thalli was restored within 72 hours and at a concentration of 10 mg L^{-1} or less, the color was normally restored within 96 hours. These results suggest that even if discoloration occurs in the seaweed fronds, it is possible to recover the color quickly by adding ammonia such that the concentration in the culture water reaches 20 mg L^{-1} of $\text{NH}_4\text{-N}$ at the seaweed farm.

These results contribute to understanding the need to monitor DIN levels at *Pyropia* farms and the likely impacts of reduced nutrient levels on *Pyropia* quality. They point to the potential for farmers to provide additional nutrients to enable rapid color recovery when discoloration is observed, supporting the continued production of high-quality *Pyropia*.

Declarations

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Figures

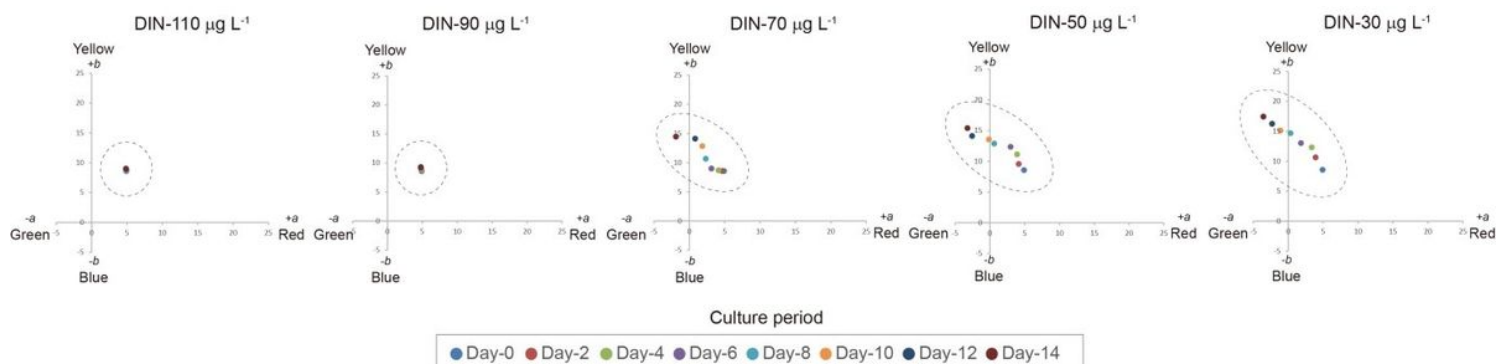


Figure 1

Location of redness (a) and yellowness (b) values on the chromaticity diagram of *Pyropia suborbiculata* obtained during 14 days culture in each dissolved inorganic nitrogen (DIN) levels.

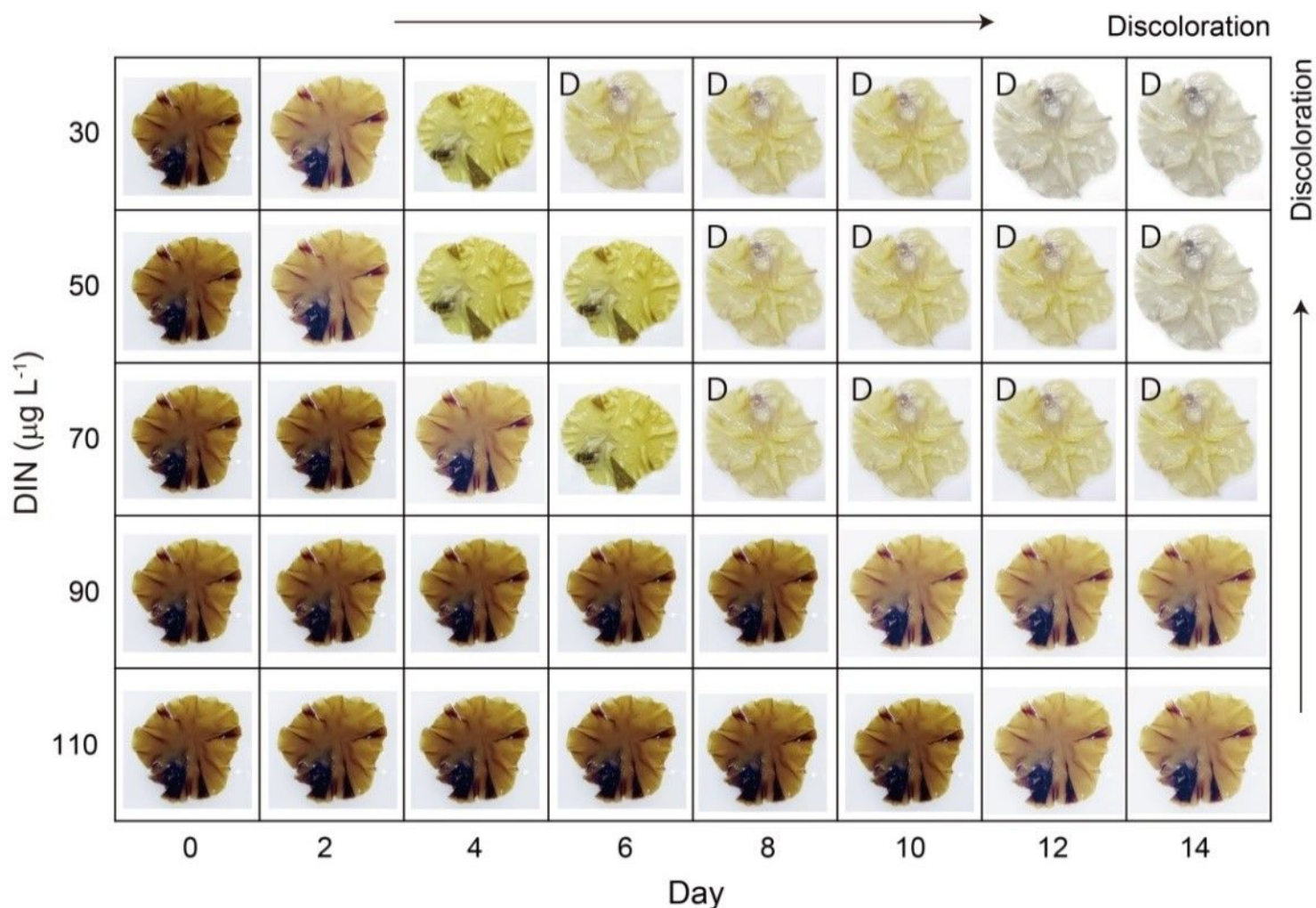


Figure 2

Discoloration induction of *Pyropia suborbiculata* obtained during 14 days culture in each dissolved inorganic nitrogen (DIN) levels. Culture conditions were 8°C, 80 $\text{mmol m}^{-2} \text{s}^{-1}$ and 12:12h L:D. D indicates discoloration.

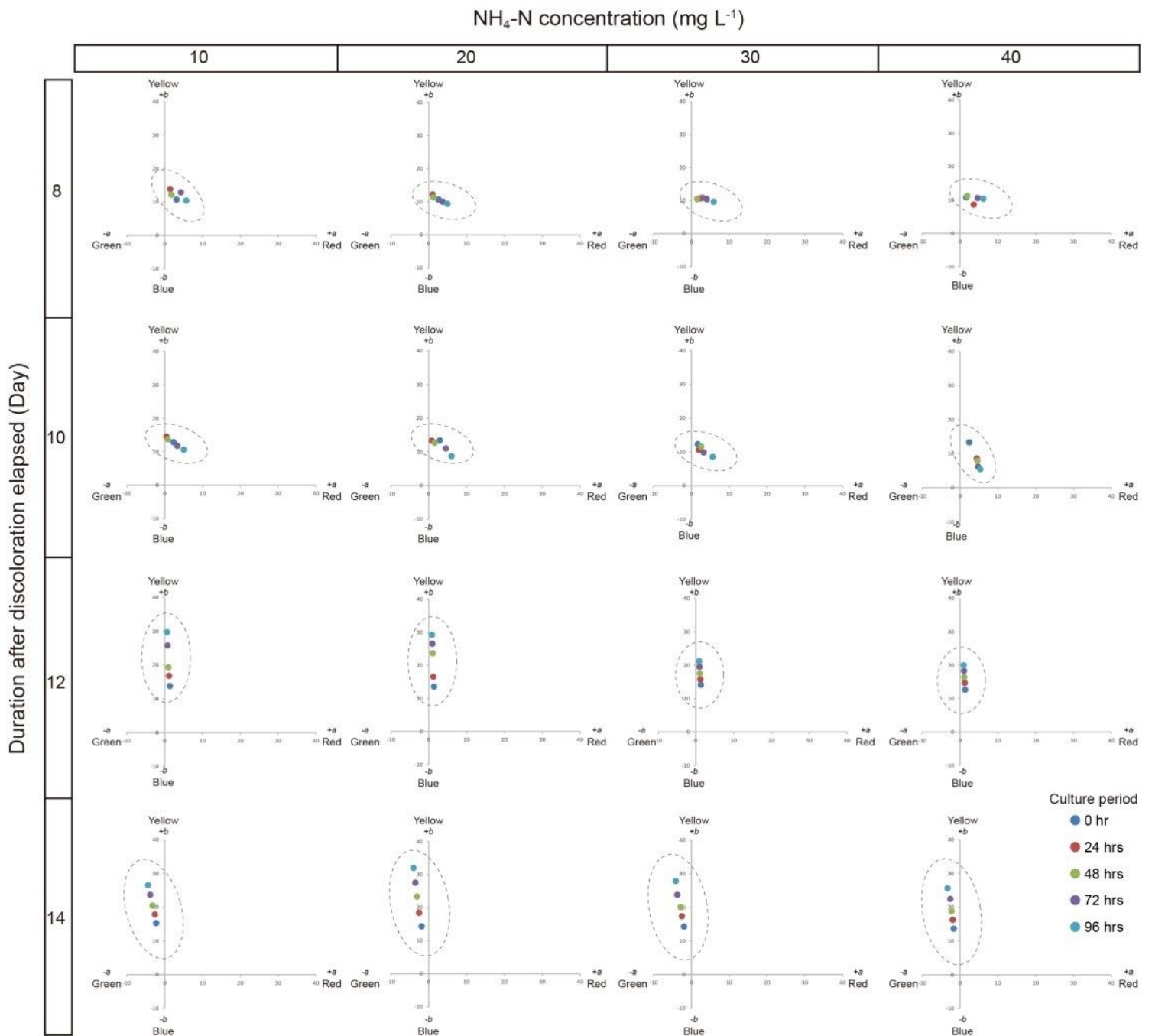


Figure 3

Location of redness (a) and yellowness (b) values on the chromaticity diagram of *Propia suborbiculata* obtained during 96 hrs culture under the conditions of the duration of discoloration elapsed and $\text{NH}_4\text{-N}$ concentrations.

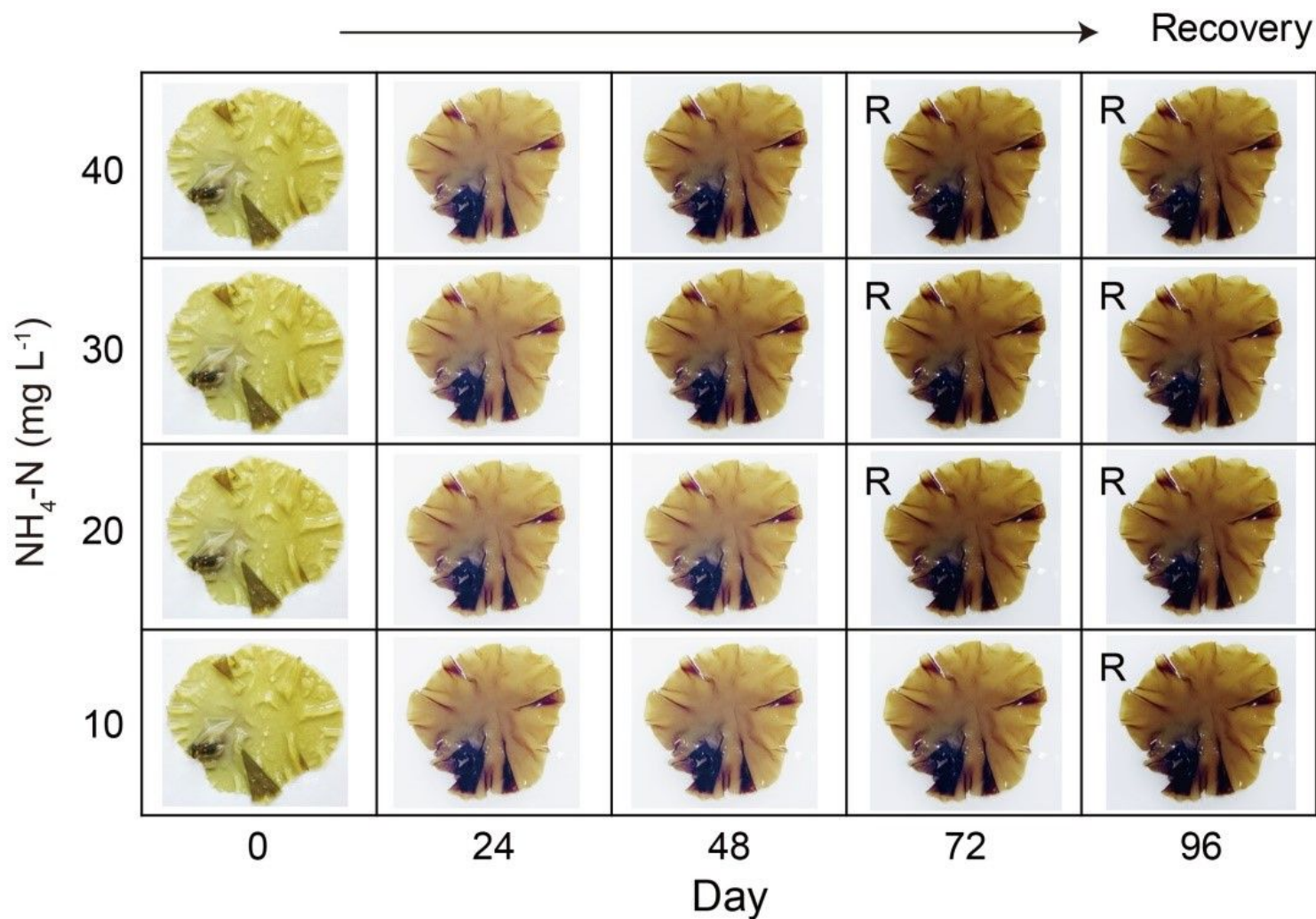


Figure 4

Recovery of discoloured *Pyropia suborbiculata* under different $\text{NH}_4\text{-N}$ concentrations during 96 hrs culture. Culture conditions were 8°C , $80 \text{ mmol m}^{-2} \text{ s}^{-1}$ and 12:12h L:D. Recovery was only possible in the thalli within 8 days of discoloration by chlorosis. Thalli older than 10 days after discoloration do not recovery even if nutrients are supplied. R expressed recovery.

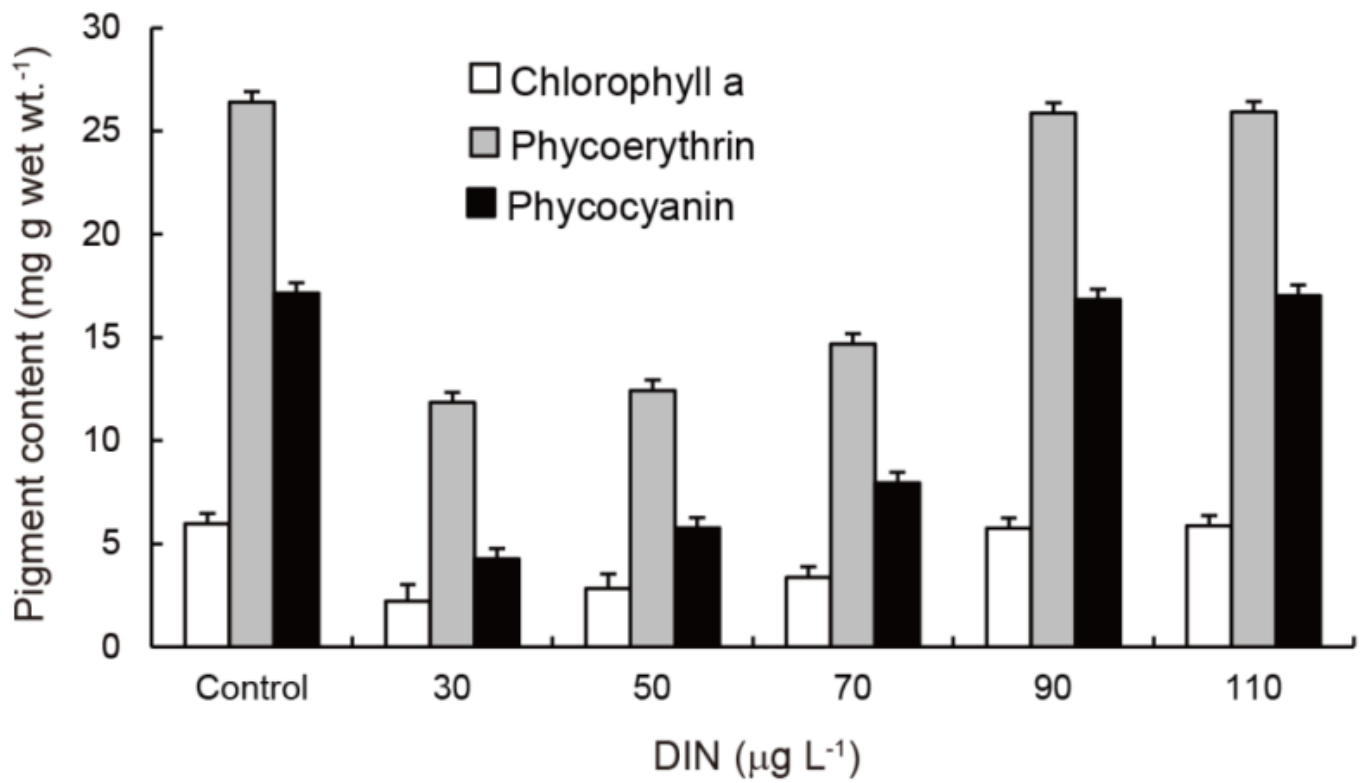


Figure 5

Photosynthetic pigment contents in *Pyropia suborbiculata* obtained after 14 days culture in each dissolved inorganic nitrogen (DIN) levels.

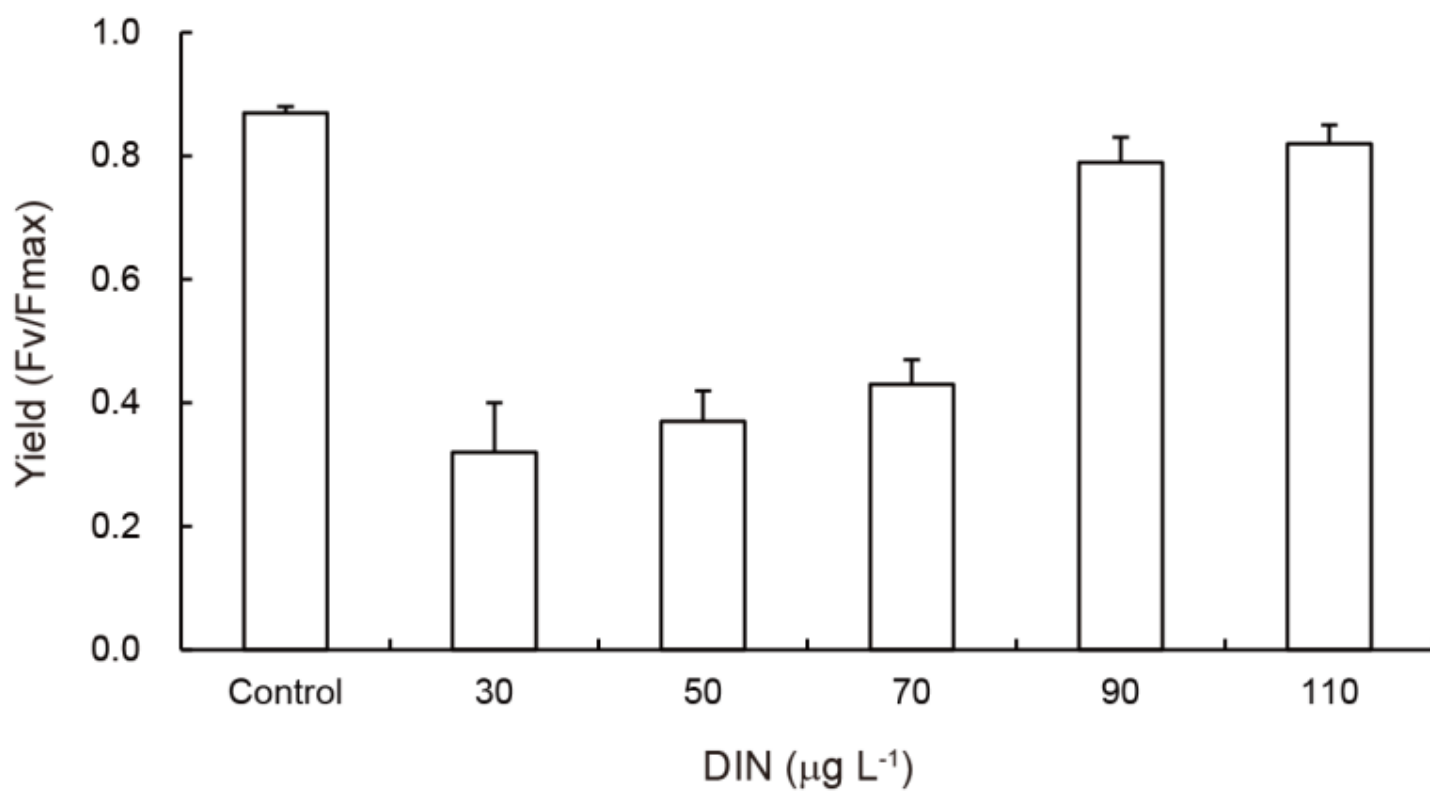


Figure 6

Photosynthetic yield in *Pyropia suborbiculata* obtained after 14 days culture in each dissolved inorganic nitrogen (DIN) levels.

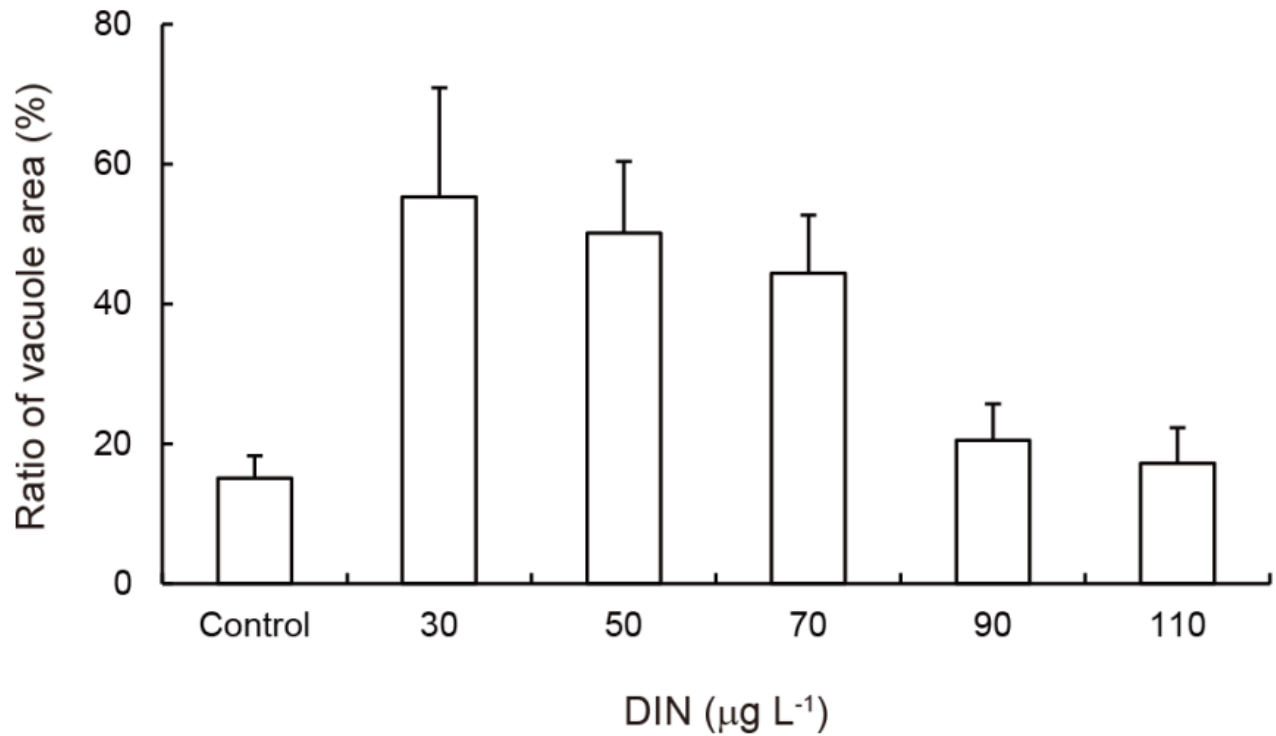


Figure 7

Ratio of vacuole in a cell area in *Pyropia suborbiculata* obtained after 14 days culture in each dissolved inorganic nitrogen (DIN) levels.

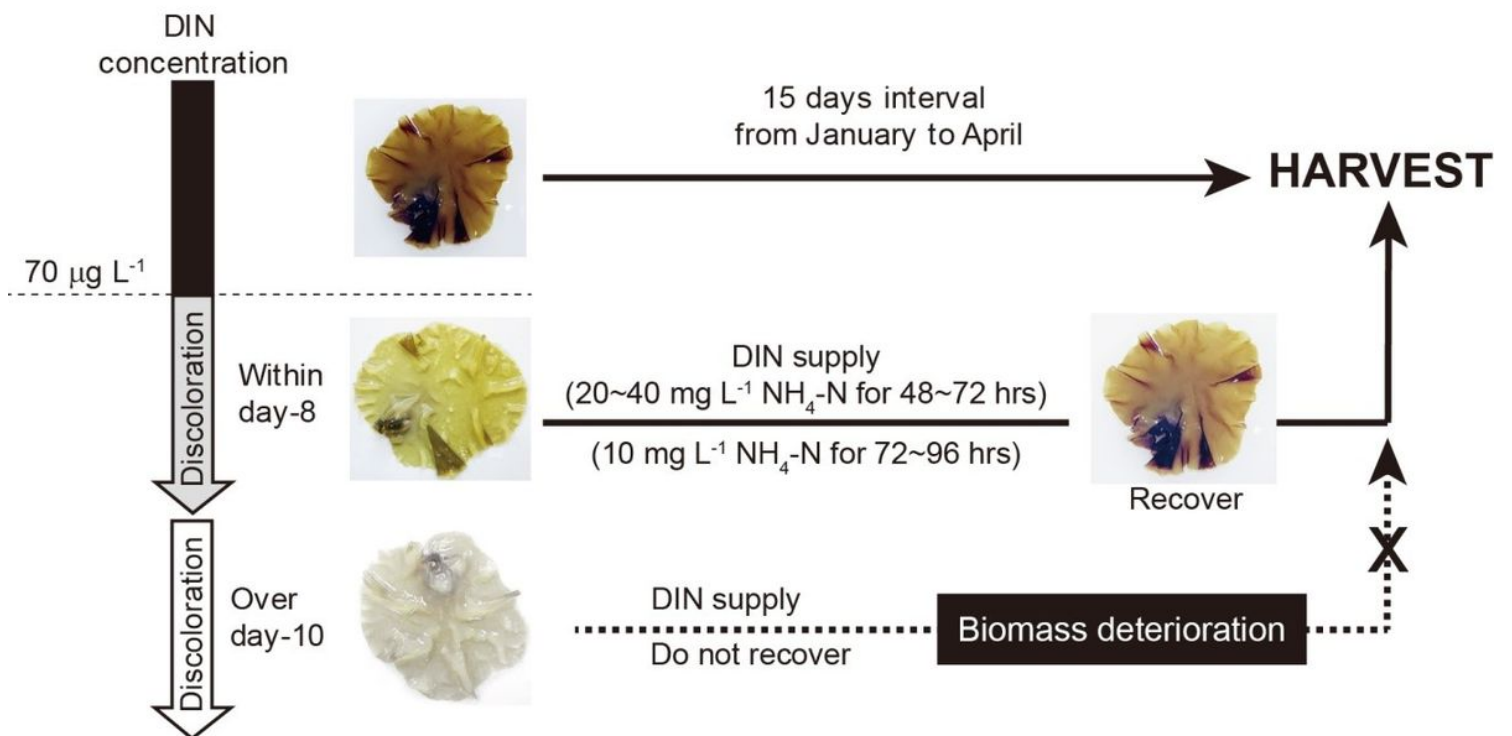


Figure 8

An illustration of discoloration and recovery of *Pyropia suborbiculata* with dissolved inorganic nitrogen (DIN) concentrations. Thalli that have been damaged by discoloration more than 10 days do not recover no matter how much nutrients were supplied.