

Environmental status of *Kappaphycus alvarezii* cultivation area following temporary eutrophication

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Abstract. Eutrophication of shallow coastal areas can lead to the high growth of macroalgae. The present research was conducted, over two time periods, to clarify marine environment status in a seaweed cultivation area following eutrophication. In the first period in June - October 2009 temporary eutrophication occurred. The second period was in June - October 2013 when a concentration of inorganic nutrients returned to normal levels. At the end of September 2009, nitrate + nitrite and phosphate concentrations were 2.4-4.2 μM and 2.59-3.45 μM , respectively. High concentration of ammonia, nitrate + nitrite and phosphate occurred at locations of disposal of fertilizers. Since July - September 2013, ammonia, nitrate + nitrite and phosphate concentration ranged between 0.3-0.6 μM , 0.4-0.7 μM and 0.16-0.58 μM respectively. There was a significant difference of ammonia, nitrate + nitrite and phosphate in 2009 compared to 2013. During May 2009, the initial growth of *Kappaphycus alvarezii* from 50 g initial weight (W_0) was 235 g in 50 days. However, in June 2013, after 50 days of cultivation weight increased from 50 to 262 g.

Key Words: *Eucaema*, macroalga, fertilizer, nitrate, phosphate.

Introduction. Indonesia is one of the Southeast Asia countries with high seaweed production. One of the provinces that have wide cultivation area and high production of *Eucaema* is Southeast Sulawesi. *Eucaema* cultivation in Southeast Sulawesi is scattered in many locations and generally uses longline (Kasim et al 2016, 2017a). In 2009, several cultivation sites in Southeast Sulawesi occurred eutrophication that emerged temporarily. Shallow coasts are common locations and prone to eutrophication. Eutrophication commonly occurs in areas with relatively high inorganic material supply and causes macroalgal (Lavery et al 1991; Valiela et al 1997; Raffaelli et al 1998; Lenzi et al 2015) and microalgal blooms (Anderson et al 2002; Graneli et al 2008). Seaweeds cultivation area is often prone to eutrophication, and often occurs in seaweed cultivation areas in eastern Indonesia in particular in the Sulawesi Region. This situation is exacerbated by fertilizer utilization to optimize growth rate of *Eucaema* spp. Utilization of fertilizer in seaweed cultivation is an unusual activity thought to be wasteful and useless. In fact fertilizer which is used in seaweed cultivation was popular during 2008-2010 in most cultivation areas in the Sulawesi Region (Kasim & Mustafa 2017). Farmers would fill a tank with 100 L of saltwater and add 100-200 g of fertilizer. Furthermore, *Eucaema* immersion for 4-5 hours before transplanted. The purpose of soaking is to spur growth and eliminate epifauna attached to the *Eucaema* thallus. After immersion farmers will discard the leftover solution containing high N and P freely to the sea. Disposal process done by 50 farmers on average in one day is equivalent to as much as 5,000 L per week. This will influence environmental conditions around the cultivation area with an increase of excess nutrients and phosphate. Inorganic nutrient are important for growth of *Kappaphycus alvarezii* (Hayashi et al 2007; Hayashi et al 2008; Bindu & Levine

2011). However, concentration of inorganic nutrient was a limiting factor for the growth of macroalgae. Research on the relationships of macroalgae and inorganic nutrients have been frequently reported. Such results suggests that the availability of phosphorus, in waters, becomes the limiting factor for the growth of macroalgae (Lapointe et al 1992; Littler et al 1991), while the relationship between the nutrient macroalgae growth has also been reported and as a limiting factor for the growth of macroalgae (Littler et al 1991; Lapointe et al 1992; McGlathery et al 1992; Delgado & Lapointe 1994). On the other hand, utilization of *K. alvarezii* in reducing nutrient concentration in the water column is also known (Doty 1973; Hayashi et al 2008). The present study attempts to confirm the environmental status during 2009 and 2013 following temporary eutrophication brought about by fertilizer intrusion surrounding the *K. alvarezii* cultivation area in Southeast Sulawesi, Eastern Indonesia.

Material and Method. This study was conducted in June – November 2009, and June – November 2013, at seaweed cultivation areas in Lakeba sub-district, city of Baubau, Southeast Sulawesi (5048'78, 2"; 122056'26 LS, 3" BT.). In 2009 most farmers used fertilizers in order to increase the growth of *K. alvarezii*, but the practice was discontinued in early 2012. We commenced measuring the environmental parameters in 2013, approximately one year after utilization of fertilizers had ceased. Observations were carried out by measuring the environmental conditions, particularly physico-chemical factors such as nitrate, phosphate, ammonia, salinity, dissolved oxygen, temperature, current velocity. In-depth interviews were conducted to obtain information related to environmental conditions at the time and prior to the study. The information obtained in the form of invisible environmental conditions before the application of fertilizers, included the amount and timing of fertilizer use. Direct observations were carried out to observe environmental conditions of the area of seaweed cultivation. Water quality measurements were carried out. Water quality measured focusing on nitrate+nitrite, phosphate, ammonia (Jones-Lee & Lee 2005). Measurement of dissolved oxygen was performed using Winkler Methods. Temperature and current velocity was measured using thermometer and current meter respectively. Salinity was measured using a refractometer. All environmental parameters were measured in every 6th day in all stations. Phytoplankton measurements were performed in every 5th day by counting abundance of diatoms and dinoflagellate (method clarified in Kasim & Mukai 2009). Epiphytic macroalgae were monitored every 8th day concerning species number and occurrence on *K. alvarezii* thallus.

Growth rate of *K. alvarezii* was determined during June to October in 2009 and 2013, cultivated in floating cages (200 x 200 x 60 cm). The outer of cage was covered with multifilament nets with a diameter of 1 cm and the upper side was fully opened to allow the control of *K. alvarezii*. Floating cages were partially submerged in the water and a small portion on the surface of the water. The depth of the cage submerged in the water was 50 cm (Kasim et al 2017a, 2018). Strings of seaweed placed in the floating cage in the middle of May 2013 where regular cleaning was done every 2-3 days to brush of the attached Epiphyte and epifauna from the outer wall of the cages (Kasim et al 2016, 2017b).

Statistical analysis. The wet weight of *K. alvarezii* was calculated in term of mean \pm standard deviation. In addition, coefficient of correlation was calculated for growth rates determined for 40 days and also for environmental parameters. Correlation coefficients between growth rate and environmental factors were calculated using simple linear models (Pearson's). Statistical analyses were performed using the SPSS Package 22.

Results and Discussion. Nitrate concentration during June to October 2009 was very high compared to in 2013. During 2013, the average nitrate concentration was below 1 $\mu\text{M/L}$ comparing to 3 $\mu\text{M/L}$ in 2009 in all stations (Figure 1). Phosphate concentration was also very high during June to October of 2009 compared to 2013.

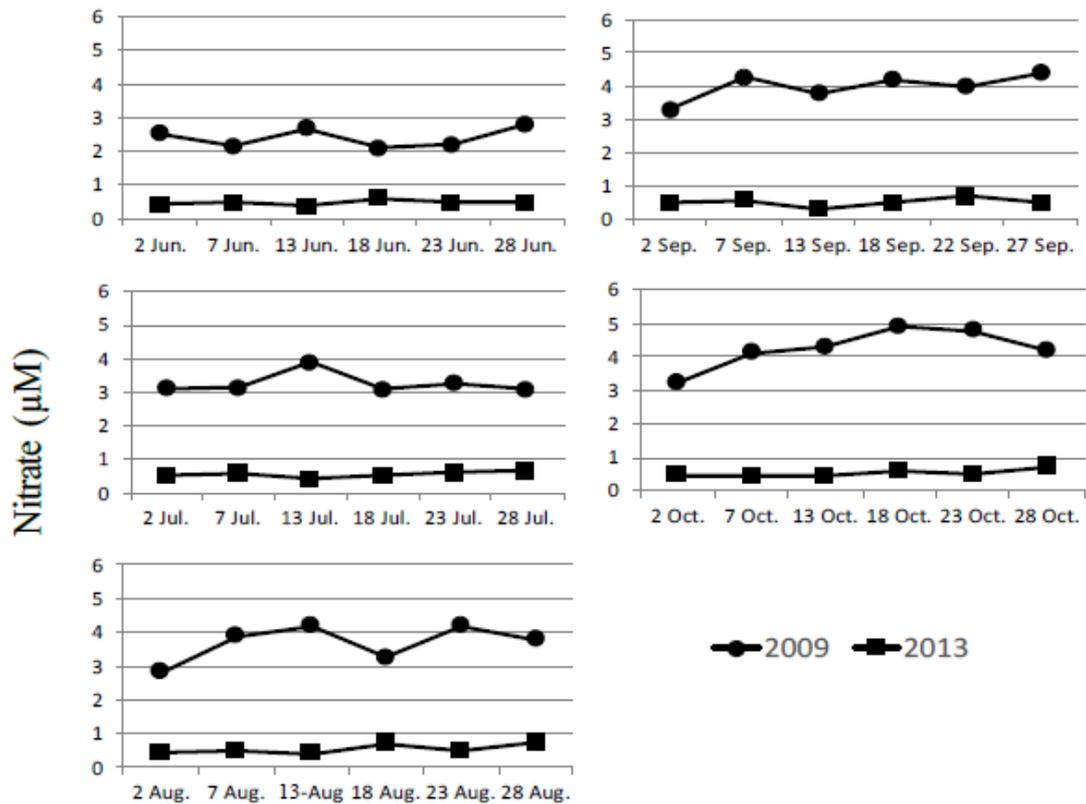


Figure 1. Nitrate + nitrite concentration during our study in 2009 and 2013.

Phosphate concentration during June – October 2009 was up to 3 µM/L on average, compared to 2013 when phosphate concentration was below 0.1 µM/L in all stations (Figure 2).

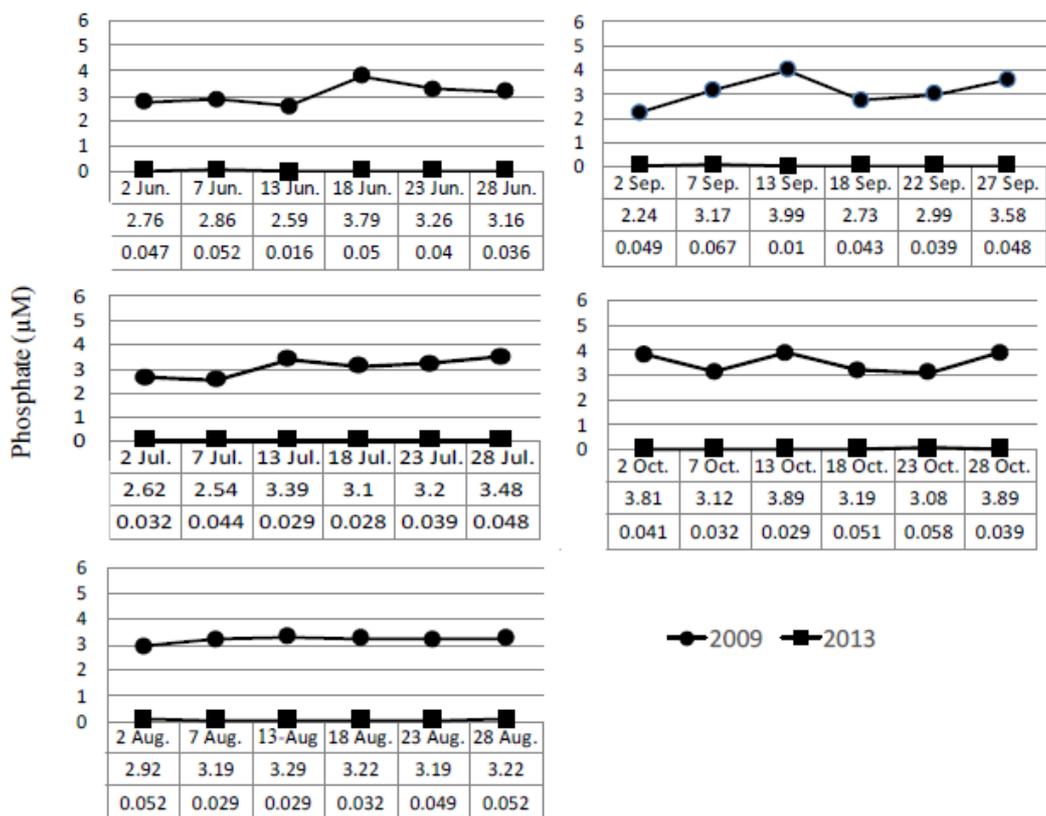


Figure 2. Phosphate concentration during our study in 2009 and 2013.

Ammonia concentration during June to October 2009 was very high compared to 2013. High ammonia in 2009 occurred in September with 4.78 $\mu\text{M/L}$ while in 2013 was only 0.47 $\mu\text{M/L}$ (Figure 3). There are negative correlations between nitrate, phosphate and ammonia concentration in 2009 and 2013 ($P > 0.95$).

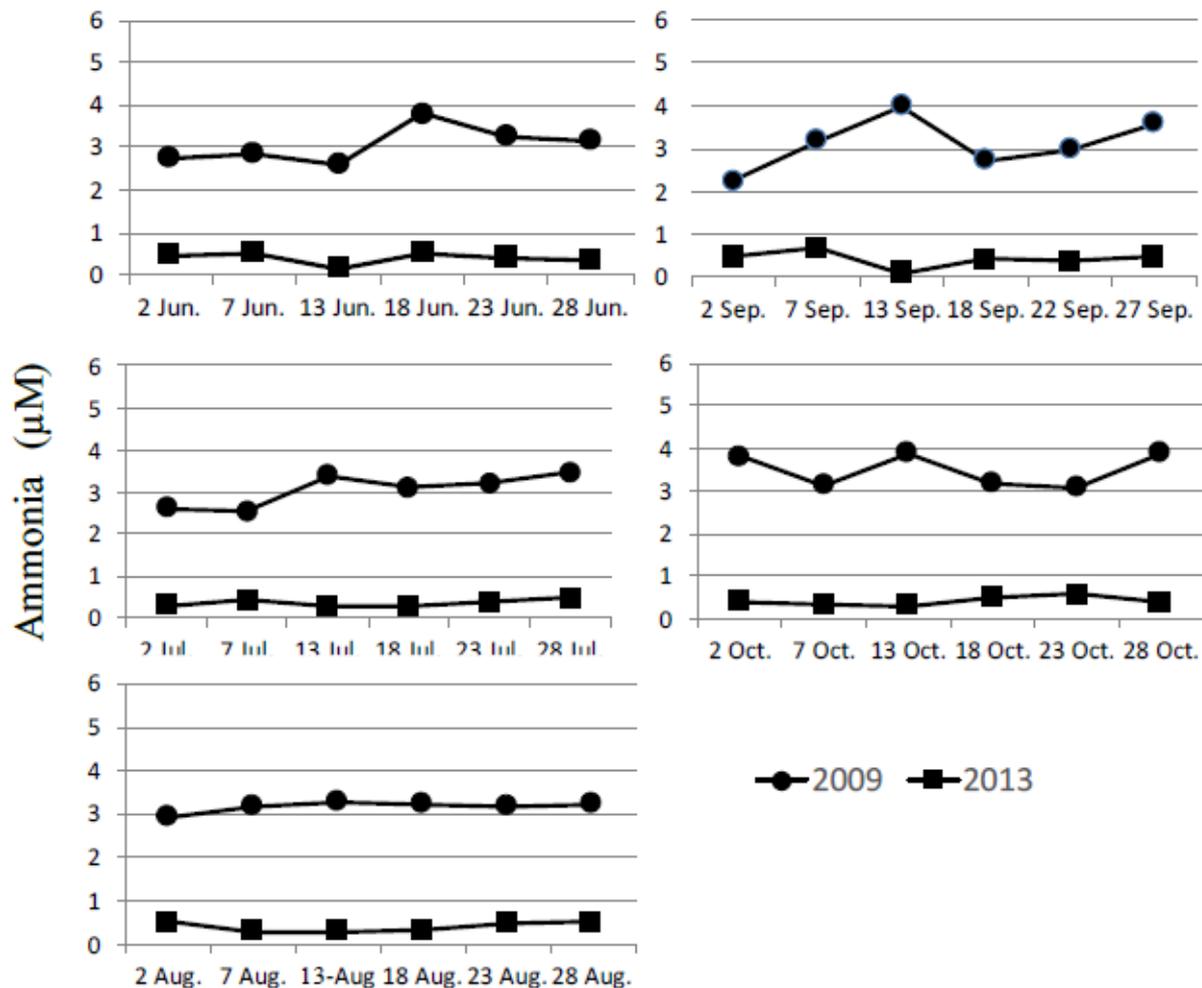


Figure 3. Ammonia concentration during our study in 2009 and 2013.

There were small differences in dissolved oxygen (DO) particularly in October 2009 and 2013. Average DO in station one in October 2009 was 5.2 ppm and 7.6 ppm in October 2013 (Figure 4). There were differences in DO concentration during June, July, and August, and similarly in September and October in both periods, 2009 and 2013.

During our research, salinity fluctuations were higher in 2009 than in 2013. Salinity was low during June to October in 2009 and high in 2013 (Figure 5). Variation of temperature was completely different in June and August in 2009 and 2013 (Figure 6). Current velocity was high during September and October in 2009 and 2013, particularly at the end of month. Mostly, current velocity in 2013 was higher compared to 2009 in all station (Figure 7).

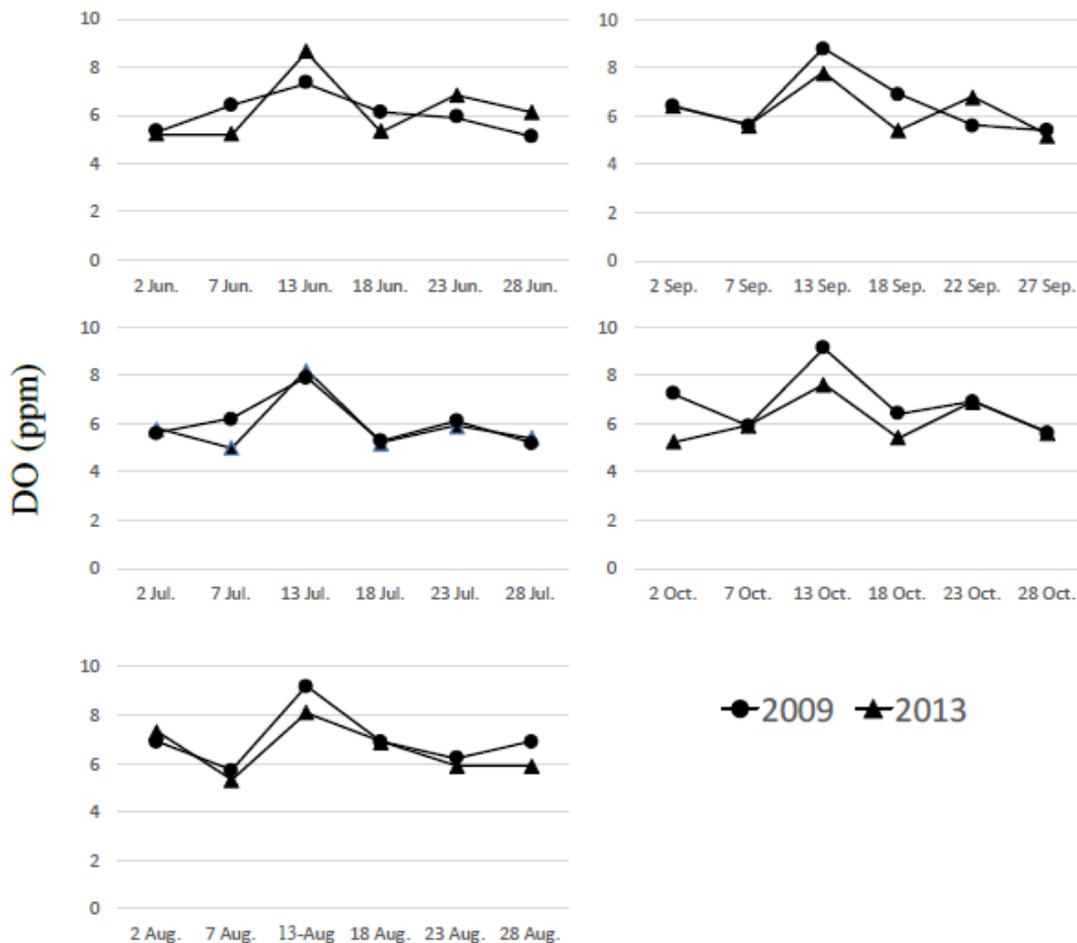


Figure 4. Comparison of dissolved oxygen during 2009 and 2013.

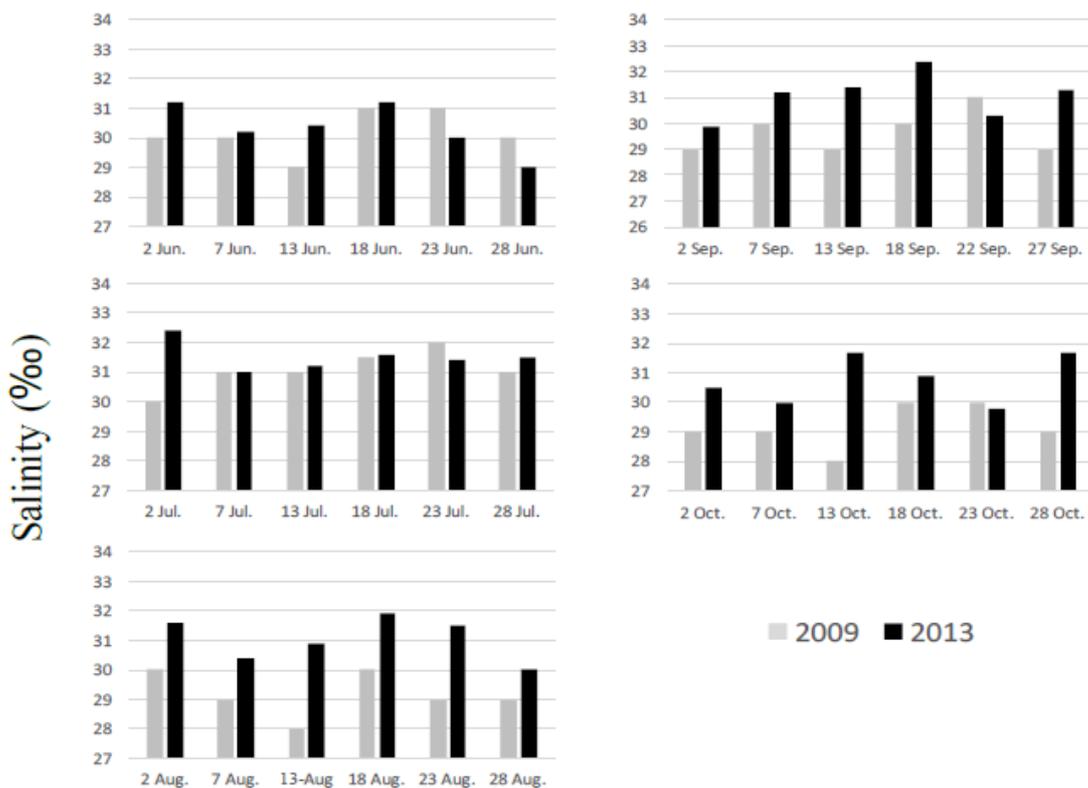


Figure 5. Salinity concentration during June – October in 2009 and 2013.

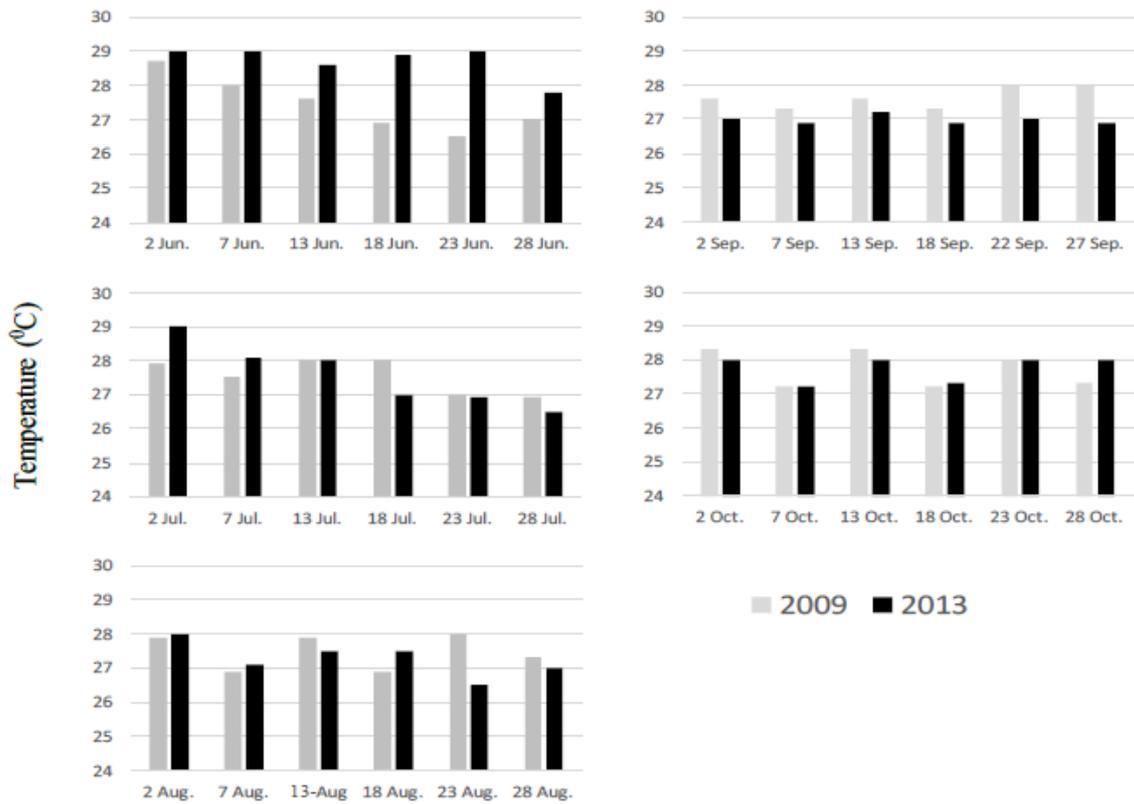


Figure 6. Water temperature during June-October in 2009 and 2013.

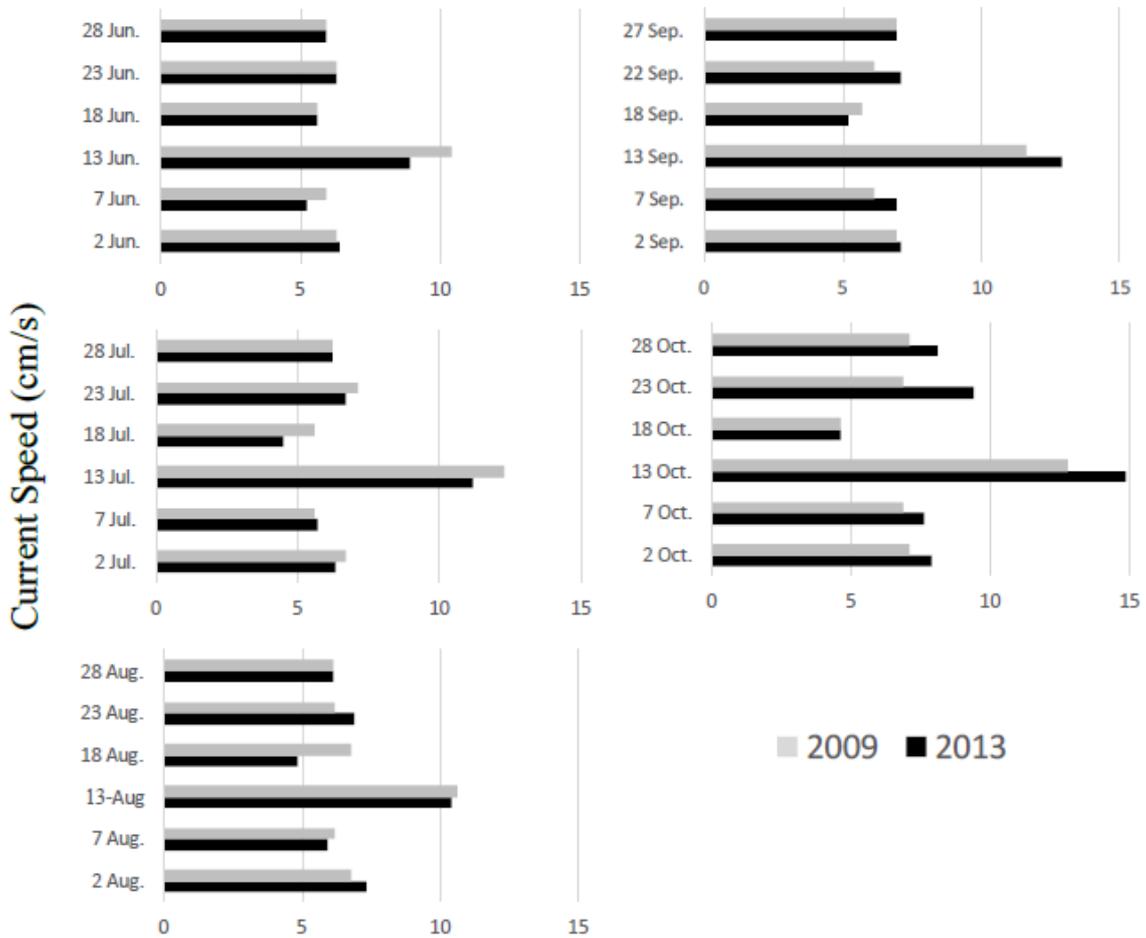


Figure 7. Current velocity during June – October in 2019 and 2013.

Growth of *Kappahycus alvarezii*. During our experiment in May 2009, growth of *K. alvarezii* reached 235 g in 50 days of cultivation from an initial weight of 50 g. However, in June 2013, wet weight of *K. alvarezii* was 262 g from 50 g during 50 days of cultivation (Figure 8). The growth rate of *K. alvarezii* cultivated in 2009 was not positively correlated with nitrate concentration and in 2013 was negatively correlated with ammonia and phosphate ($p>0.05$) (Table 1).

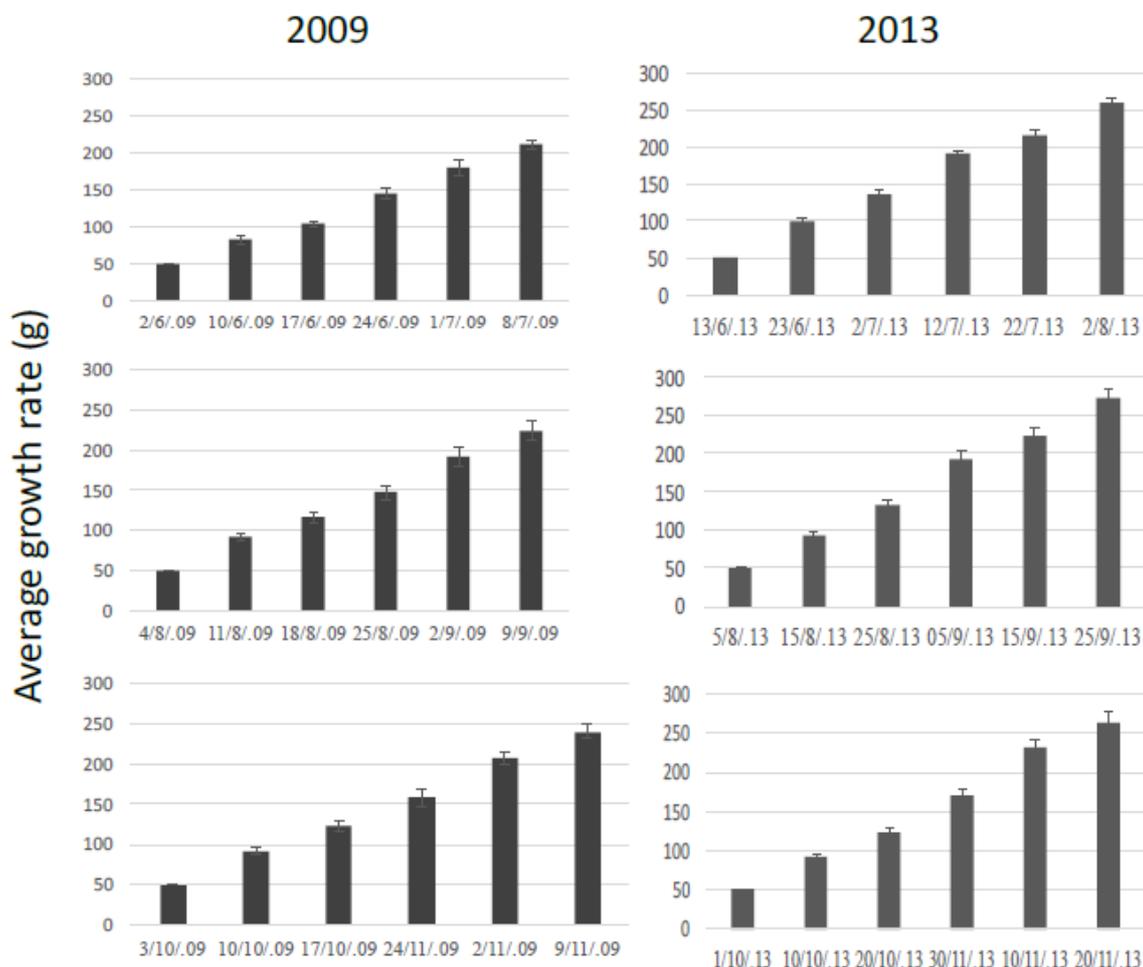


Figure 8. Growth of *Kappahycus alvarezii* during 2009 and 2013.

Table 1
Correlation coefficient between growth of *Kappahycus alvarezii* and environmental factors cultivated in 2009 and 2013

Specification	Growth rate		Nitrate		Ammonia		Phosphate	
	2009	2013	2009	2013	2009	2013	2009	2013
Growth rate	2009	0.988**	0.182	0.001	-0.064	-0.196	0.369	-0.206
	2013		0.086	0.009	-0.023	-0.163	0.346	-0.191
Nitrate	2009			-0.123	0.22	-0.015	0.27	-0.196
	2013				0.172	0.278	-0.192	0.530*
Ammonia	2009					0.35	-0.265	0.236
	2013						0.161	0.132
Phosphate	2009							-0.164
	2013							0.514

** indicate positive correlation and "-" indicate negative correlation; correlation is significant at the 0.05 level; ** Correlation is significant at the 0.01 level; * Correlation is significant at the 0.05 level.

Environmental condition. During 2009, the input of inorganic nutrients was very high particularly from July to October. High levels of nitrate, ammonia and phosphate in 2009 was led to eutrophication in this region. Chislock et al (2013) mentioned that increased nutrient content of the water is a major cause of eutrophication. Balance of nitrogen-to-phosphorus in waters may change due to eutrophication. This condition brings about a strong competition between macroalgae with other plants. Macroalgae are usually successful in such competition and grow quite rapidly, and some successful species eventually become dominant. Increased nutrient levels could cause stress for some algae and some other algae resulting in allocchemical to be dominant on other species (Graneli et al 2008). Increased concentrations of nutrients and phosphorus can cause high production of macroalgae especially in eutrophic areas (Lenzi et al 2015). In fact, *Chaetomorpha* bloom cover surroundings of the *K. alvarezii* cultivation areas (Figure 9).

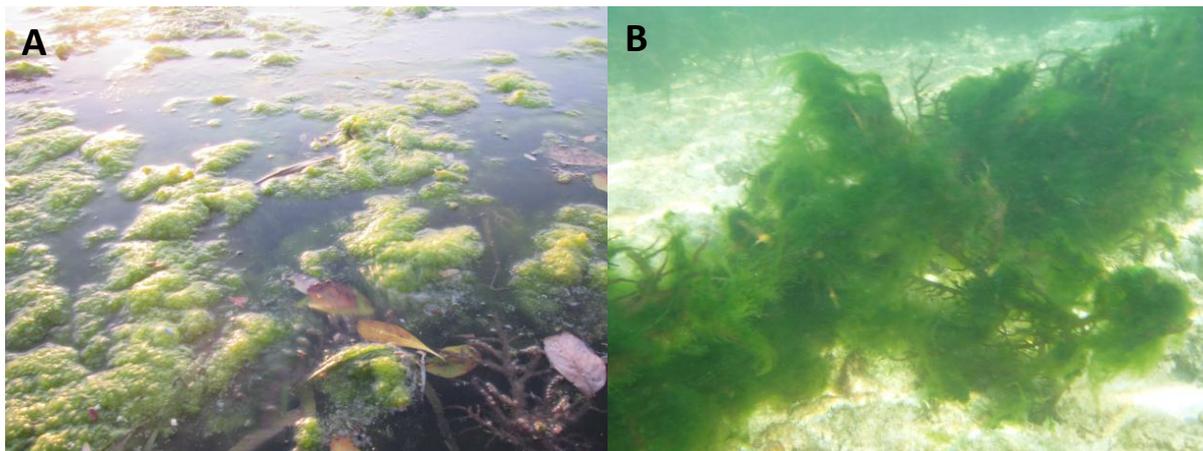


Figure 9. *Chaetomorpha* sp. blooming at *Kappaphycus alvarezii* cultivation site (A. appearance from upper side. B. *Chaetoceros* sp. cover *K. alvarezii* at longline cultivation).

The above mentioned conditions impede growth of *K. alvarezii*. According to our observation, *K. alvarezii* do not grow well, being under an average value of a good growth for this species. This kind of phenomenon occurred from August to September while nitrate concentration was high in the region. Increasing concentrations of N and P in the water of Mediterranean coastal lagoon can significantly increase growth of *Chaetomorpha linum* (Menéndez et al 2002). Annual concentration of dissolved inorganic nitrogen (DIN) is directly correlated with the growth rate of *Ulva* spp. Growth of *Ulva* spp. increases with increasing concentrations of DIN (Teichberg et al 2010). Blooming macroalgae in some shallow areas are not desirable because not only creates worse coastal environment but also threaten harvest of cultivated commodities. Heavy blooming not only disrupts the ecosystem, but also drastically damages the environment (Duarte 1995; Valiela et al 1997; Raffaelli et al 1998). Increased concentrations of nitrate and phosphate potentially increase algae growth in marine aquaculture area. These conditions are boosted by an increase in the concentration of phytoplankton in areas near sources of nutrients as a cause of eutrophication (Anderson et al 2002). Increased concentrations of nitrate, phosphate and ammonia in the surrounding research site in 2009 when eutrophication occurs, could increase the growth of microalgae. There are many cases of excessive nutrient enhancement, known as eutrophication area really can increase the potential for the blooming of phytoplankton such as Chesapeake Bay and the Albemarle-Pamlico Estuarine System Japan Inland Sea, the Black Sea and the coastal waters of China (Anderson et al 2002).

During our experiment, other environmental parameters monitored did not varied during June – September 2013. Temperature, salinity and the current velocity ranged between 28-29°C, 31-34‰, and 0:04 to 0:14 m/sec, respectively. The most prominent physical factors that impact the growth of *K. alvarezii* are salinity and current velocity. Salinity will greatly affect the viability of seaweed metabolism thus lowering salinity will

affect the viability of *K. alvarezii*. When salinity is below 26‰, *K. alvarezii* will experience stress and can be easily affected by ice-ice disease. Some thallus will be damaged and will not grow well. Sulu et al (2003) explained that growth rate of *Euclima denticulatum* is related to salinity. This species tends to be able to grow well in the range of salinities above 30‰ with a temperature range of 25–29°C.

During our experiment in 2013, concentration of nitrate and phosphate was between 0.048-0.068 ppm and 0.102-0.302 ppm, respectively. Nitrite ranged between 0.025-0.053 ppm, dissolved oxygen (DO) ranged between 2.90 and 3.94, salinity between 31-34 ‰ and pH between 6.5-7.5. Salinity will greatly affect the metabolic processes of the seaweed. The low salinity will affect the durability of seaweed on the environment. Seaweed is only able to adjust to salinity between 26-34‰ and if there is a decrease or increase in salinity will cause stress which affects the appearance of ice-ice disease.

Correlation between growth rate and environmental conditions. During the study, the growth of *K. alvarezii* in 2009 and 2013 showed similar growth rate (Table 1). Even though on average, total growth rate during 2009, from 50 g (initial weight), reached only 243 g, compared to year 2013 when was able to reach up to 274 g after 40 days. This condition explains that the increase in nutrients such as nitrate and phosphate in the water column has no significant effect on the daily growth rate of *K. alvarezii*. Intrusion of high inorganic nutrient only stimulates blooming of phytoplankton and epiphytic macroalga which cover *K. alvarezii*. Epiphytic macroalga such as *Chaetomorpha* sp. compete with *K. alvarezii* for growth. This indicates that growth rate of *K. alvarezii* is not addressed to variability of environmental condition during our research. Cultivation in subtidal region resulted significantly higher fresh weight (12.5 ± 0.9 kg fresh Wt m⁻²) than in the intertidal region. Rao et al (2011) reported that daily growth rates of *K. alvarezii* during 45 days cultivation in Northwest coast India was 3.64-13.98% day⁻¹. Hurtado-Ponce (1992) reported a daily growth rate of *K. alvarezii* in Panagatan Cays, Caluya, Antique Philippines of 2.3-4.2% day⁻¹. Research conducted on the Yucatan Peninsula, Mexico, reported daily growth of *K. alvarezii* in a range of 2.0-7.1%/day (Munoz et al 2004). Growth of Eucheumatoids in April and May is a good season for *K. alvarezii* in Bongao, Southern Philippines; *K. alvarezii* growth rate can reach 300% of the biomass after a cultivation of 4-7 weeks (Villanueva et al 2011). In Vietnam, growth rate of *K. alvarezii* cultivated by longlines method at a depth of 0.5 to 1 m seems good during January-August, with an average daily growth of 6.14-6.26% day⁻¹. However, the growth rate increased in May-June in the range of 9.14-10.8% day⁻¹ respectively (Ohno et al 1994).

From early June 2013, farmers began to use floating cages for *K. alvarezii* cultivation and also stopped using fertilizers. This will enhance growth rate of *K. alvarezii*. Morphologically, seaweed *K. alvarezii* looked healthy. Thallus shape was fully and presented no damage. Four floating cages were observed, and all seaweeds showed a healthy condition. Luhan & Sollesta (2010) reported that waters with a high level of brightness will produce brighter colored and good growth of *E. denticulatum*. However, the morphology of this species will be highly dependent on environmental conditions. Environmental conditions with a fairly high level of brightness as well as the availability of sufficient nutrients will provide good growth opportunities for this species (Doty 1985; Trono 1990). Furthermore, Ask & Azanza (2002) explains that the increasing of growth rates of *K. alvarezii* is related to the available nutrients, salinity and water current velocity. During our experiment in 2013, morphologically *K. alvarezii* showed bright color with abundant branching. Within 2 months, *K. alvarezii* had large thallus and dark brown color. Woo (2000) reported that old branches of seaweed *Kappaphycus striatus* were cultured for a period of more than 4 weeks showed strong thallus and abundant branching. This is strongly related to environmental factors on reef bay Kane Ohe Hawaii. *E. cottoni* found spread in the area around coral reefs showed excellent growth with the support of environmental factors (Rodgers & Cox 1999).

Conclusions. There was a significant difference in ammonia, nitrate + nitrite and phosphate concentrations in 2009 compared to 2013. Initial growth of *K. alvarezii* from 50 g initial weight (Wo) reached 235 g in 50 days during May 2009. However, this is different from the June 2013 observation, when after 50 days of cultivation weight increased from 50 to 262 g. Temporary eutrophication occurred during August-September 2009, particularly on the study site.

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