*ICE-ICE D*ISEASE OCCURRENCE IN SEAWEED FARMS IN BAIS BAY, NEGROS ORIENTAL AND ZAMBOANGA DEL NORTE

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Date Submitted: October, 2009
Date Revised: November, 2009
Word Count: 4,025

Abstract

Incidence and degree of infection of four forms belonging to carrageenophytes, Kappaphycus alvarezii brown and green, and Eucheuma denticulatum brown and green were investigated in the established three farms in Bais Bay, Negros Oriental Philippines, on April 2007 to January 2008. Additional samples were also taken on December 2007 in Jose Dalman and Rizal, Zamboanga del Norte. Physical and chemical parameter like rainfall, salinity, temperature, and tide level were obtained to examine their relationship with the disease. As revealed in the two way ANOVA analysis, the occurrence and degree of infection differed significantly between species, strain, time (months) and farms. K. alvarezii has higher incidence and infection, as well as the farm situated near the community that is exposed frequently during low tide.

Keywords: *carrageenophytes, ice-ice disease, Kappaphycus alvarezii, Eucheuma denticulatum*

Introduction

Initially, ice-ice disease in *Kappaphycus/Eucheuma* was thought of as mainly a non-infectious disease which could be triggered by unfavorable environmental condition such as extreme temperature, irradiance and salinity, and opportunistic bacterial pathogens, *Vibrio* sp. (P11) and *Cytophaga* sp. (P25) (Largo et al., 1999). These findings suggest that the whitening phenomenon is caused by both abiotic and biotic factors acting in combination. When the seaweed is under stress, it emits a moist organic substance that attracts bacteria in the water and induces the "whitening" and hardening of the seaweed branches. Uninfected parts remain healthy while infected ones undergo depigmentation and eventually lead to plant breakage by any force of nature.

The problem of ice-ice disease has not only affected the seaweed farmers but also the nation as a whole. Most recent statistics reveal that farming in Zamboanga City suffered the drastic decline in aquaculture production with negative escalation of 42.8 percent because of *ice-ice* disease, as a result of the ailing seaweed sector. *Ice-ice* disease

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and epiphyte infestation dropped in annual seaweed yield by 22 percent (BAR, 2003). According to Trono (1993), ice-ice disease leads to a major decrease in seaweed production and reduced yield of carrageenan compared to the healthy crop ranging from 25 to 40 %; Mendoza *et al* (2002), revealed that ice-ice also leads to the decline of carrageenan yield, viscosity and gel potency of infected thalli.

Local farmers particularly in Bais Bay, attribute this disease to the temperature increase and slow water current in the cultivation ground, happening around March to May and occasionally in October, plus pollution of anthropogenic origin near the farms. Reduced salinity during rainy periods, however, concurring with low tide and coupled with slow water movement, is another perfect coalition leading to the ice-ice disease. These are as far as changes in physico-chemical factors are concerned (Largo, 2006).

The problem of *ice-ice* infection seems to have worsened in recent years, without any significant solution, while on the other hand people in coastal areas who rely on eucheumatoid farming, especially in the central production sites like in Zamboanga, Tawi-Tawi, Bohol and Cental Visayas are affected (Largo, 2006).

This study was conducted to establish the incidence and infectivity of *ice-ice* disease of *Kappaphycus alvarezii* and *Eucheuma denticulatum,* as well as the environmental causes that activated the disease.

Research Method and Design

Site Description. Three farms in Bais Bay were instituted in particular: two farms (Farms 1 & 2) located in Okiot and one farm (Farm 3) situated near Talabong Mangrove. Each has an area of about 500 m^2 were assumed to have different conditions. Farm 1 (Fig 1A), used off bottom monoline method, located near community (09°35’58.8” N; 123°09’01.6” E), with a distance from shore about 500 m.

The water depth in this farm ranged from 0 m at high low tide to 120 m at high high tide. Therefore, this farm usually exposed to the light intensity during low tide. The substrate was sandy silt with seagrasses, *Thallasia hemprichii* and *Enhallus acoroides.* Farm 2 (Fig. 1B), used the floating raft method, located offshore (09°36’03.2” N; 123°09’01.8” E), with a distance from shore about 800 m. This farm always submerged with the water, about 1 m deep during high low tide to 2.5 m during high high tide.

The substrates was sandy with very few of *Enhallus acoroides* and *Thallasia hemprichii.* Farm 3 (Fig.1C), employed the off bottom monoline method, located offshore near Talabong mangrove area (09°35’19.3” N; 123°09’16.7 E”), with a distance from shore about 800 m.

The condition of this farm was similar with that of farm 1; the water depth ranged from 0 m at high low tide to 150 m at high high tide. Therefore, this farm usually exposed to light intensity during low tide.

The farm substrate was characteristically sandy with seagrass communities compose mainly of *Thallasia hemprichii* and *Enhallus acoroides* In each farm, two strain of *Kappaphycus alvarezii*, brown (Kabr) and green (Kagr); and *Eucheuma denticulatum*, brown (Edbr) and green (Edgr) were planted randomly. In farm #1 and #2, all strain and species were planted together; while in farm #3 the species were separated.

Additional samples via line floating method were taken from Jose Dalman and Rizal, Zamboanga del Norte, for comparison. In Jose Dalman, only *K. alvarezii* brown giant was planted; while in Rizal three strains of *K. alvaresii*: brown, green and giant brown were planted.

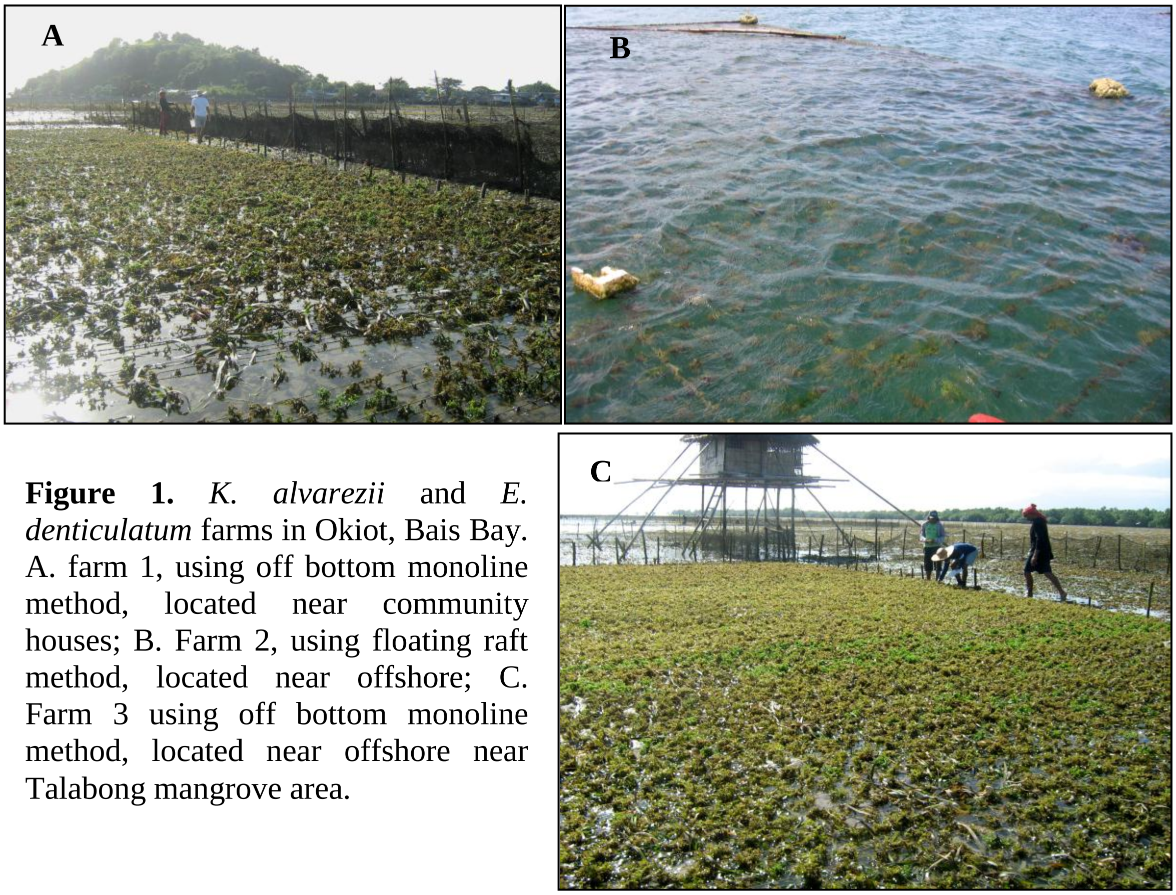


Figure 1. *K. alvarezii* and *E. denticulatum* farms in Okiot, Bais Bay. A. farm 1, using off bottom monoline method, located near community houses; B. Farm 2, using floating raft method, located near offshore; C. Farm 3 using off bottom monoline method, located near offshore near Talabong mangrove area.

Sampling Design. Sampling was executed in Bais Bay every two months, starting April 2007 to January 2008, but only once in Zamboanga del Norte, on December 2007.

In each of the three farms in Bais Bay, five cultivating ropes were randomly chosen. A cultivating rope is where bunches of seaweeds are being tied. Generally, a rope contains 100–120 bunches. The frequency of occurrence was observed every 10^{th} bunch of seaweed per cultivating rope. For *K.* alvarezii, ten bunches were monitored in each line for a total of fifty bunches. However, for *E. denticulatum*, since this was not commonly farmed, each line has only 60-70 bunches. Thirty bunches were monitored.

Data Analysis. To calculate for the incidence, the quantity of infected bunch was divided by the total number of bunches examined for every farm.

$$\% \ Incidence = \frac{No.\ of\ infected\ bunches}{Total\ No.\ of\ bunches} \ X\ 100\%$$

To compute the degree of infection, the number of infected branches was divided by total branches in the bunch.

$$\% \ Degree\ of\ Infection = \frac{No.\ of\ infected\ branches}{Total\ No.\ of\ branches} \ X\ 100\%$$

The data were then summarized using mean and standard error. Two-Way Analysis of Variance was utilized by means of the SPSS software program in comparing the incidence and the degree of infection between time (month), strain, and farm. Post Hoc Test (Tukeys HSD) was also made to see where the difference was located. The Pearson correlation was also used to correlate between salinity and rainfall.

Physical and Chemical Parameters. Physical and chemical parameters such salinity and temperature were taken in each farm for every observation. Three readings were taken for each parameter in each farm per sampling. Secondary data such as rainfall and tide level were also taken from PAGASA (2007) and DENR (2007).

Results

Site Conditions. Temperature was low to high between 32.55°C ± 0.04 in October in farm 1 to 29.77°C ± 0.33 in August in Farm 2. Temperature was found to be highest in Farm 1 in all observations, compared to Farm 2 and 3. Temperature was highest in April and October, mean 32.2°C ± 0.05 and 32.2°C ± 0.16 respectively, and lowest in August, mean 30.07°C ± 0.08 (Fig. 2.5). Salinity range between 33.97‰ ± 0.03 on January in Farm 3 to 29.27‰ ± 0.33 on August in Farm 3. Salinity was found higher in Farm 3 for every observation, compared to Farm 1 and 2 (Appendix 4). Salinity found highest in April, mean 33.42‰ ± 0.03 and lowest in June, mean 29.96‰ ± 0.03. Sea high during low tide found highest in June, mean 24.44 cm ± 7.17, and lowest in October, mean 0.64 cm ± 3.18. On the other hand, Rainfall found highest in June, mean 10.17 mm ± 3.51, and lowest in April, mean 0.81 mm ± 0.49 (Fig 2).

Pearson correlation analysis between salinity and rainfall showed a very low correlation (R = 0.187) which was not significant (p = 0.181) (Fig 3).

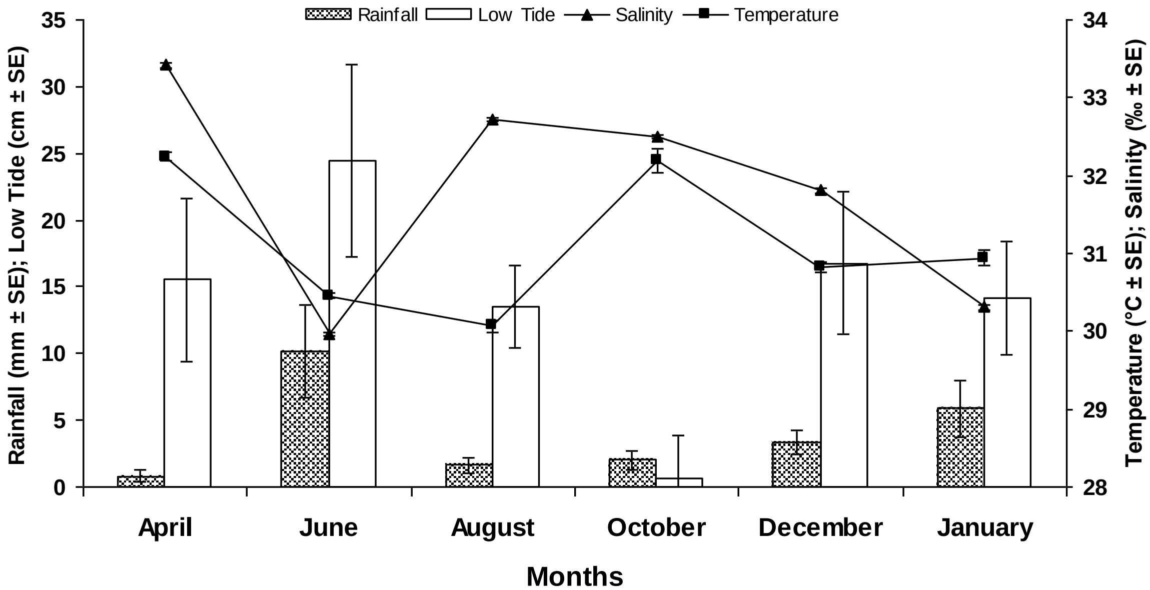


Figure 2. Mean physical and chemical parameters during sampling in Bais Bay, Negros Oriental. Bars indicate Standard Error.

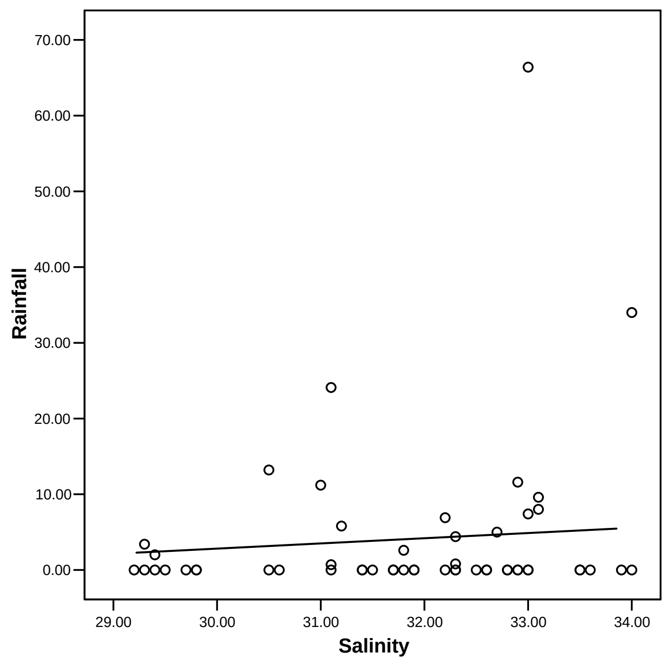


Figure 3. Correlation between salinity and rainfall

Incidence of ice-ice. The incidence of ice-ice disease was observed to vary between species, strain, time (months), and Farm (Fig. 4.). Incidence ranged between 2.22% ± 1.11 to 73.33% ± 3.53. In terms of time (month), October, April and December recorded the highest incidence of *ice-ice* disease (mean = 55.67% ± 4.26; 55.33% ± 4.33

and 52.00% ± 11.62, respectively) while August and January the lowest (mean = 21.61% ± 1.44 and 14.67% ± 2.17, respectively). With regard to species, *K. alvarezii* has the highest incidence (mean 42.50% ± 1.53) than *E. denticulatum* (mean 29.815% ± 2.17). In terms of strain, *K. alvarezii* brown had the highest incidence (mean 45.78% ± 1.53), followed by *K. alvarezii* green, (mean 39.22% ± 1.53); *E. denticulatum* green (mean 31.85 ± 2.17) and *E. denticulatum* brown (mean 27.78 ± 2.17).

When related with the environmental factors (Fig. 2 and 3), the incidence was elevated (in average) when temperature was high (in April and October) and low tides were at minimum (in October). The incidence also reached middle ranges when rainfall, salinity, temperature and sea level subsist in middle ranges (in June). When the temperature and salinity were low and rainfall and low tides were in the middle ranges, the incidence was lowest (in January).

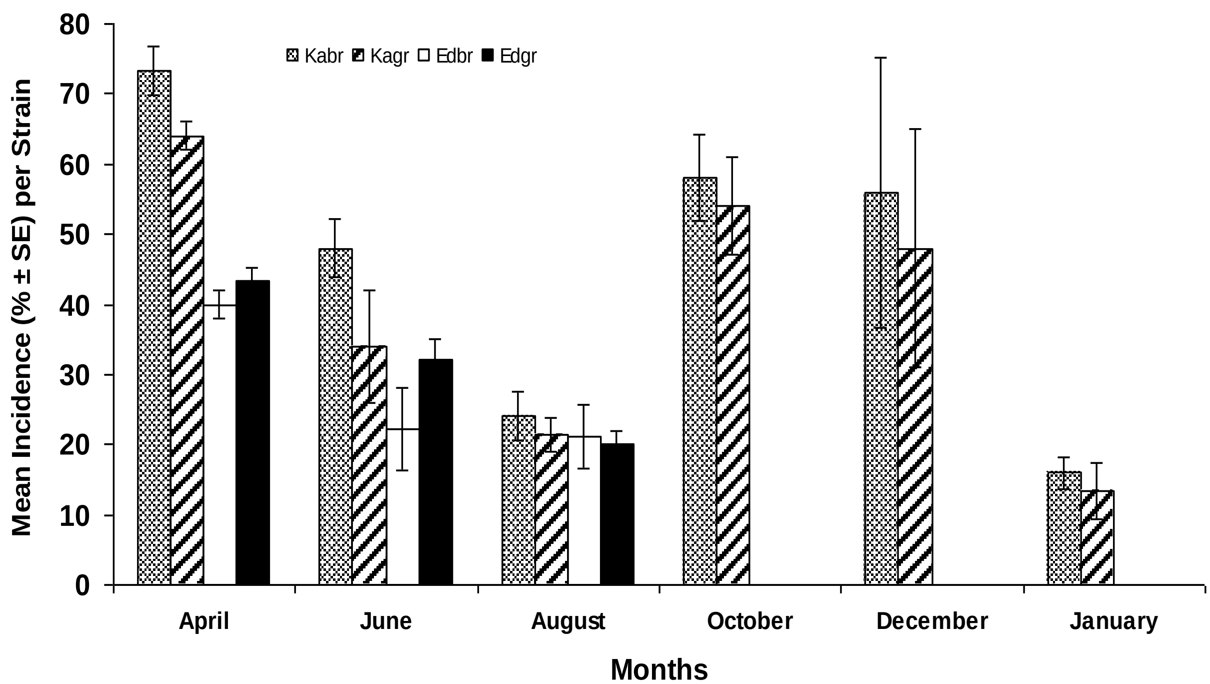


Figure 4. The incidence of ice-ice disease in *K. alvarezii* and *E. denticulatum* in time (month) and strain. Kabr, *K. alvarezii* brown; Kagr, *K. alvarezii* green; Edbr, *E. denticulatum* brown; Edgr, *E. denticulatum* green. Bars indicated Standard Error.

In terms of Farm, the highest incidence was observed in Farm 1 (46.63% ± 1.53), followed by Farm 2 (38.93 ± 1.53) and Farm 3 (29.26 ± 1.53). This condition occurred in almost all months, especially in October, December and January (Fig. 5).

Two-way analysis of variance with 95% confidence level showed significant differences in incidence between time (month) [F(5,216) = 62.61, p = 0.000]; strain

[F(3,216) = 18.33, p = 0.000]; and Farm [F(2,216) = 31.61, p = 0.000], in addition, there is significant interaction among month-strain [F(9,216) = 3.16, p = 0.001]; and month-Farm [F(10,216) = 5.83, p = 0.000).

Post hoc analysis using Tukey HSD test indicated the significant difference between means in terms of time, strain and Farm. In terms of time (month), the mean incidence for April was significantly higher than in June, August and January. The mean incidence in June, in turn, was significantly varied from April, October, December, January and August. The mean incidence in August was significantly different from April, December, June and October. The mean incidence in October was significantly different from August, January and June. The mean incidence in December was significantly different from August, January and June. While the mean incidence in January is significantly different from April, December, June and October.

In terms of strain, *K. alvarezii* brown was significantly different from all other strains, as well as *K. alvarezii* green. While *E. denticulatum* brown and *E. denticulatum* green, only have significant difference with *K. alvarezii* brown and green, but not with each other. Finally, Farms 1, 2, and 3 were significantly different from each other.

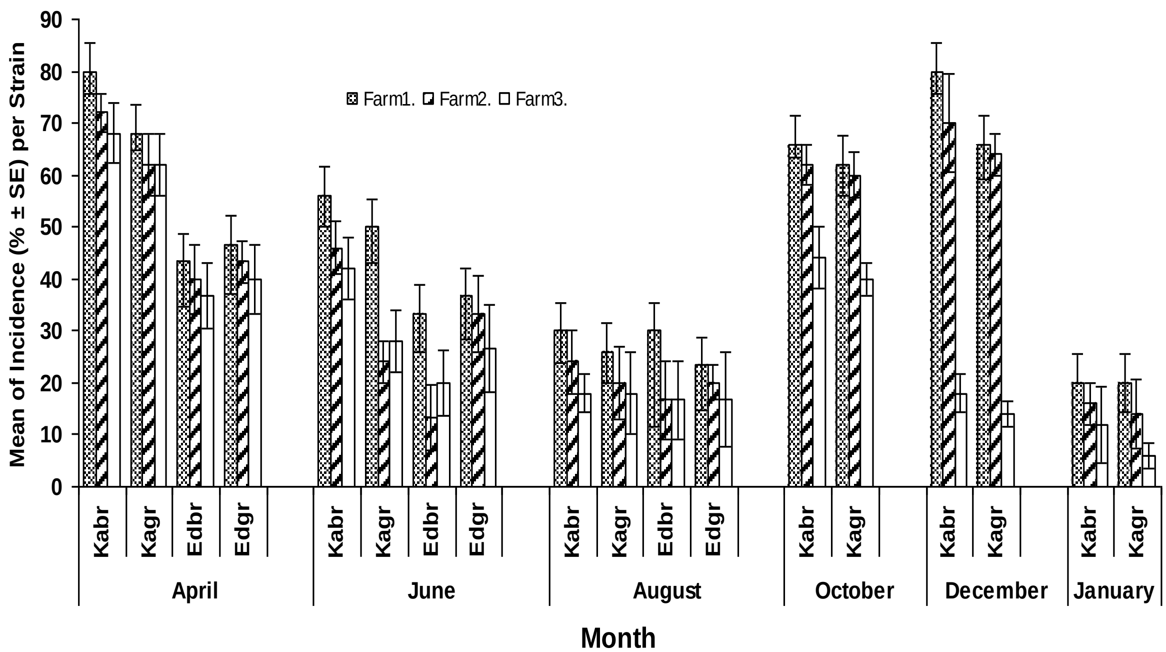


Figure 5. The incidence of ice-ice disease of *K. alvarezii* and *E. denticulatum* in different farms in Bays Bay. Kabr, *Kappaphycus alvarezii* brown; Kagr, *K. alvarezii* green; Edbr, *E. denticulatum* brown; Edgr, *E. denticulatum* green. Bars indicated Standard Error.

Degree of infection. The degree of infection of *ice-ice* disease observed in *K. alvarezii* and *E. denticulatum* also varied in terms of strain, time (months), and Farm. It ranged between 0.13% (± 0.07) to 29.31% (± 1.89). Fig. 5 showed that December, October and April comprised the highest degree of infection, mean = 23.49% ± 2.57; 18.82% ± 2.69 and 16.92% ± 2.42, respectively; while January and August comprised the lowest, mean = 2.225% ± 0.79 and 3.17% ± 1.03, respectively. In terms of species, *K. alvarezii* has the higher mean (15.53% ± 2.27) than *E. denticulatum* (4.37% ± 1.81). For the strain, *K. alvarezii* brown was higher (mean=16.40% ± 2.24), then *K. alvarezii green* (14.65% ± 2.30). On the other hand, *E. denticulatum* green has higher mean (4.42% ± 1.31) than *E. denticulatum* brown (4.31% ± 2.31).

When the degree of infection was correlated to environment conditions (Fig 3 and 4), the highest mean degree of infection occurred in April, when the rainfall was at the minimum while salinity and temperature were at the maximum. In January, the lowest scale of infection was observed, as all environmental factors were in the moderate arrays. No data from October to January for *E. denticulatum.*

In terms of Farm, the highest mean degree of infection was observed in Farm 1 (15.90% ± 2.13), followed by Farm 2 (12.33% ± 2.57) and Farm 3 (7.55% ± 1.65). In every observation (April – January), the degree of infection in Farm 1 was always high compared to other Farms, especially in December (Fig. 6 and 7).

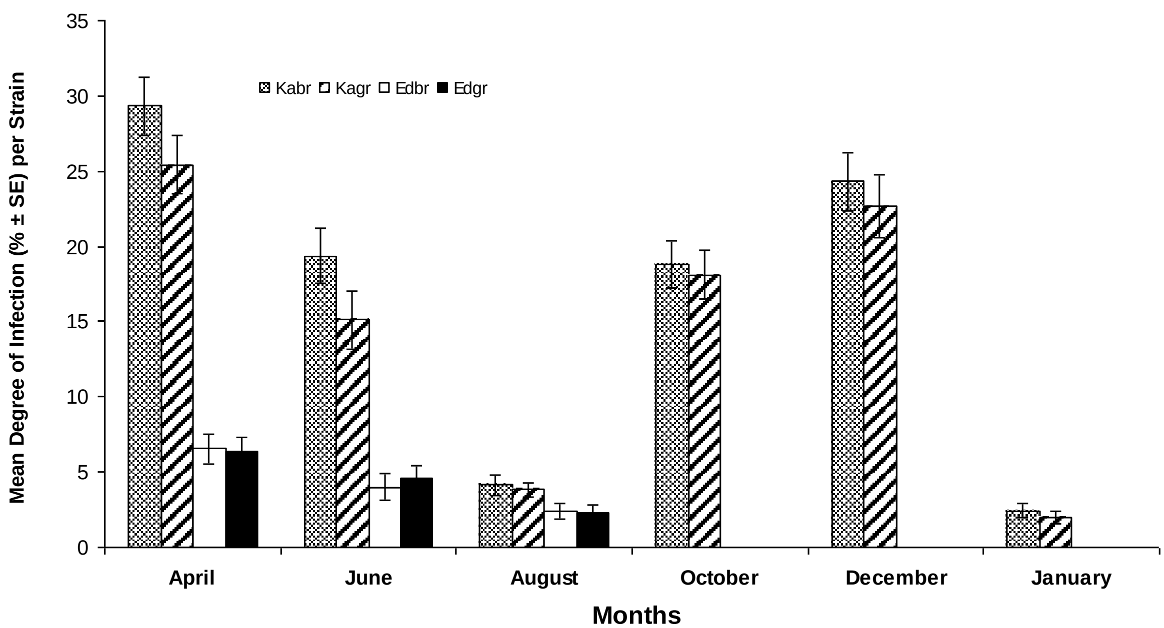


Figure 6. The degree of infection of ice-ice disease of *K. alvarezii* and *E. denticulatum* in time (month) and strain. Kabr, *K. alvarezii* brown; Kagr, *K. alvarezii* green; Edbr, *E. denticulatum* brown; Edgr, *E. denticulatum* green. Bars indicated standard error.

Two-way analysis of variance with 95% confidence level showed significant differences in infection between time (month) [F(5,2286) = 97.48, p = 0.000]; strain [F(3,2286) = 64.83, p = 0.000]; and Farm [F(2,2286) = 44.29, p = 0.000], in addition, there was significant interaction among month and strain [F(9,2286) = 10.14, p = 0.000]; and month and Farm [F(10,2286) = 19.32, p = 0.000). Using Cohen's (1988) criterion, the effect size (strength of association) of infection for the time (month) was large; while for the strain, Farm, interaction between month-Farm and month-strain were moderate.

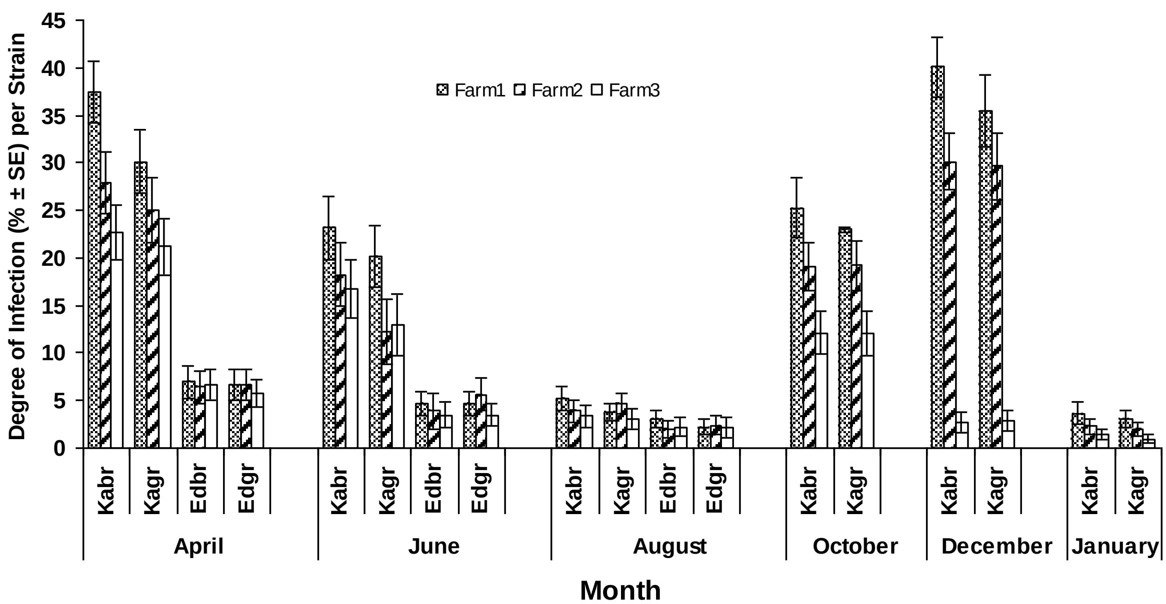


Figure 7. The mean degree of infection of ice-ice disease of *K. alvarezii* and *E. denticulatum* in different farms in Bais Bay. Kabr, *K. alvarezii* brown; Kagr, *K. alvarezii* green; Edbr, *E. denticulatum* brown; Edgr, *E. denticulatum* green. Bars indicated standard error.

Post hoc comparisons by means of the Tukey HSD test specified the significant difference among means in terms of time, strain and Farm. In terms of time (month), April was significantly different from June, August, December and January; June was significantly different from April, August, October, December and January; August was significantly different from April, December, June and October; October was significantly different from August, December, January and June; December was significantly different from April, August, October, January and June; and January was significantly different from April, December, June, and October.

In terms of strain, *K. alvarezii* brown and *K. alvarezii* green were significantly different from *E. denticulatum* brown and *E. denticulatum* green and vice versa, but not significantly different from each other. This also happened with *E. denticulatum* brown and *E. denticulatum* green. In terms of Farm, each Farm was significantly different from each other.

For the samples from Zamboanga del Norte, the highest incidence and degree of infection were observed in *K. alvarezii* brown Farmed in Jose Dalman, mean = 66.67% ± 4.55 and 13.61% ± 1.25, respectively; while the lowest was observed in *K. alvarezii* brown Farmed in Rizal, mean = 2.22% ± 1.41 and 0.09% ± 0.06, respectively (Fig 8).

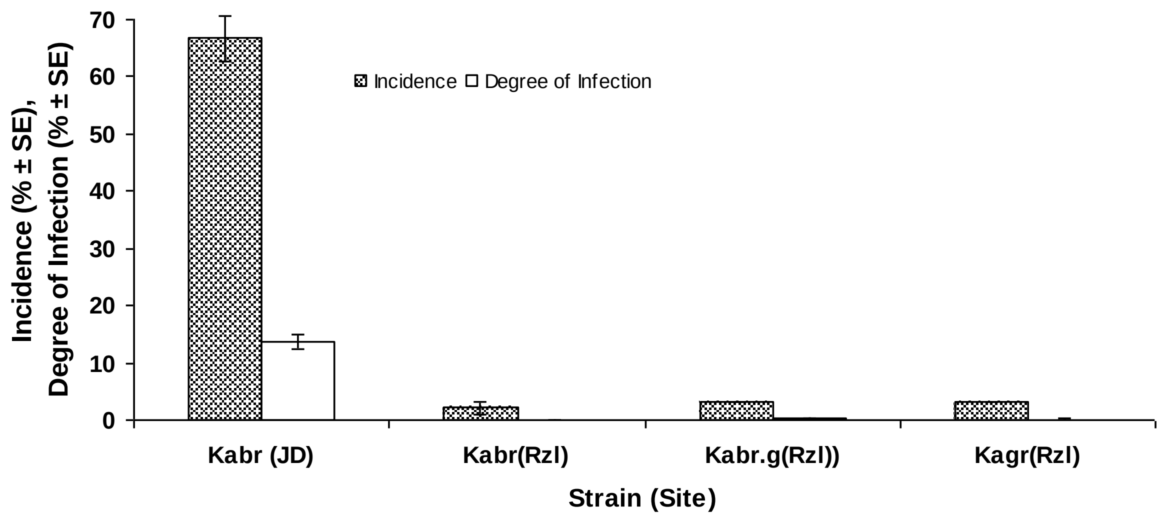


Figure 8. Mean incidence and degree of infection of *K. alvarezii* farmed in Zamboanga del Norte. Kabr, *K. alvarezii* brown; Kagr, *K. alvarezii* green; JD, Jose Dalman; Rzl, Rizal. Bars indicated Standard Error.

Discussion and Conclusion

Based on the Post hoc test, the incidence and the degree of infection of *ice-ice* disease were significantly different in terms of species/strain, time (month), and Farms, indicating varying resistance to the disease. The one with the least incidence and infection were the most resistant to ice-ice disease. Result showed that *E. denticulatum* was more resistant than *K. alvarezii*, while between strains, the incidence and the infection gave varying results. The higher susceptibility of *K.* alvarezii compared to *E. denticulatum* can be due to the differences in carrageenan types and genetic makeup. In addition, the higher susceptibility may be exacerbated by the surface texture of the thalli based on field observations. *E. denticulatum* thallus has a smoother surface compared to *K. alvarezii*. The rough thallus surface of *K. alvarezii* enables the dirt, epiphytes, parasites, and other microorganisms to easily attach to it (Fig 9). The more they can affix and establish themselves on the thalli surface, the more they can develop into a good medium for pathogens to breed and establish and later will permeate to the cortex and medulary stratum of thalli, making the thalli to become weaker. In addition, dirt and epiphytes can also decrease light absorption of the thalli. These conditions can make the thalli prone to bacterial infection.

According to Largo (1995), Uyenco (1981) and Trono (1999), crowding of plants due to high planting density and the obstruction due to the presence of epiphytes in the immediate vicinity of each thallus may produce an artificial shading effect that is detrimental. Thus, one of the factors associated with high *ice-ice* occurrence in the field is the high incidence of epiphytes. In addition, the mutual effect of stress and biotic agents, such as opportunistic bacteria are prime factors of the *ice-ice* disease (Largo, 1999). The infection of the seaweed by these pathogens was found by Largo to depend initially on the bacteria's ability to establish themselves on the seaweed surface. However, he found this bacteria's ability to be influenced by the constituents of the bacterial community, probably with other concomitant micro-organisms, like epiphytes.

The other factor is environmental condition. Seaweed farming is good at some point in August and January, when seaweeds are in good condition and contain high resistance to *ice-ice* disease in terms of time (month). On the other hand, April, October and December are the months when seaweeds are susceptible to *ice-ice* disease. This observation could be associated with the water quality, such as circulation of nutrient, current speed/water movement, light intensity, pollution, temperature and salinity. Unfortunately, salinity and temperature data and some secondary data like rainfall and tidal fluctuation are only covered by this study. The months of August and January encompass a lower mean temperature, high sea level, moderate mean salinity, and rainfall and incidence appeared at the minimum. October and April appeared to have the highest incidence when the farms have the highest mean temperature, lowest sea level, highest rainfall and lowest salinity.

When the sea level was below the minimum, the plants were more exposed to the air. The mutual effects of air and light exposure which augmented the ambient temperature, stressed the seaweeds which resulted to seaweed bleaching. This outcome in which high temperature in the summer months (occasionally reaching more than 30°C in some places during low tides), coupled with elevated light intensity and low water movement are the prime factors that trigger ice-ice development in the cultivation ground conforms with the findings of Largo (2006). According to Ask & Azanza (2002), temperature of up to 33-35°C causes wide-scale whitening that lead to total damage of the branches. Incidence and infection, however, were also elevated in Farm 1 and 2 where pollution was high for the period of December. According to the farmers, the sugar mill factory released their waste products into the Bay, mainly in Okiot in the months of May and December. This detail explicated that water contamination appeared to be one of the significant factors that triggers *ice-ice* disease incidence.

In terms of Farms, the major disparities on the incidence and infection of *ice-ice* disease among Farms imply the influence of various environmental conditions, hence, water quality. Temperature was consistently high in Farm 1 while salinity was consistently low (a river delta was nearby). It was also more exposed to the high light intensity during low tide than the other farms. On the other hand, the pollution from human activity was higher due to the small distance from the communities. These factors made the incidence and degree of infection of *ice-ice* disease to be higher in Farm 1.

The degree of infection during the month of April was higher than what was obtained by Briones (2004) in Bais Bay, especially for *K. alvarezii* as documented in this study. The infection in Briones's study ranged from 17.29 – 33.69% for *K. alvarezii;* and 2.78 – 8.66% for *E. denticulatum;* while in this study, *K. alvarezii* ranged from 21.195 to 37.42% and 5.825 to 7.01% for *E. denticulatum*. This increase could be related to the changes of environmental condition in the Bay, mainly, increasing amount of pollution due to increasing human activities near the Farms; and increasing temperatures as an effect of global warming.

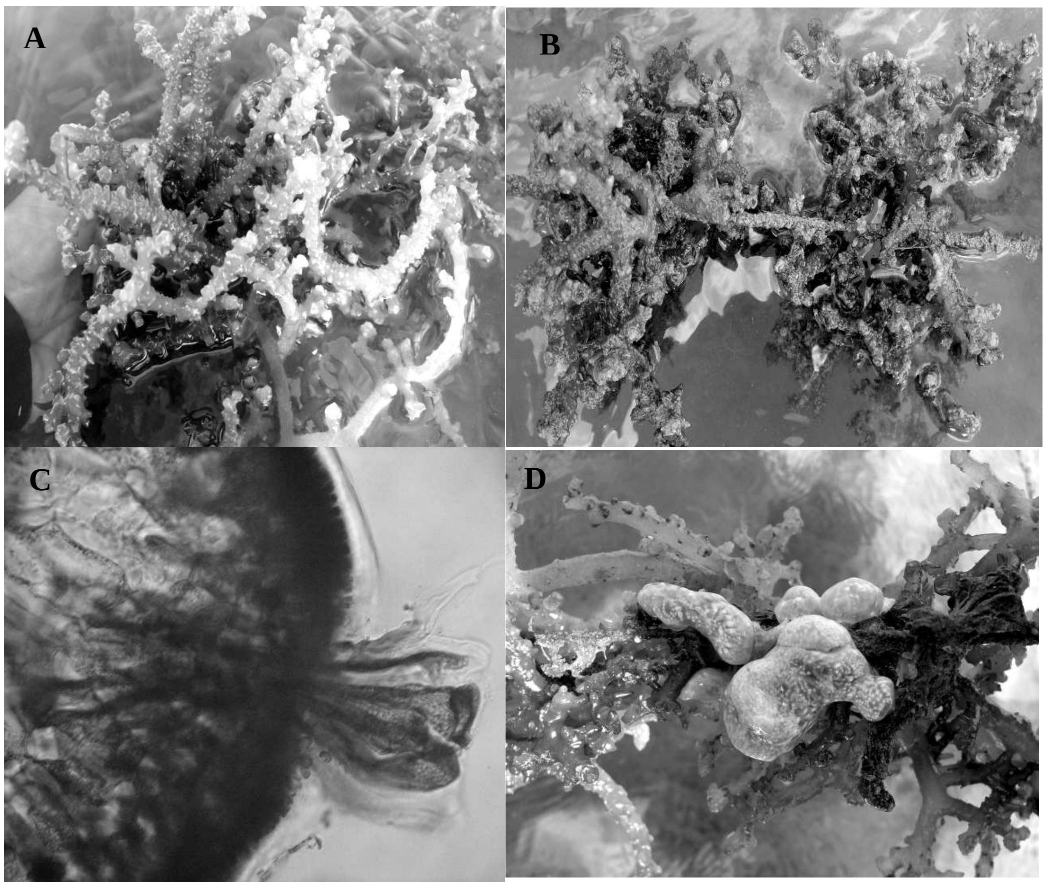
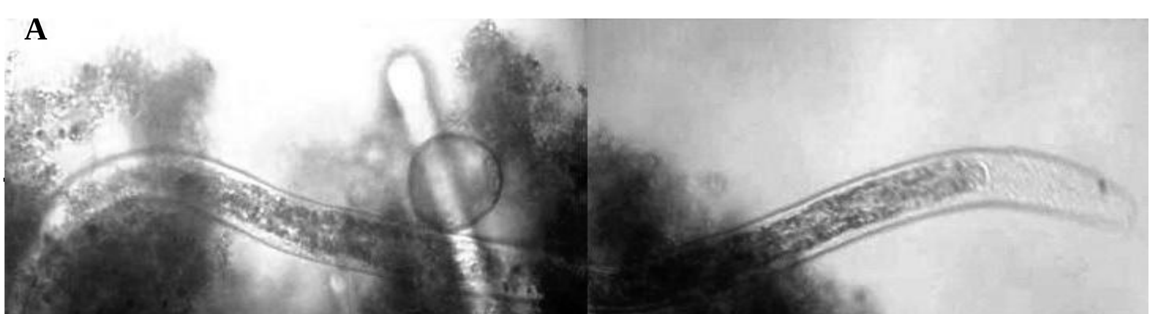


Figure 9. The thalli conditions of *E. denticulatum* and *K. alvarezii* in farming areas. A. bleached thalli of *E. denticulatum*; B. *K. alvarezii* thalli loaded with dirt, silt and epiphytes; C. epiphytes penetrating the thallus; D. Tunicates attachedto the thalli during high incidence of ice-ice disease in October.



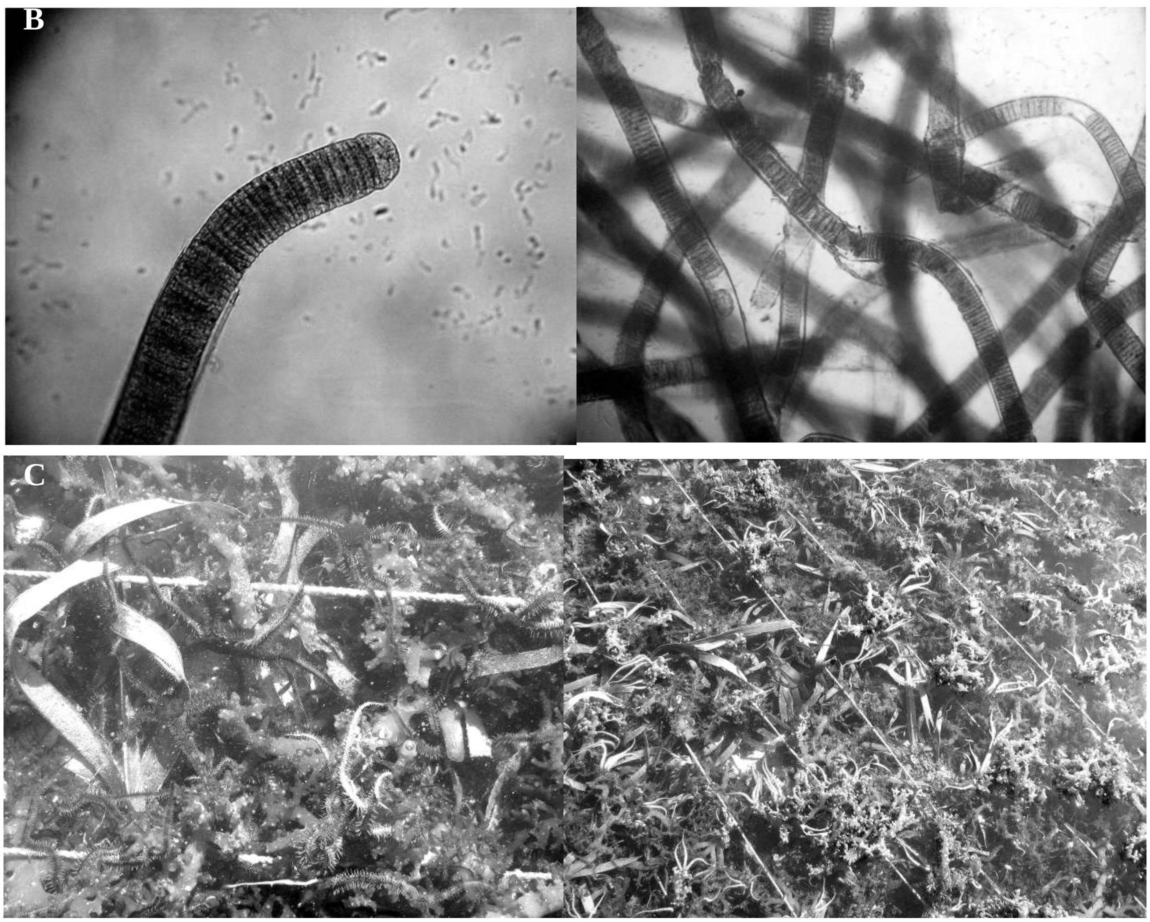


Figure 10. Namatode (A) and *Lyngbya* sp. (B) found in the culture of infected thallus in Laboratory. C, Brittle stars found associated with the healthy *K. alvarezii* during the lowest incidence of ice-ice on January.

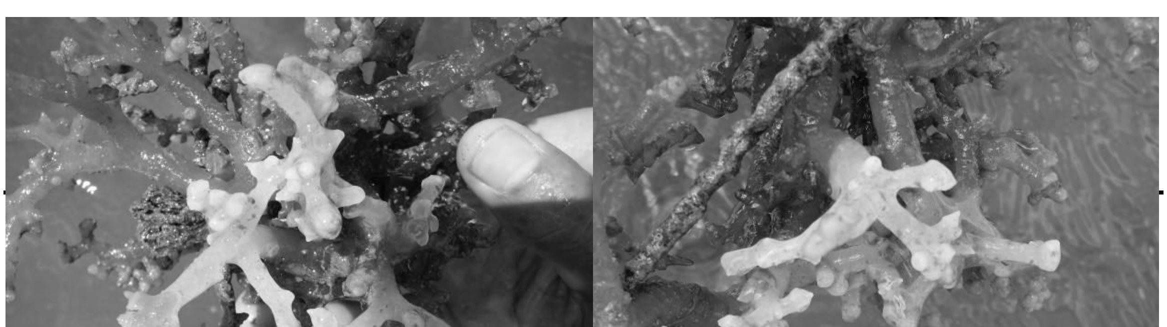


Figure 12. Plants with wide-scale whitening of *ice-ice* disease caused by high temperature.

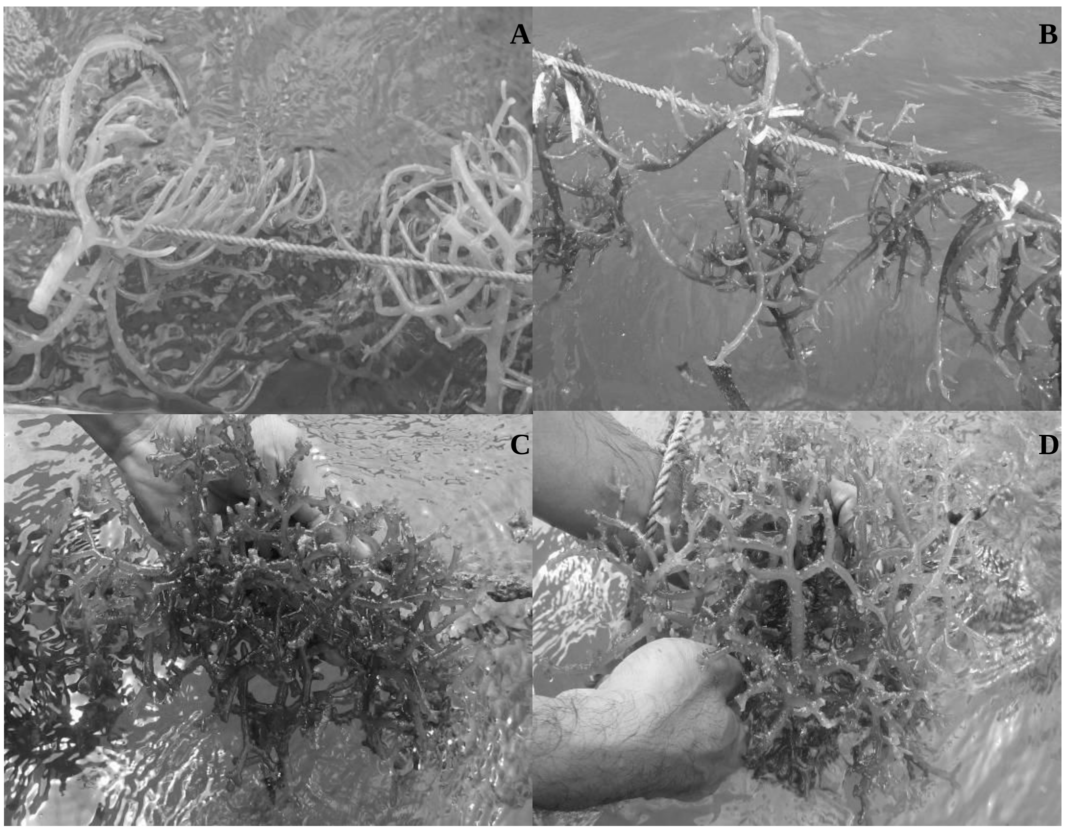


Figure 13. Different strains with different resistance to ice-ice disease, farmed in Zamboanga del Norte. A: *K. alvarezii* from Jose Dalman, its ice-ice incidence is high; B. *K. alvarezii* brown giant from Rizal. C, D. *K. alvarezii* brown and green from Rizal**.**

Briones (2004) recorded water temperatures between 29.6 to 32°C, while in this study, temperatures were more uniform, ranged from 32.07 to 32.33°C. For the plants in Zamboanga del Norte, result showed that different strains have different resistance. *K. alvarezii* from Rizal, were more resistant than *K. alvarezii* from Jose Dalman (Fig 13).

Kappaphycus alvarezii and *Eucheuma denticulatum* showed different resistance to *ice-ice disease*, according to time (month), strain and location. *Eucheuma denticulatum* was more resistant and less susceptible. Moreover, the occurrence of *ice-ice* disease is influenced by environmental conditions especially temperature and light intensity.

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