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CHAPTER 8

Kappaphycus (Rhodophyta) Cultivation: Problems and the Impacts of Acadian Marine Plant Extract Powder

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1 Introduction

Kappaphycus is one of the most significant, economically valuable red seaweeds, cultivated in tropical and sub-tropical waters. This alga demands a relatively high market value globally, due to applications of the kappa carrageenan colloid that is industrially extracted from the biomass. Carrageenan is widely used in food, pharmaceuticals, nutraceuticals and for aquaculture applications.

The first successful commercial cultivation of *Kappaphycus* (previously called *Eucheuma*) was recorded from the southern Philippines. It took more than five years of field trials from 1967 to the early 70s in order to domesticate *Kappaphycus* for reliable commercial cultivation. The first commercial quantities of "cottonii" produced from extensive cultivation

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were obtained in 1974 with a total production of 8,000 t. Dramatic increases in production were achieved and the Philippines was the leading producer of *Kappaphycus* for 33 years until it was overtaken by Indonesia in 2008. It was in 1978, when *Kappaphycus* farming first saw successful adoption in Indonesia under the initiative of the Copenhagen Pectin Factory. Due to these successes, *Kappaphycus* farming has also grown commercially in East Africa, Fiji Is., India, Malaysia, Vietnam, Cambodia, Myanmar and southern China, although their volumes are minimal when compared to Indonesia and the Philippines. On the other hand, Latin American (Argentina, Colombia, Brazil, Equador, Venezuela, Mexico, and Peru) and the Caribbean countries (St. Lucia, Jamaica, and Panama) are even more recent entrants to *Kappaphycus* cultivation. Most information comes from published pilot-plot, demonstration farms and scientific studies, as such, only relatively small commercial quantities are produced presently.

The success of seaweed aquaculture in the Philippines and Indonesia has been dominated by various species and strains of two major genera: viz. *Kappaphycus alvarezii* (i.e., the '*cottonii*' type and source of kappa carrageenan) and *Eucheuma denticulatum* (the '*spinosum*' type and source of iota carrageenan). Once these species were domesticated, they comprised a relatively small genetic stock and their propagation has been mainly vegetative, i.e., repeated cutting of young branches from the same plant. The same method was practiced by the seaweed farmers from the start of farming and continues to present times. It is surprising that although there have been attempts to use cultivars developed from spores and tissue culture, the application of this relatively mainstream technology is still very much in its infancy, as applied to carrageenophyte cultivation. Furthermore, *in situ* proving and testing through field trials are still required to test the viability of the 'new plants' developed.

Not unlike the development of vegetatively developed terrestrial crops, the early years of commercial farming of *Kappaphycus* were beset with problems, of which 'ice-ice' malaise and macro-epiphytism were predominant. These problems still persist today. Epiphytic Filamentous Algae (EFA) infestations are a more recent problem which adversely affected the quality and quantity of the seaweed. One of the most notable negative effects of severe EFA infestation by *Neosiphonia* (a filamentous red alga), with a concomitant 'ice-ice' malaise incidence, is the scarcity, or the lack of availability of good quality propagules for re-planting purposes. Recent reports showed that dipping the cultivars in Acadian Marine Plant Extract Powder (AMPEP), a commercial extract of the brown seaweed *Ascophyllum nodosum* resulted in more vigorous 'seedlings' and increased resistance to 'ice-ice' malaise, epiphytes and particularly *Neosiphonia* infestation. Good aquaculture practices and improved and consistent management practices have, likewise, contributed to improved productivity and production.

The socio-economic impacts of seaweed farming on coastal communities have been overwhelmingly positive. Family-unit-type operations are far more advantageous than the initial corporate-owner-operator style farms, especially in remote areas where coastal communities are faced with a limited number of opportunities for alternative economic activities. There are few published articles, but several anecdotal reports state that seaweed farming has improved the economic status of coastal fisherfolk. However, the profitability of seaweed farming during 'ice-ice' outbreaks and *Neosiphonia* infestations, has been severely negatively impacted because the carrageenophyte crops have been damaged which in turn affected productivity and importantly the carrageenan quality and quantity, such that there have been significant downturns in availability of crops for commercial harvest.

A limited number of papers have shown the impact of 'ice-ice' outbreaks and *Neosiphonia* infestations on seaweed farming. Surface seawater temperature, salinity, water movement and light intensity, all acting in combination, play significant roles in *Kappaphycus* health and growth. It is not conclusively established if these changes are in fact related to global climate change in surface seawater temperature and/or other environmental and/or biological variables. The economic impact of the decline of *Kappaphycus* in some areas, especially the Philippines, requires more intensive and regional investigation to establish cause and effects. There is an important need to sustain carrageenophyte farming due to the many inter-linked economic and coastal development benefits which can be derived, if practiced and maintained in a sustainable manner. The key players along the value chain must work in harmony providing increased and sustained efforts to continue to provide the extraction industry with this high-value, natural colloid. If this is not the case, the hydrocolloid industry could switch to other sources of raw materials with similar or competing rheological properties.

The consumption and utilization of seaweed worldwide is associated with a myriad of products that generate near US$ 8 billion per year (FAO 2010). Almost 90 percent is food products for human consumption; the remainder is for the hydrocolloid industry focused on agar, carrageenan and alginates. Macroalgae for direct or indirect consumption (i.e., coloring, flavoring agents and biologically active compounds sold as dietary supplements) have been gaining market share mainly due to the shift of men and women of traditional uses of seaweed in their daily lives. A good example is the United States and certain countries of South America which in the last 15 years had an 83 percent increase in domestic consumption of products derived from algae, much of which has been provided by the popularization of Asian food (McHugh 2003, FAO 2010).

Seaweed cultivation has expanded over the years, fueled by the growing demand for macroalgal raw material by an industry that initially relied on the exploitation of natural beds (Pickering et al. 2007). This demand necessitates a search for new technologies to improve macroalgal production, seeking more effective ways to produce biomass and also more resistant individual cultivars (Zemke-White and Ohno 1999, Ask and Azanza 2002).

Records of the first "crops" of *K. alvarezii* date back to the 1960s in southern Mindanao, Philippines using seedlings derived from individuals collected from natural beds (Bindu and Levine 2011). After four decades, the cultivation of *K. alvarezii* and *Eucheuma denticulatum* (N.L. Burman, F.S. Collins and Hervey) is responsible for 88 percent of the world's production of kappa (*K. alvarezii*) and iota (*E. denticulatum*) carrageenan, producing 120,000 dry tons/year. The main producing countries are the Philippines, Indonesia and Tanzania (Ask 2001, Hayashi et al. 2010, Bindu and Levine 2011). However, *K. alvarezii* crops can be found in over 20 countries located at latitudes near 10° which allow ideal conditions for the cultivation of the species (Hayashi et al. 2010).

2 History of *Kappaphycus* Farming

In the mid 1960s, experimental farms for *Kappaphycus* cultivation were first established in southern Philippines by the Marine Colloids Philippines, University of Hawaii, Bureau of Fisheries and Aquatic Resources and the University of the Philippines. In 1972, commercial quantities of *Kappaphycus* (then called *Eucheuma* and "*cottonii*") were harvested from the cultivation areas. A continuous supply of dried '*cottonii*' has been exported (Doty and Alvarez 1981) from that time to the present day. Vegetative propagation has been the only widely used method employed for developing biomass for cultivation. Young, robust and healthy branches were cut from a selected 'mother plant' and then tied to soft plastic rope previously wound around a cultivation rope (i.e., monofilament rope, polyethylene rope, or flat binder). Five methods of culturing *Kappaphycus* were introduced in the Philippines in 1973 (Doty 1973, Ricohermoso and Deveau 1979): (1) off-bottom monoline, presently called fixed-off bottom, (2) broadcast, (3) floating bamboo, (4) net system, and (5) the tubular net. However, the off-bottom monoline, single floating-raft and hanging long-lines (adapted in shallow waters) are the most popular methods adopted in most recent times, not only in the Philippines (Hurtado-Ponce et al. 1996, Hurtado and Agbayani 2000, Hurtado et al. 2008) but also in other countries such as Fiji (Luxton et al. 1987), Hainan, China (Wu et al. 1989), India (Mairh et al. 1995, Eswaran and Jha 2006, Kaliaperumal et al. 2008, Bindu and Levine 2011), Indonesia (Adnan and Porse 1987, Firdausy and Tisdell 1991, Luxton 1993), Kiribati

(Luxton and Luxton 1999), Madagascar (Mollion and Braud 1993), Tanzania (Lirasan and Twide 1993), and Vietnam (Ohno et al. 1996). Over time and with gains in experience, innovations were introduced, e.g., the free swing method (Fig. 1a; Hurtado et al. 2008), multiple, raft long-lines (Fig. 1b; Hurtado and Agbayani 2002, Hurtado 2007); and the 'spider web' (Fig. 1c; Hurtado 2007, Hurtado et al. 2008) which are adapted for practices in deeper waters. Table 1 summarizes the history of *Kappaphycus* farming and its introduction to other tropical and sub-tropical countries.

The Americas and the Caribbean countries are new entrants to *Kappaphycus* farming. Optimal conditions for farming can be found in these countries that could possibly provide a great potential, if correctly administered. However, the biggest concern of the introduction in these countries is the potential environmental risk of a non-native species. A series of bio-invasions have been attributed to the introduction of *K. alvarezii* for farming purposes in Hawaii (Rodgers and Cox 1999, Conklin and Smith 2005) with later reports in Venezuela (Barrios 2005) and India (Chandrasekaran et al. 2008). However, other researchers found that the 'invasiveness' of this species is restricted to sites that are already affected by anthropogenic environmental perturbations (Pickering et al. 2007). Moreover, the lack of culture and tradition of seaweed farming, and poor government incentives have unfortunately not led to a concerted effort to solve the problems. Thus academia has taken up the challenge of generating alternatives in biotechnology and simple reliable techniques to ensure that eucheumatoid cultivation in the Americas and Caribbean countries remains possible. Since these countries are new to *Kappaphycus* cultivation, farming practices of each country will be presented briefly.

2.1 Brazil

The exotic species *K. alvarezii* is the main macroalga that is commercially cultivated in Brazil (Pellizzari and Reis 2011, Góes and Reis 2011). Its success is explained by the relatively easy management techniques which are employed (i.e., fragmentation of the thallus) and the species' cultured resilience to local abiotic factors that resulted in high growth rates (Ask 1999, Ask and Azanza 2002).

The introduction of *K. alvarezii* into Brazil took place at Ubatuba Bay, São Paulo State by Dr. Édison José de Paula and his research group from University of São Paulo. It was responsibly introduced as a seedling clone from Japan which had originated in the Philippines. After a quarantine procedure of 10 months, a series of tests were conducted, not only with *K. alvarezii* (Paula et al. 2002), but also with *Kappaphycus striatum* (F. Schmitz) Doty ex P.C. Silva, which produced viable tetraspores and was removed from the sea in order to avoid environmental risks (Bulboa et al. 2007).

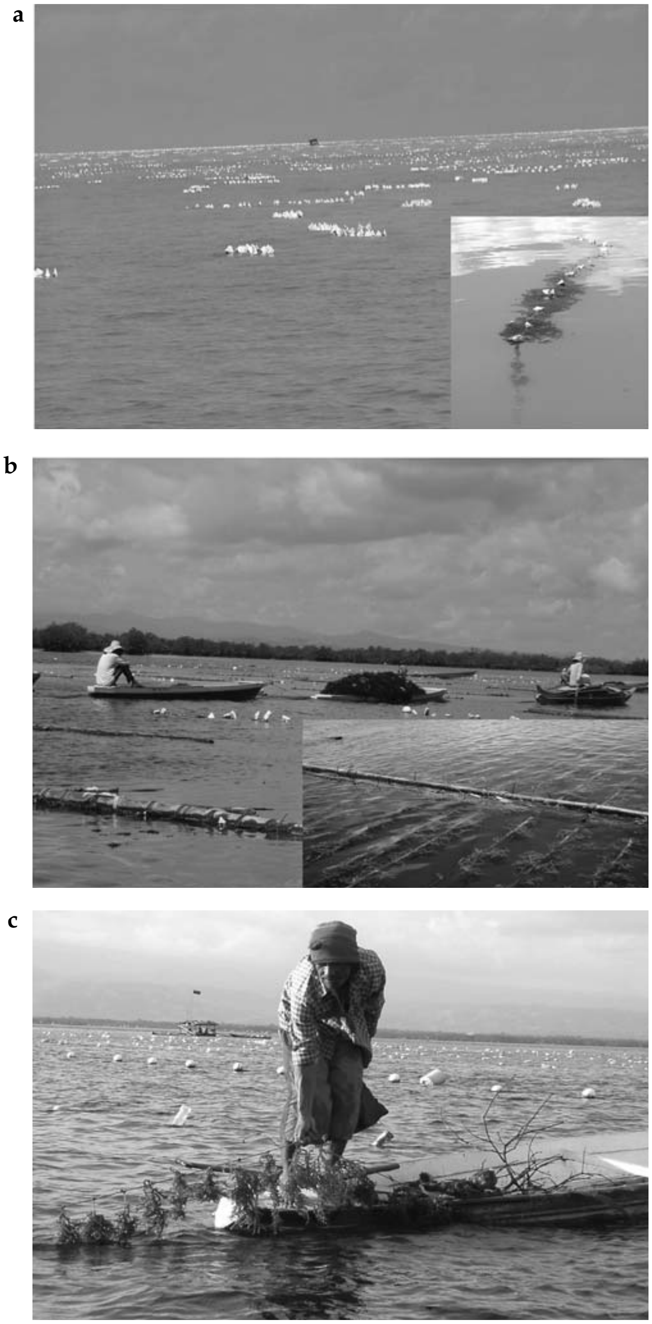

Figure 1. (a-c) Innovations in the cultivation of *Kappaphycus* in deeper waters (Photos courtesy of A.Q. Hurtado): (a) free swing; (b) multiple raft long line; (c) spider web.

Table 1. History of *Kappaphycus* farming and its introduction to other tropical and sub-tropical countries.

Year of introduction	Year of commercialization	Country	Culture technique	Reported by
1967	1971	Philippines (Sulu archipelago)	off-bottom monoline, broadcast, floating bamboo, net and tubular	Doty 1973, Doty and Alvarez 1981, Parker 1974
			off-bottom monoline, hanging long-line	Neish and Barraca 1978, Ricohermoso and Deveau 1979
1969	1978	Indonesia	floating rafts	Soerjodinoto 1969, Soegiarto 1979, Adnan and Porse 1987
1976	1982	Fiji Islands	off-bottom monoline	Booth et al. 1983, Luxton et al. 1987, Prakash 1990
1977	1986	Kiribati	off-bottom monoline, long line	Why 1985, Uan 1990
1978	1978	Sabah, Malaysia	off-bottom monoline	Doty 1980
1984	1989	Solomon Islands	off-bottom monoline	Smith 1990, Kronen et al. 2013 (in press)
1985		Micronesica		Doty 1985
1985	1989	Zanzibar & Pemba Is, Tanzania, East Africa	off-bottom monoline	Mshigeni 1985
				Eklund and Patterson 1992, Lirasan and Twide 1993
				Msuya et al. 2007, Shechambo et al. 1996
1985	1986	Hainan, China	hanging long line	Wu et al. 1989
1989/1995	1998	Tamil Nadu, India	bag and net methods	Mairth et al. 1995, Krishnan and Narayana 2013 (in press)
			raft method	
1990	1991	Madagascar	off-bottom monoline	Mollion and Braud 1993

Table 1. contd....

Table 1. contd.

Year of introduction	Year of commercialization	Country	Culture technique	Reported by
1991	Data not available	Colombia	floating rafts	Rincones 2006
1993	1998	Vietnam	off-bottom monoline (lagoon)	Ohno et al. 1995, 1996
			pond cultivation	
			raft cultivation (off-shore)	
			fixed-off bottom	Pham 2009, Le 2011
			floating rafts	
1995	1998	Brazil	floating rafts	Paula et al. 2002, Castelar et al. 2009
1996	Data not available	Kenya	netting bags	
1996	Data not available	Venezuela	floating rafts	Rincones and Rubio 1999, Smith and Rincones 2006
1999	2000	Cambodia	hanging long line	Cambodia Ministry of Fisheries
1999	2002	Gulf of Mexico	fixed off-bottom, floating system	Munoz et al. 2004
2002	2008	Panama (Colon)	floating rafts	Batista 2006, 2009
2007	2008	Myanmar	hanging long line	The Myanmar Times, July 23–29, 2008
2010	Data not available	Equador	off-bottom monoline (enclosed tanks)	Equator Ministry of Fisheries and Aquiculture
2011	2011	Saint Lucia, Saint Martin & Grenadines	floating rafts; off-bottom	Rincones and Sepulveda, pers. communication

The first commercial farming in Brazil was located at Ilha Grande Bay, Rio de Janeiro State by Miguel Sepúlveda, in 1998, after a quarantine period in tanks at the University of Santa Catarina University, using seedlings which were brought from Venezuela (Castelar et al. 2009). In 2004, another farm was installed in Sepetiba Bay, Rio de Janeiro State, by Sete Ondas Biomar Cultivo de Algas Marinhas Ltda., in an enterprise that occupied over 20 ha; which became the biggest farming site for *Kappaphycus* in Latin America. However, this enterprise was not long-lived and shut down operations in 2008 due to high operational costs. After that, small farms were transferred to the southern coast of Sepetiba Bay (Góes and Reis 2011). Presently, most of the commercial cultivations are at Ilha Grande Bay, to the south of Rio de Janeiro State, and belong to private companies with no attached government initiatives.

In Brazil, the cultivation techniques used for *K. alvarezii* are comprised of floating rafts made with PVC tubes (Figs. 2a-e), differing from the most widely used techniques in cultivation of this species in other countries, i.e., the off-bottom systems (Hayashi et al. 2010). The adaption of these Brazilian techniques is mainly due to higher water turbidity and muddy bottom substrata at the cultivation sites, making the off-bottom techniques impossible.

Góes and Reis (2011) discussed the use of a novel culture technique, i.e., the tubular net (Fig. 2b). The floating rafts of PVC are retained; however tubular networks stuffed with *K. alvarezii* seedlings replaced the traditional 'tie-tie'. This technique showed no difference in daily growth rates of seedlings or carrageenan yield and quality when compared to the 'tie-tie', but saved time and had lower costs and provided ease of management.

2.2 Colombia

Kappaphycus alvarezii was introduced in Colombia in 1991, through an FAO funded program supervised by Prof. Germnán Bula Meyer. The Cuban seedlings, which originated from Venezuela, were quarantined and acclimatized in tanks before being used for cultivation (R.E. Rincones, personal communication).

Presently, branches of the original FAO program, now funded by the non-governmental organization Fundación Terrazul at Cabo de La Vela in the La Guarija Peninsula, works with Native American Wayuu communities on experimental farms using floating rafts and tubular nets in unused shrimp breeding tanks. However, these attempts to create a semi-closed cultivation system, to lower the risks of bio-invasions, seemed to be ineffective since *K. alvarezii* was reported to occur along the La Guarija coastline since 2004 (Rincones 2006).

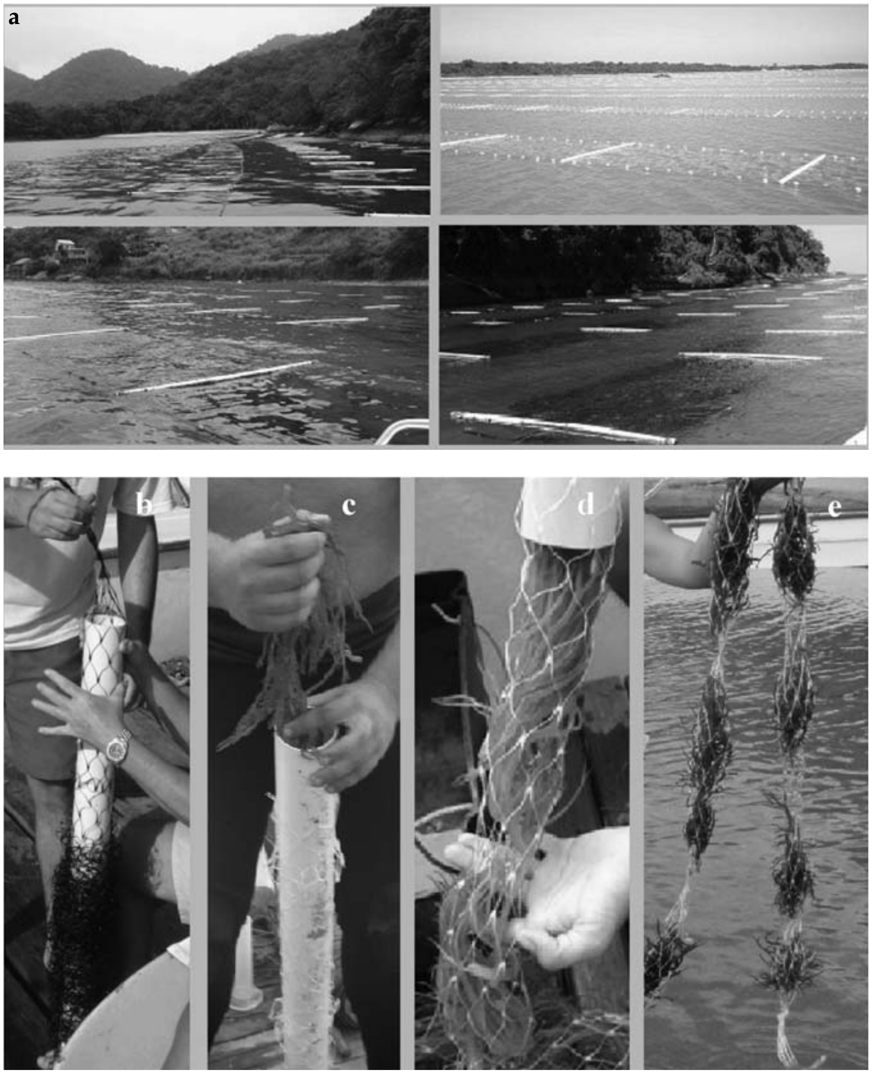

Figure 2. (a-e) Cultivation methods in Brazil, South America (Photos courtesy of RP Reis and R.R. Loureiro): (a) PVC raft; (b) tubular net; (c-d) stuffing of seedling into the tubular net; (e) tubular net with *Kappaphycus* 'seedlings'.

Color image of this figure appears in the color plate section at the end of the book.

2.3 Equador

Kappaphycus seedlings were introduced by a Brazilian seaweed consultant into Ecuador to be part of an enterprise called Ecuaalgas S.A. which was supported by the local government through the Ministry of Fisheries and Aquiculture (MAGAP). The cultivation is still restricted to the enclosed disused shrimp breeding tanks where their acclimation and quarantine process took place, being set in the off-bottom method.

In these tanks, *K. alvarezii* is afflicted by 'ice-ice' and consequent epiphyte infestation creating major seedling losses with no apparent solution (Sepúlveda, personal communication).

2.4 Mexico

Muñoz et al. (2004) proposed the cultivation of *K. alvarezii* at the Yucatan Peninsula obtaining very promising initial results. This activity did not receive government support, even though the project was aimed towards a sustainable cultivation program conducted by fishermen and coastal communities through social cooperatives.

The seedlings used were originally from Brazil and were quarantined following the rules of the Official Mexican Norm (NOM). The farming practice was mostly the off-bottom cultivation technique; there were no reports of problems with either herbivores or epiphyte infestations. The main cause of crop losses in this region seemed to be due to 'ice-ice', which was most frequently observed when the seawater temperatures exceeded 30°C. This is possibly a problem that could be solved by making use of floating rafts (Robledo, personal communication).

2.5 Panama

Alongside Brazil and the Caribbean countries of Saint Lucia, Saint Martin and Grenadines, Panama is one of the few that has commercial farms of *K. alvarezii* in the Americas having exported its production to Europe and Asia (Vega and Rincones, personal communication).

K. alvarezii seedlings, from Venezuela, were originally introduced by the Smithsonian Tropical Research Institute group with no previous quarantine procedure. The material was for experimental purposes in the Colón Province, the location of the only commercial site in Panama; activities were supported by private companies, such as Panamá Seafarms and Bansistemas S.A., overseen by the local government through the Panama Authority of Aquatic Resources (ARAP). Moreover, in the Bocas del Toro Province, experimental studies were being conducted using the same floating rafts and 'tie-tie' system used in the Colón Peninsula farms (Batista 2006, 2009).

When the seedlings were originally brought to the Colón Peninsula, infestations of epiphytes (i.e., red, green and brown macroalgae) seemed to be a major concern negatively influencing *K. alvarezii* daily growth rates and causing crop losses (Batista 2009). Today, there are no reports of epiphytes, however, the occurrence of 'ice-ice' and losses due to herbivores seem to be of great concern to the local farmers (R.E. Rincones, personal communication).

2.6 Saint Lucia, Saint Martin and Grenadines

With no apparent government support, and relying on private companies and non-governmental organizations, such as Ashton Multipurpose Cooperative AMCO and the Sustainable Grenadines Project, these Caribbean countries were one of the few to develop fully functional *K. alvarezii* farms, with the original stock coming from Venezuela. These seedlings were introduced in 2011, with no apparent quarantine procedure (R.E. Rincones and Sepúlveda, personal communication).

A myriad of available cultivation techniques were employed and varied according to the farming site, and ranging from floating rafts to off-bottom long-lines (both of which utilized 'tie-tie'). There are no reports of epiphyte infestations. 'Ice-ice' and herbivores are issues in these countries, as in most of the Americas, and continue to be the biggest impediment to productivity (R.E. Rincones, personal communication).

2.7 Venezuela

K. alvarezii was legally introduced to Venezuela; the seedlings originated from the Philippines in 1996. A six week acclimation and quarantine process was applied. The project had full government support, through the National Institute of Agropecuary Investigations (INIA) and the Rural Development, Innovation and Capacitation Foundation, Venezuela conducted experimental studies with *K. alvarezii* in the Araya Peninsula and Cubagua Isle (Rincones and Rubio 1999, Smith and Rincones 2006).

Those studies provided the information necessary for the government to permit the farming of an exotic species along the Araya Peninsula (Sucre State), using floating rafts with tubular nets and 'tie-tie'; techniques varied depending on the cultivation site. Tubular nets were more common in places with the greatest water motion so as to avoid crop losses. So far no reports of complications due to epiphyte infestations have been were reported from the region, while a small degree of 'ice-ice' and herbivore grazing were the only problems reported (R.E. Rincones, personal communication).

3 Global Production of '*Cottonii*' and '*Spinosum*'

The first successful commercial cultivation of *Kappaphycus* (previously called *Eucheuma*) was recorded from the southern Philippines. It took more than five years of field trials from 1967 to the early 70s in order to domesticate *Kappaphycus* for reliable commercial cultivation. The first commercial quantities of '*cottonii*' produced from extensive cultivation, were obtained in 1974 with a total production of 8,000 t. Thereafter, the Philippines continued to cultivate and export dried *Kappaphycus* to the present. Figure 3 shows the production of '*cottonii*' (*Kappaphycus*) and '*spinosum*' (*Eucheuma*) from 2002–2009. The '*cottonii*' production of the Philippines first showed signs of decline in 2003. Production was regained the following year, however, it steadily decreased thereafter. From the start of commercial seaweed farming, the Philippines retained the status of number one producer in the world for '*cottonii*'—*Kappaphycus*. However, at the same time, Indonesia's proximity to the Philippines made for easy and rapid transfer of seaweed farming technology and seedlings. Through the initiative of the Copenhagen Pectin Factory in 1978, the introduction and successful adoption of *Kappaphycus* farming in Indonesia was made possible. The geographic location of Indonesia, occupying about 65 percent of the total shoreline within the 10° latitude of the Coral Triangle creates an ideal environment for the adoption of seaweed farming in coastal waters. The Philippines has

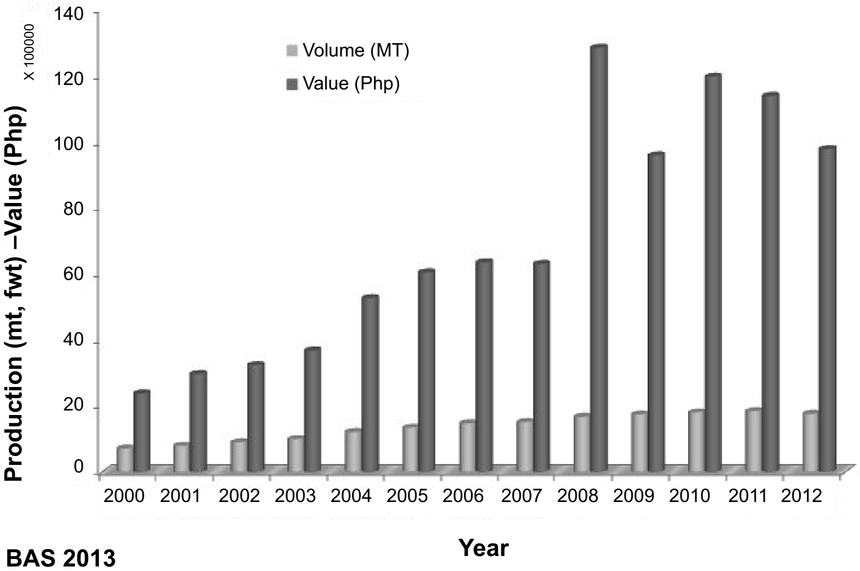

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Figure 3. Production (dwt, MT) of 'cottonii' in the Philippines from 2000–2012.

only 15 percent of the total area of the Coral Triangle and the remaining 20 percent is divided among Malaysia, Papua New Guinea, Timor Leste and the Solomon Islands (I.C. Neish, personal communication). The ideal geographic location of Indonesia, coupled by a receptive, iterative approach to seaweed farm development, accompanied by decentralized policies of the Indonesian government, in concert with traditional 'adat' forms of village government, paved the way for the rapid increase in Indonesian production of cultivated seaweeds (I.C. Neish, personal communication). In addition, strong market linkages and the all important assistance by the Business Development Services (BDS), played significant roles in the rapid and extensive development of seaweed farms. The combined effects were such that by 2008, Indonesia became the world's number one producer of *Kappaphycus*.

Since *Kappaphycus* had also been transplanted to a number of other tropical and sub-tropical countries from as early as the mid'80s, commercial farming has also become a successful enterprise, after several years of research and trial farming in East Africa, Fiji Is., India, Malaysia, Vietnam, Cambodia, Myanmar and southern China. However, their volumes, even today, are minimal when compared to Indonesia and the Philippines (Fig. 4). On the other hand, some Latin American countries (e.g., Colombia, Brazil, Equador, Venezuela, Mexico and Peru) and the Caribbean countries

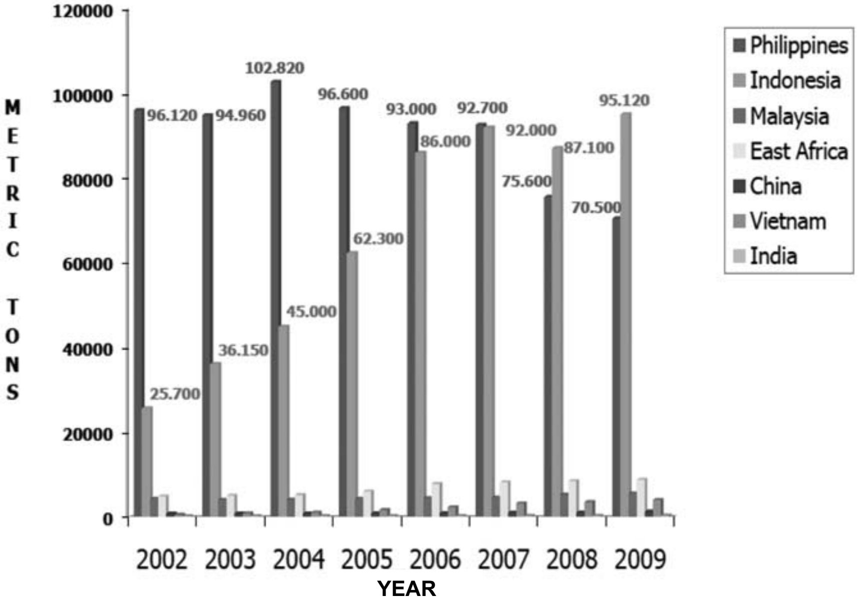

Figure 4. Global production of 'cottonii' (dwt, MT) from the major producing countries.
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(e.g., St. Lucia, Jamaica and Panama) are even more recent entrants to those countries practicing *Kappaphycus* cultivation. Most information on these activities comes from published pilot-plot, demonstration farms and scientific studies; as such, only small commercial quantities are produced presently, but the scale could increase if there is sufficient demand for the supply of raw materials.

4 Two Major Problems of *Kappaphycus* Cultivation

4.1 Lack of Availability of Good Quality Propagules (=Seedlings)

Successful seaweed aquaculture in the Philippines and Indonesia has been dominated by a relatively small number of species and, in particular, strains of just two major genera: viz. *Kappaphycus alvarezii* and *K. striatum* (Figs. 5a-b; i.e., the '*cottonii*' type and source of kappa carrageenan) and *Eucheuma denticulatum* (Fig. 6; the '*spinosum*' type and source of iota carrageenan). After these species were domesticated, they formed a relatively limited genetic stock and their subsequent multiplication has been exclusively through vegetative propagation, i.e., relatively simple, repeated cutting of young branches from the same plant. The same method of cropping and ensuring the availability of new material for the next harvest has been practiced and perpetuated by the seaweed farmers to the present time. It is somewhat surprising that although there have been attempts to use

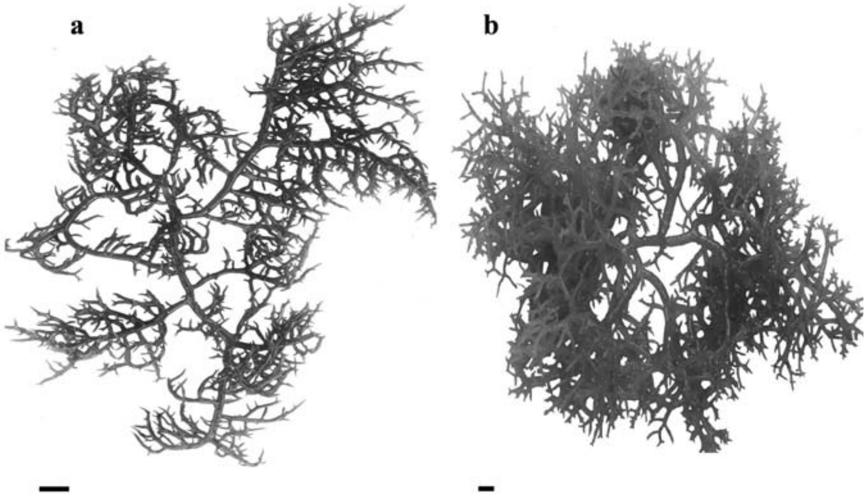

Figure 5. Two major species of *Kappaphycus*, 'cottonii' type (a) *alvarezii* and (b) *striatum*, commonly used in cultivation (bar = 1 cm, Photos courtesy of A.Q. Hurtado).

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Figure 6. 'Spinosum' type *Eucheuma denticulatum* (bar = 1 cm, Photo courtesy of A.Q. Hurtado).

Color image of this figure appears in the color plate section at the end of the book.

cultivars, as developed from spore production (see: Azanza-Corrales et al. 1996, Azanza and Aliaza 1999, Bulboa et al. 2007, 2008, Luhan and Sollesta 2010) and also tissue culture (see: Dawes and Koch 1991, Dawes et al. 1993, 1994, Hurtado and Cheney 2003, Muñoz et al. 2006, Hayashi et al. 2007, Hurtado and Biter 2008, Hurtado et al. 2009, Yunque et al. 2011, Yong et al. 2011), the application of these relatively mainstream technologies are still very much in their infancy, as applied to carrageenophyte cultivation. Furthermore, *in situ* proving and testing through field trials are still required to test the viability of the 'new plants' developed. As a consequence, the simple method of vegetative propagation prevails; unfortunately this technique has only perpetuated the limited genetic stock of the same limited number of strains or cultivars.

4.2 Occurrence of Disease and Epiphytes

Not unlike the development of vegetatively developed terrestrial crops, the early years of commercial farming of *Kappaphycus* were beset with problems, of which 'ice-ice' malaise and macro-epiphytism were predominant. These problems still persist today. Epiphytic filamentous algae (EFA) infestation was first described by Ask and Azanza (2002) as numerous species of filamentous algae that attach to the cortical layer of the host thalli. Recent EFA infestation is dominated by the filamentous red alga *Neosiphonia* previously called *Polysiphonia,* which adversely affected the quality and quantity of the seaweed. The most notable negative effects of severe EFA infestation by *Neosiphonia* with a concomitant 'ice-ice' malaise incidence is the resultant scarcity of good quality propagules for re-planting purposes.

'Ice-ice' Malaise

The early years of commercial farming of *Kappaphycus* in the Philippines and elsewhere were beset with problems, of which 'ice-ice' (Fig. 7) and macro-epiphytism (Fig. 8) were predominant. The outbreaks of 'ice-ice' malaise were most pronounced in areas with lower water quality, slow water exchange (almost stagnant), low salinity and extreme (low or high) temperatures (Doty 1987, Largo et al. 1995b). The 'ice-ice' phenomenon appeared with a greening of the normally pink/red tissue followed by whitening, like 'ice' of necrotic tissue as decay of the surface tissue lead to softening of entire or partial branches, which finally resulted in fragmentation of the thallus and considerable loss of biomass. Bacteria (e.g., *Pseudomonas, Cytophaga-Flavobacterium* complex, *Vibrio, Xanthomonas* and *Achromobacter*) have been isolated from the affected areas of tissue (Uyengco 1977, Uyengco et al. 1981, Largo et al. 1995a, Butardo et al. 2003), but no specific causative bacterial species have been linked to the 'ice-ice' malaise. Hence, it is interpreted as an indication of "stress" rather than a pathogen-induced disease (i.e., the bacteria are secondary rather than primary, causative instigators of the disease). It is likely that the bacteria

Figure 7. A branch of *Kappaphycus* with an 'ice ice' (bar = 1 cm, Photo courtesy of A.Q. Hurtado).

Color image of this figure appears in the color plate section at the end of the book.

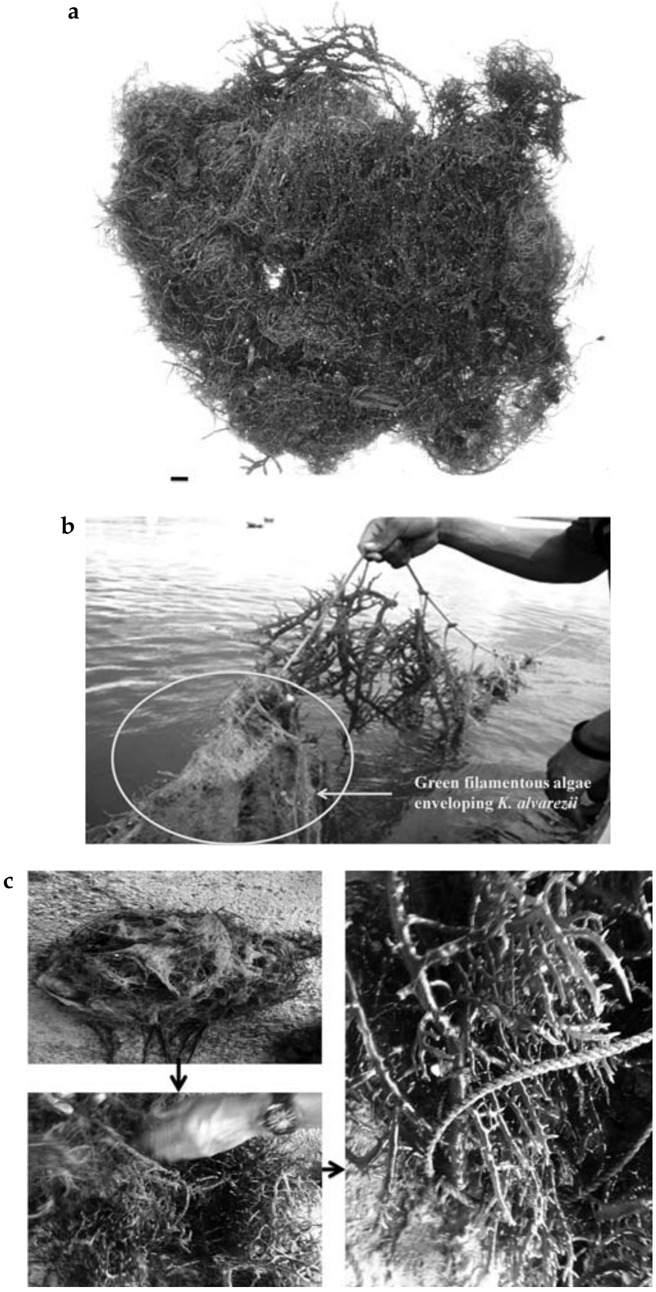


Figure 8. (a-c) Examples of macro-epiphytes (Photos courtesy of A.Q. Hurtado).

Color image of this figure appears in the color plate section at the end of the book.

are able to effectively invade the alga only at periods when the (stressed) seaweed is less able to produce and release extracellular products which may be inhibitory to the biofilm, fouling microorganisms (Uyengco et al. 1981).

The incidence and degree of infection of 'ice-ice' was observed to vary significantly between color morphotypes of *K. alvarezii* and *E. denticulatum*, time (season) and geographical location (Tisera and Naguit 2009). *Eucheuma denticulatum* was found to be more resistant to 'ice-ice' than *K. alvarezii* in Negros Oriental and Zamboanga del Norte, Philippines. High water temperature and low salinity during April and October gave rise to the higher incidence of 'ice-ice', as claimed by the authors.

Most reports on the incidence and degree of infection of 'ice-ice' by *K. alvarezii* and *E. denticulatum* were mainly due to the interplay of ecological factors and secondary infections by pathogenic marine bacteria (Uyengco 1977, Uyengco et al. 1981, Doty 1987, Largo et al. 1995a,b) and most recently by marine fungi (Table 2; Solis et al. 2010). The ability of marine fungi (e.g., *Aspergillus ochraceus, A. terreus* and *Phoma* sp.) to produce seaweed-degrading enzymes, i.e., carrageenases and cellulase, and the preference of these fungi for carrageenan as a carbon source, supports their potential as causative agents of 'ice-ice' malaise (Table 2; Solis et al. 2010).

An understanding of the significance of the 'ice-ice' problem should be viewed not only from the interplay of the ecological or environmental conditions of where the seaweed is grown, but more importantly, on the underlying cell physiology and cell wall responses to the surrounding environment. The early works of Collen et al. (1995), Mtolera et al. (1995, 1996) and Pedersen et al. (1996) on *E. denticulatum*, *E. platycladum* and other marine algae are the only known reports which demonstrated the role of water quality in the production of stress-induced hydrogen peroxide (H_2O_2) and volatile halogenated compounds by seaweeds susceptible to 'ice-ice'. The production of H_2O_2, as an oxidative burst, is probably a part of the chemical defense mechanisms of *E. denticulatum* (Collen et al. 1995). A burst of H_2O_2 might mimic the algal responses to the grazing activity of fish or invertebrates. Mtolera et al. (1995) showed that continuous production of H_2O_2 in *Eucheuma* increased with increasing irradiance and pH. In the field, *Eucheuma* spp. are rarely found subjected to epiphytes and pathogens (Mtolera et al. 1996), which is perhaps due to the production of H_2O_2; this may explain how the surface of the alga is maintained clean of epiphytes and pathogens. H_2O_2 produced in seaweeds induces other reactions inside the cell and cell walls, and the formation of other strong oxidants such as hypochlorite, hypobromite and mono-chloroamine; these reactions can take place with the help of halogenated peroxides that are present in the algae (Collen et al. 1995). Seawater contains halide ions, in high concentrations and these may serve as substrates in halogenating reactions. A burst of hydrogen peroxide from *E. denticulatum* may create a release of HOCl, HOBr and

Table 2. List of marine-derived fungus isolated from healthy and infected *K. alvarezii* and *K. striatum* farmed in Calatagan, Batangas, Philippines (orange and green varieties) (Solis et al. 2010).

Taxon	**No. of isolated MDF strains**						
	Kappaphycus alvarezii		***Kappaphycus striatum* (orange variety)**		***Kappaphycus striatum* (green variety)**		**Total no. of strains**
	Healthy host	**Infected host**	**Healthy host**	**Infected host**	**Healthy host**	**Infected host**	
Scopulariopsis brumptii Salv.-Duval	0	0	1	0	0	0	1
Cladosporium sp. 1	0	0	1	0	0	0	1
Phoma nebulosa (Pers.) Mont.	0	0	1	0	0	0	1
Cladosporium sp. 2	1	0	0	4	5	0	10
Phoma lingam (Tode) Desm.	0	3	0	3	3	0	9
Aspergillus terreus Thom	0	1	0	3	2	0	6
Eurotium sp.	0	1	0	8	0	0	9
Phoma sp.	0	6	0	8	0	0	14
Aspergillus sydowii (Bainier et sartory) Thom et Church	4	4	0	2	0	0	10
Curvularia intermedia Boedijn	0	9	0	9	0	0	18
Cladosporium sp. 3	0	12	1	2	0	0	15
Fusarium sp.	0	7	0	7	5	0	19
Fusarium solani (Mart.) Sacc.	1	7	0	0	0	1	9
Aspergillus ochraceus G. Wilh.	0	0	0	6	0	0	6
Aspergillus flavus Link	0	0	5	1	0	1	7
Penicillium sp.	2	0	0	0	0	0	2
Penicillium purpurogenum Stoll	0	0	0	1	0	0	1
Engyodontium album (Limber) de Hoog	1	1	4	0	0	0	6
Total	**9**	**51**	**13**	**54**	**15**	**2**	**144**

chloramines in response to certain pathogenic microorganisms or physical damage by grazers and, as such, could be a very efficient chemical defense (Pedersen et al. 1996). However, the defense mechanism of H_2O_2 production can, in itself, be damaging to the alga particularly if the algal density is high (compared to the water volume into which the strong oxidants diffuse), as might be found in a high density seaweed farm. Field observations indicate that *Kappaphycus alvarezii* is more easily subjected to 'ice-ice' and epiphyte infestation as compared to *E. denticulatum* (Hurtado, personal observation). This was noted especially in high density cultivation areas, particularly where support structures such as bamboo rafts and spider webs were in close proximity to one another. Such intensive arrangements may impede water movement, and act as substrata for the development of epiphytic communities. Further research into these preliminary observations will be required to develop management action plans for healthier cultivation practices. Healthy *Kappaphycus* or *Eucheuma* thalli/seedlings have a greater chance of maintaining efficient chemical defense mechanisms when exposed to lower water quality, with slow exchange, low salinity and low or high temperatures (Uyengco et al. 1981, Largo et a. 1995a,b, Tisera and Naguit 2009). It is ironic that the intensively cultivated stressed seaweeds may become victim to their own defense mechanisms, such that they may eventually perish.

The earliest practice of cultivating *Kappaphycus* in shallow areas, especially in seagrass beds (Doty and Alvarez 1981), proved to be detrimental, both to the seagrass bottom and the cultured seaweed. The interference of seagrass with the uptake of CO_2 by *Eucheuma* resulted in poor growth (Collen et al. 1995). In addition, sometimes, the sea grasses were cut during construction of the seaweed farm to accommodate the stakes and cultivation lines; this in turn had negative impacts on the growth and population diversity of the associated benthic ecosystem for the support of fish nurseries, invertebrates and zooplankton.

Epiphytes

The characteristics of epiphytes and the extent of their damage at the host/epiphyte interface was classified into five groups (B. Kloareg, personal communication, Leonardi et al. 2006):

1. **Type I**: Epiphytes weakly attached to the surface of the host and not associated with any host tissue damage. The contact between the host and the epiphyte was so close that the interface was indistinct
2. **Type II**: Epiphytes strongly attached to the surface of the host but not associated with any host tissue damage

3. **Type III**: Epiphytes penetrate the outer layer of host cell wall without damaging the cortical cells
4. **Type IV**: Epiphytes penetrate the outer layer of the host cell wall, and are associated with the host's cortical disorganization
5. **Type V**: Epiphytes invade the tissue of the host, growing intercellularly, and are associated with destruction of cortical and (in some cases) medullary cells

The question is posed as to, at which point does an epiphyte, which invades the tissues of the host, become a parasite?

Epiphytes and grazers create large problems for seaweed farmers in general. Hard substrata are often limiting in the sea, and seaweed thalli are attractive surfaces for the settlement of epiphytic algae and animals. Epiphytism may be problematic since it can deprive the cultured seaweed of sufficient irradiance required for photosynthesis, as well as decrease the amount of available nutrients and carbon dioxide, or dissipation of oxygen. Taken together, these factors may reduce growth rate and quality of the cultivated material. *In vitro*, Loureiro et al. (2010) observed that the use of AMPEP was efficient in improving the daily growth rate of *K. alvarezii* and eliminated some epiphytes like *Ulva* and *Cladophora*. In Brazilian commercial cultivations, the same response was observed using AMPEP to improve growth and carrageenan yield (Reis, personal field and laboratory observations). The most common macro-epiphytes of *Kappaphycus* are the green (e.g., *Ulva*) and red (e.g., *Acanthophora, Hypnea, Hydrocanthus, Chondrophycus*) algae which can entirely envelope the surface of the thalli (see Figs. 8a-c). The negative effects of macro-epiphytism bring more working hours to the farmers, requiring the removal of the attached seaweeds. The poor practice of some farmers to just throw away the removed epiphytised plants within the existing farms, brings more problems to the entire cultivation area. The discarded covered crop can be carried by the current from one area to another only to act as a vector for the dispersal of the epiphytes; the problem becomes a vicious circle. To combat this problem, the pruned seaweed segments must be brought on land, sundried and perhaps even used as soil conditioners for agricultural or horticulture plants.

Endophytic Filamentous Algae (EFA) (Fig. 9) infestation is a more recent problem that adversely affected the quality and quantity of *K. alvarezii* commercial production. In Brazilian cultivation the main problem is with the calcareous organisms that can sink the rafts and consequently increase costs to clean the rafts (Marroig and Reis 2011). The most notable adverse effect of severe EFA infestation was by *Neosiphonia,* with a concomitant 'ice-ice' malaise incidence, which resulted in the scarcity or even the lack of available good quality propagules for re-planting purposes. Furthermore, there were signs of slowing growth rates which decreased the capacity for

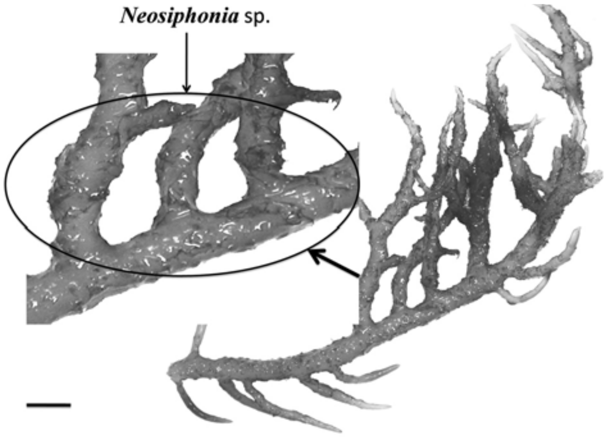


Figure 9. Photo of *Neosiphonia* sp. (bar 1 cm, Photo courtesy of A.Q. Hurtado).

regeneration, leading to a significant decrease in both carrageenan yield and also molecular weight of the carrageenan extracted (Mendoza et al. 2002). A similar situation was reported in *Chondrus crispus,* a carrageenophyte of temperate waters when infected with the endophytic green alga (*Acrochaete heteroclada* and *A. operculata*). Sporophytes of *Chondrus crispus* became completely endophytised, resulting in severe damage of the host tissue, with secondary bacterial infections, and eventual disintegration and death of the thallus (Correa and McLachlan 1991, 1992, 1994, Bouarab et al. 2001, Potin et al. 2002, Weinberger et al. 2005).

The survey work of Hurtado (2005a) revealed that the earliest report of the red algal epiphyte *Neosiphonia* (first recorded as *Polysiphonia*), infestation in the Philippines was in 1994 in Sitangkai, Tawi-Tawi, Philippines. The author reported that *Neosiphonia* infestations (Table 3a) also occurred in Iloilo and Antique (1997), Zamboanga City (1998), Calaguas Is., Camarines Norte (2000), Zamboanga del Norte, Palawan and Quezon (2002), and Bohol (2003). These early incidences of *Neosiphonia* infestation did not concern the Philippine seaweed industry until a severe infestation was experienced in Calaguas Is., Camarines Norte in 2000 (Critchley et al. 2004). As a result, a high percentage of *Neosiphonia* infestation in combination with 'ice-ice' occurred due to overcrowding in the cultivation areas and localized slow water movement (Hurtado et al. 2006, Hurtado and Critchley 2006). The problem of *Neosiphonia* infestation became so severe in some areas as to limit cultivation activities. The Sulu Archipelago, a region of large areas of ideal sites for *Kappaphycus* farming, was severely affected by this outbreak. This resulted in cultivation activities relocating to areas which remained unaffected, especially in the Visayas and Luzon. Seaweed farmers in Sitangkai allotted an exclusion area in order to promote the recovery

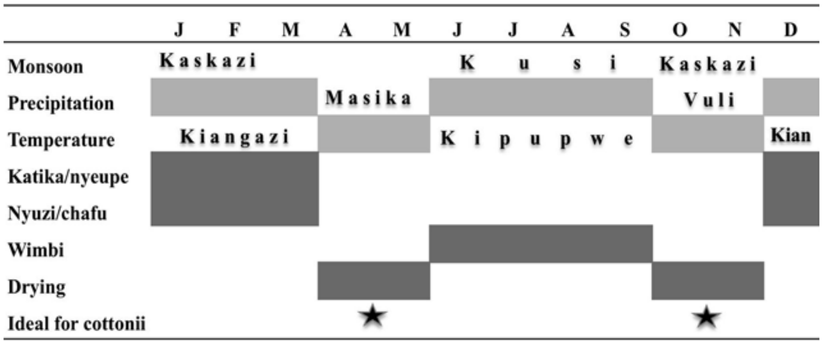
Table 3a. Seasonality of good growth of *K. alvarezii*, ‘ice-ice’ and *Neosiphonia* incidence in some major producing areas in the Philippines (Critchley et al. 2004, Hurtado 2005, Hurtado and Critchley 2006, Hurtado et al. 2006, Hurtado 2008).

	J	**F**	**M**	**A**	**M**	**J**	**J**	**A**	**S**	**O**	**N**	**D**
Bohol												
good growth	■	■	■						■	■	■	■
‘ice-ice’		▓	▓									
Neosiphonia sp.			░	░	░							
Camarines Norte												
good growth	■	■	■					■	■	■	■	■
‘ice-ice’		▓	▓	▓	▓						▓	
Neosiphonia sp.			░	░	░							
Palawan												
good growth	■	■	■	■				■	■	■	■	■
‘ice-ice’			▓	▓	▓							
Neosiphonia sp.			░	░	░							
Panay												
good growth	■	■	■							■	■	■
‘ice-ice’			▓	▓	▓							
Neosiphonia sp.			░	░	░							
Zamboanga del Norte												
good growth	■	■	■	■				■	■	■	■	■
‘ice-ice’				▓	▓							
Neosiphonia sp.				░	░							
Sibutu, Tawi-Tawi												
good growth	■	■	■	■			■	■	■	■	■	■
‘ice-ice’				▓	▓							
Neosiphonia sp.			░	░	░							
Sorsogon												
good growth	■	■	■	■							■	■
‘ice-ice’			▓	▓	▓							
Neosiphonia sp.								░	░			

Table 3b. Seasonality of best growth of *K. alvarezii*, 'ice-ice' and *weeds* (epiphytes) incidence in some major producing areas in Indonesia (I.C. Neish, Personal communication).

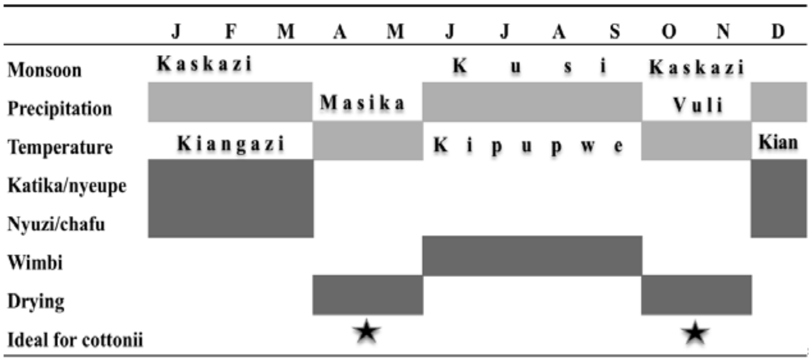
	J	F	M	A	M	J	J	A	S	O	N	D
Sulsel												
'*cottonii*' best			■	■	■	■	■	■	■	■		
'ice-ice'	░	░	░	░	░	░		░	░	░	░	░
weeds (epiphytes)	▓	▓	▓	▓	▓				▓	▓	▓	▓
Bali												
'*cottonii*' best				■	■	■	■	■	■	■		
'ice-ice'	░	░	░	░	░				░	░	░	░
weeds (epiphytes)	▓	▓	▓	▓	▓			▓	▓	▓	▓	▓
NTT Maumere												
'*cottonii*' best	■	■	■	■	■	■	■	■				
'ice-ice'						░	░	░	░	░		
weeds (epiphytes)						▓	▓	▓	▓	▓		
NTT Lembata												
'*cottonii*' best	■	■	■	■	■	■						■
'ice-ice'						░	░	░	░	░	░	
weeds (epiphytes)						▓	▓	▓	▓	▓	▓	
NTT Timor												
'*cottonii*' best			■	■	■	■	■	■	■	■	■	■
'ice-ice'	░	░										░
weeds (epiphytes)	▓	▓										▓
SultraButon												
'*cottonii*' best	■	■	■	■								
'ice-ice'	░	░										░
weeds (epiphytes)	▓	▓	▓									▓
SultraMuna												
'*cottonii*' best	■	■	■			■	■	■	■			■
'ice-ice'				░	░							
weeds (epiphytes)	▓											

Table 3c1. Farmers' perception of seasonal problems at Muugoni, Unguja, Zanzibar. A black box denotes the time farmers recognize the problem: (1) katika/ngeupe = 'ice-ice', (2) nyuzi/chafu = EFA and nuisance algae, (3) wimbi = waves. The Table also shows the monsoon, precipitation and temperature readings in the area (Davis 2011).



	J	F	M	A	M	J	J	A	S	O	N	D
Monsoon	Kaskazi					Kusi				Kaskazi		
Precipitation	░	░	░	Masika		░	░	░	░	Vuli		░
Temperature	Kiangazi			░	░	Kipupwe				░	░	Kian
Katika/nyeupe	▓	▓	▓									▓
Nyuzi/chafu	▓	▓	▓									▓
Wimbi						▓	▓	▓	▓			
Drying				▓	▓					▓	▓	
Ideal for cottonii				★						★		

Table 3c2. Farmers' perceptions of seasonal problems in Jambiani, Unguja, Zanzibar. A black box denotes the time farmers recognize the problem. (1) katika/nyuepe = 'ice-ice', (2) mashava = EFA and nuisance algae, (3) wimbi = waves, (4) unumba = sea urchins. The Table also shows the monsoon, precipitation and temperature readings in the area (Davis 2011).



	J	F	M	A	M	J	J	A	S	O	N	D
Monsoon	Kaskazi					Kusi				Kaskazi		
Precipitation	▒	▒	▒	Masika		▒	▒	▒	▒	Vuli		▒
Temperature	Kiangazi			▒	▒	Kipupwe				▒	▒	Kian
Katika/nyeupe	▨	▨	▨									▨
Nyuzi/chafu	▨	▨	▨									▨
Wimbi						▨	▨	▨	▨			
Drying				▨	▨					▨	▨	
Ideal for cottonii				★						★		

of *Kappaphycus* from 'ice-ice' and *Neosiphonia* (A.Q. Hurtado, personal observation). Indonesia had a lot more areas suitable for *Kappaphycus* available than the Philippines; hence, the farmers transferred their farms to places which were still free from *Neosiphonia* infestation. In areas which have a history of a number of years of cultivation, 'ice-ice' and *Neosiphonia* (Table 3b; I.C. Neish, personal communication) infestation problems have also occurred; one such example is Tanzania (Tables 3c1-2; Davis 2011).

The cultivation of *Kappaphycus alvarezii* and *K. striatum* has been extensive in the Southeast Asian region, notably the Philippines, Indonesia and Malaysia. It is in these regions where *Neosiphonia* infestation and 'ice-ice' have been most severely experienced. *K. alvarezii* cultivation in Sabah, Malaysia was reported to be susceptible to *Neosiphonia* infestation during the dry months (March–June and September–November, Vairappan 2006). Here, the infective organism was dominated by *Neosiphonia savatieri* (Hariot) M.S. Kim *et* I.K. Lee, with 80–85 percent cover of *Kappaphycus* during the peak season. The author further claimed, that the emergence of epiphytes in late February/March coincided with large increases in salinity (from 28–34 ppt) and temperature (from 27–31°C). However, the opposite was observed in the second occurrence of epiphytes and 'ice-ice' in September– November when both salinity and water temperature decreased from 29–27 ppt and 30–25°C. Five notable epiphytes (Fig. 10) were recorded in Malaysia in the following order of abundance: *Neosiphonia savatieri* > *N. apiculata* > *Ceramium* sp. > *Acanthophora* sp. > *Centroceras* sp. (Vairappan 2006). *Neosiphonia apiculata* earlier identified as *Polysiphonia* sp. in the Philippines (Hurtado et al. 2006, Hurtado and Critchley 2006) was also recorded in Indonesia, Philippines and Tanzania (Vairappan et al. 2008).

The growth and development of *Neosiphonia savatieri* (Figs. 11A-D), as described by Vairappan (2006) started as black spots in the surface cuticle on *K. alvarezii.* Tissue cross-sections of the black spots revealed the presence of epiphyte tetraspores embedded between the outer cortical cells. These black spots increased in size, disturbing the host tissue and the vegetative epiphyte emerged after 3–4 weeks and grew to become reproductively mature. The

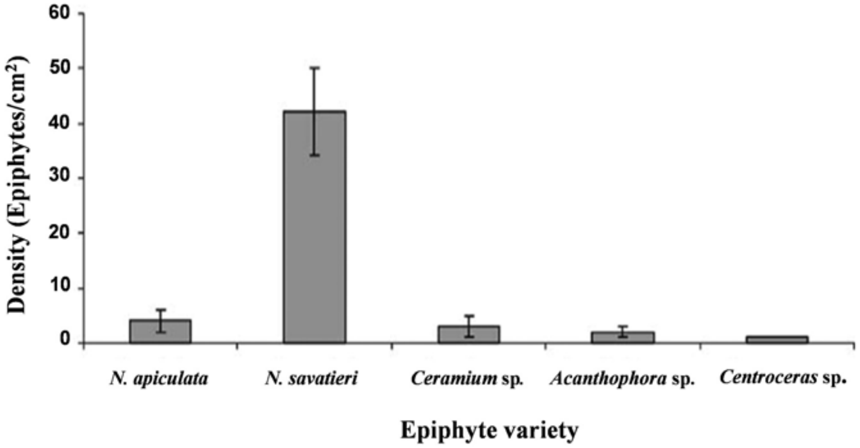


Figure 10. Five notable epiphytes recorded in Malaysia (Vairappan 2006).

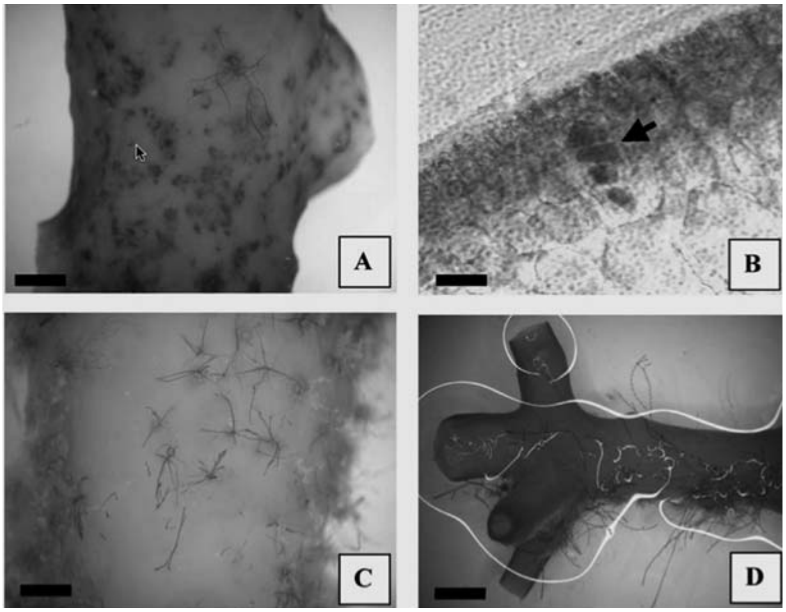


Figure 11. (A-D) Growth and development of *Neosiphonia savatieri* (Vairappan 2006).

presence of a secondary bacterial infection was reported from Scanning Electron Microscopy (Figs. 12a-f), and was observed to further contribute to the disintegration of the infected tissue, leading to fragmentation of the thallus and loss of material from the cultivation lines (Vairappan et al. 2008).

Unfortunately, there is paucity of information on how the endophytes first attach to and subsequently penetrate the host's tissues. Epiphytic filaments may enter the host's internal tissues through wounds (Apt 1988), an observation which was not seen in *Undaria pinnatifida* (Gauna et al. 2009) and *K. alvarezii* (Vairappan 2006, Hurtado and Critchley 2006). Spores of *Neosiphonia* may behave like the spores of *Laminarionema elsbetiae* (Peters

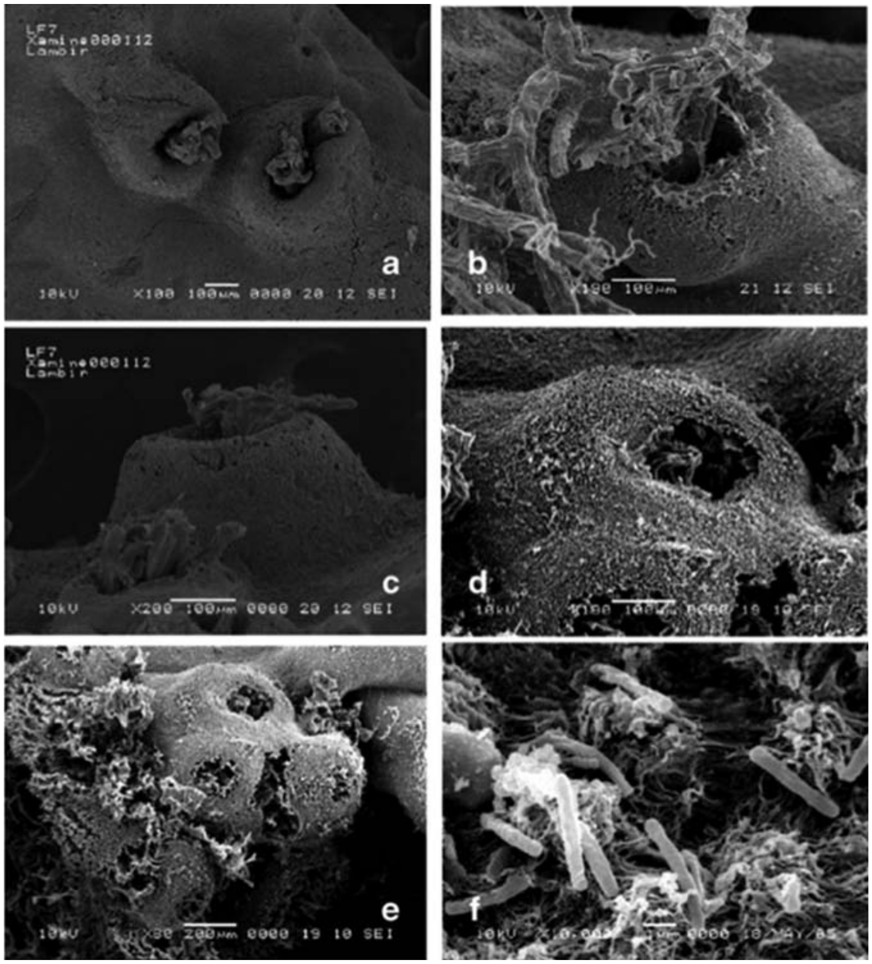


Figure 12. (a-f) Secondary bacterial infection in *Kappaphycus* through an SEM photography (Vairappan et al. 2008).

and Ellerstsdottir 1996) as specialized infective agents that attach to, and penetrate, the host surface and require neither specific wounds nor an opening for a successful invasion of the host.

The reduction of biomass in cultivated *K. alvarezii* populations could be possibly explained by the infection of *N. savatieri* if there was also damage to the oxygen evolving-complex (OECs), which could thereby decrease active reaction centers (RCs) and the plastoquinone (PQ) pool resulting on a significant reduction in the Performance Index (PI) of Phytosystem II (PSII) further leading to reduced photosynthetic activity of *K. alvarezii* (Pang et al. 2011). Such a phenomenon occurred in southern China when the *K. alvarezii* farms were infested with *N. savatieri.* Excessive growth of *N. savatieri* on the surface of the *K. alvarezii* reduced water motion nearby, as well as the gas and nutrient exchange between the *K. alvarezii* and the seawater. Thus, *N. savatieri* limited N, P, CO_2, O_2 and exchange of mineral elements required for growth. At times of heavy infestation, the host *K. alvarezii* becomes nutrient-deficient, resulting in stunted growth while *N. savatieri* has sufficient nutrient and gaseous exchange and proliferates rapidly. Likewise, light becomes limiting to *K. alvarezii* due to the endophytic *Neosiphonia.* Though both seaweeds produce oxygen through photosynthesis during daytime, oxygen consumption through respiration during night-time becomes insufficient especially for the *K. alvarezii.*

5 The Impact of AMPEP on the Growth and Vigor of *K. alvarezii*

Many efforts have been made to prevent 'ice-ice' and solve the problems of declining cultivated biomass. One such effort was the development of cultivation methods using the fluctuation system, instead of the off-bottom method, traditionally used in Asian countries. Whereas the off-bottom method can be accessed only during low tides, the technique of deep water, floating lines, can be accessed consistently, and is not dependent on tides. However, the material cost of operating in deep water is more expensive and the use of boats for crop management is an additional requirement. These costs can be a challenge for poor coastal communities, which constitute the majority of *K. alvarezii* producers (Msuya and Salum 2007).

Seaweed and seaweed extract have been used extensively in agricultural crops such as lettuce (Crouch et al. 1990), tomatoes (Crouch and van Staden 1992), potatoes (Kowalski et al. 1999), barley (Rayorath et al. 2008), canola (Ferreira 2002) bean, wheat, and maize (Blunden et al. 1997), spinach (Fan et al. 2011), carrot (Jayaraj et al. 2008), cucumber (Jayaraj et al. 2011) and horticulture as a source of nutrients, bioactive compounds and biostimulants. The report of Craigie (2011) on seaweed extract stimuli in plant science and agriculture presented a comprehensive account of the many potential applications of seaweed extract. *Ascophyllum nodosum*, a brown alga abundant in the temperate waters of the North Atlantic (Ugarte et al. 2006, Ugarte and Sharp 2001) is one of the economically important seaweeds which are used in the production of liquid or powder extracts (Craigie 2011).

There are only few reports on the use of seaweed extract, as applied to aquatic plants or seaweeds. Kelpak and Acadian Marine Explant Powder (AMPEP) extracted from *Ecklonia maxima* and *Ascophyllum nodosum*, respectively, are the only known seaweed extracts used in the cultivation of another seaweed. Kelpak has been used effectively for the tips of *Gracilaria gracilis* when grown under laboratory conditions, and for *Ulva* grown on a pilot-scale, as a natural feed to abalone, using a 1:2500 concentration in combination with the effluents of turbot fish, demonstrated the highest growth, suggesting that Kelpak could be used in commercial seaweed mariculture (Roberstson-Anderson et al. 2006).

5.1 Development of **Kappaphycus** *Varieties Plantlets Through Tissue Culture Techniques*

Acadian Marine Plant Extract Powder (AMPEP) extracted from *A. nodosum* has the following properties (Table 4). Extract from *A. nodosum* either in powder or liquid form has been used extensively for the benefit of land plants. However, recently, AMPEP has been found to work efficiently and economically for the benefit of seaweeds (Hurtado et al. 2009, Loureiro et al. 2010, Borlongan et al. 2011, Yunque et al. 2011). The use of AMPEP in the tissue culture of three different varieties of *K. alvarezii* and one variety of *K. striatum* proved to be an efficient medium for the regeneration of young plants, for the production of seed stock for nursery and out-planting purposes (Hurtado et al. 2009). The authors further claimed that the addition of Plant Growth Regulators (i.e., Phenyl Acetic Acid (PAA) + zeatin) to AMPEP hastened shoot formation of the explants, as compared to AMPEP when used singly at lower concentrations (0.001–0.10 mg/L). To prove further that AMPEP was an efficient and economical major source of culture media in tissue culture techniques, optimal media concentrations with, or without PGRs, pH-temperature combinations, and explant density: media volume combinations were determined to improve the production of *Kappaphycus* plantlets. *Kappaphycus alvarezii* var. tambalang purple (PUR), Kapilaran brown (KAP), Vanguard brown (VAN), Adik-Adik (AA), Tungawan green (TGR), and *K. striatum* var. Sacol green (GS) were used as explants. Based on the shortest period for shoot emergence and the economical use of AMPEP, the optimum enriched media were 3.0 mg/L AMPEP and 0.1 mg/L AMPEP+PGR 1 mg/L each PAA and zeatin for PUR;

Table 4. Physical and chemical properties of Acadian Marine Plant Extract Powder (AMPEP) extracted from the brown alga *Ascophyllum nodosum*.

Physical data	
Appearance	Brownish-black crystals
Odor	Marine odor
Solubility in water	100%
pH	10.0–10.5
Typical analysis	
Maximum moisture	6.50%
Organic matter	45–55%
Ash (Minerals)	45–55%
Total nitrogen (N)	0.8–1.5%
Available phosphoric acid (P2O5)	1–2%
Soluble potash (K2O)	17–22%
Sulfur (S)	1–2%
Magnesium (Mg)	0.2–0.5%
Calcium (Ca)	0.3–0.6%
Sodium (Na)	3–5%
Boron (B)	75–100 ppm
Iron (Fe)	75–250 ppm
Manganese (Mn)	5–20 ppm
Copper (Cu)	1–5 ppm
Zinc (Zn)	25–50 ppm
Carbohydrates	Alginic acid, mannitol, laminarin

1.0 mg/L AMPEP+PGR for KAP and GS, 0.1 mg/L AMPEP+PGR for VAN; and 3.0 mg/L AMPEP and 0.001 mg/L AMPEP+PGR for AA and TGR. Results showed that the addition of the PGR to low concentrations of AMPEP hastened shoot formation. pH–temperature combinations for the most rapid shoot formation were determined for the brown (KAP) and purple (PUR) color morphotypes of *K. alvarezii* var. tambalang and the green morphotype of *K. striatum* var. sacol (GS) cultured in 1.0 mg/L AMPEP+PGR. The brown morphotype produced the most number of shoots at pH 7.7 at 20°C after as short as 20 days. Purple *K. alvarezii* showed an increased shoot formation at pH 6.7 at 25°C and the green *K. striatum* morphotype at pH 8.7 at 25°C. These results simply indicated that each variety responded differently to the pH-temperature combinations tested. The optimal number of explants added to the culture media was also determined for Tungawan green (TGR), Brown (KAP), and Tambalang purple (PUR) varieties of *K. alvarezii* in 1.0 mg/L AMPEP+PGR. The number of explants and the volume of the culture media combination were also tested. The highest average number of shoots formed occurred in two explants: 1 mL culture media (2:1) for KAP and PUR (35 percent and 17 percent, respectively) and 1 explant: 2 mL culture media for the TGR (100 percent) with a range of 0.5–3.0 mm shoot length after 40 days in culture. The earliest shoot formation was observed after 21 days for the brown and 9 days for both the green and purple color morphotypes of *Kappaphycus*, in all densities investigated. This indicated that within the range tested, the density of explants did not have a significant effect on the rate of shoot formation but did influence the average number generated from the culture. The rate of production of new and improved *Kappaphycus* explants for a commercial nursery stock was improved through the use of AMPEP with optimized culture media pH, temperature, and density conditions (Yunque et al. 2011). These findings parallel those of Rayorath et al. (2008) on the effect of *A. nodosum* extract to induce amylase activity, independent of gibberellic acid (GA_3) which may act in concert with GA-dependent amylase production leading to enhanced germination and seedling vigor in barley. Similar effects of *A. nodosum* extract have been reported for the model plant *Arabidopsis thaliana* during early root and shoot formation (Rayorath et al. 2008).

The viability of laboratory generated, and hatchery reared, plantlets of different varieties of *Kappaphycus* developed from tissue culture techniques, using AMPEP for out-planting purposes is encouraging as demonstrated from the successful initial works at Southeast Asian Fisheries Development Center, Aquaculture Department, Tigbauan, Iloilo, Philippines (Figs. 13a-d) and the National Seaweed Technology Development Center, Cabid-

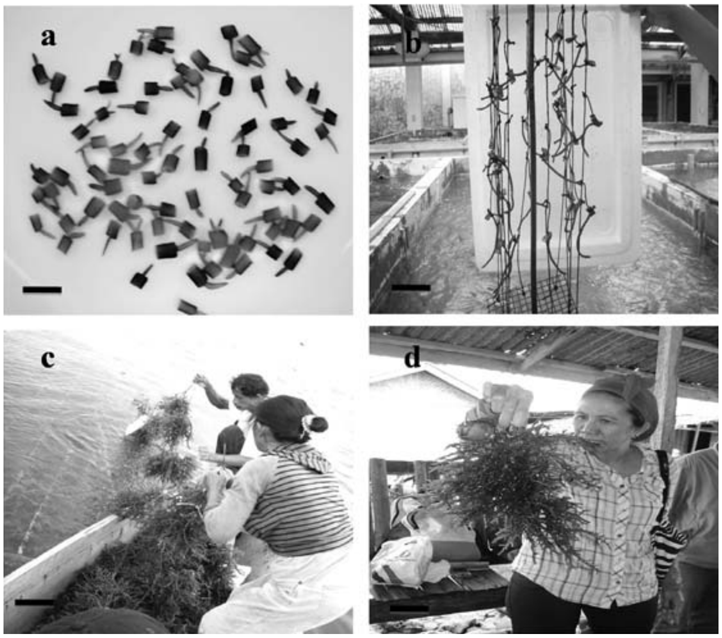


Figure 13. (a-d) Plantlet regenerants using tissue culture techniques at SEAFDEC-AQD, Tigbauan, Iloilo, Philippines (bar = 1 cm, Photos courtesy of A.Q. Hurtado).

an, Sorsogon, Philippines (Figs. 14a-d). Mass production of plantlets of *Kappaphycus* varieties using AMPEP is thus encouraged to develop good quality propagules for commercial cultivation; this should be scaled-up to warrant pilot-testing and demonstration farming of the 'new and improved' *Kappaphycus* plantlets.

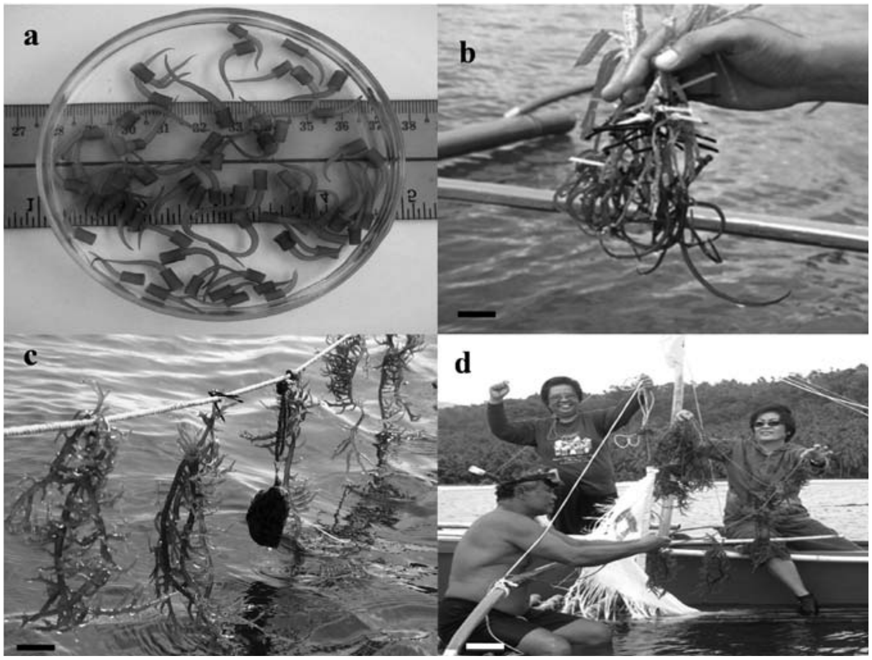


Figure 14. (a-d) Plantlet regenerants using tissue culture at NSTDC Cabid-an Sorsogon, Philippines (bar = 1 cm, Photos courtesy of I.T. Capacio).

Color image of this figure appears in the color plate section at the end of the book.

5.2 Growth of Kappaphycus *in Commercial Nurseries and Mariculture*

The use of AMPEP in commercial nurseries in Mindanao and Palawan, Philippines proved to accelerate the growth of *K. alvarezii* var. tambalang and *K. striatum* var. duyan-duyan. The study of Hurtado et al. (2012) showed that the average monthly growth rate of *K. alvarezii* (yellowish brown) ranged from 0.8 to 6.7 percent/day between July and January, respectively (Fig. 15). Significant differences ($P<0.05$) were observed between the various months of culture. Declining growth rates were observed from February to July while increasing growth rates were observed from August to October, with a slight decrease in November (but reaching its highest growth rate in January). The authors further reported that dipping the three color morphotypes of *K. alvarezii* for 30 min at the lower AMPEP concentrations of 0.01–0.1 g/L resulted in higher growth rates compared to the controls. The active compounds within the seaweed extract must be applied as small doses to be effective, as also claimed by Robertson-Anderson et al. (2006). The proliferation of young multiple shoots (Fig. 16) of the three color morphotypes of *K. alvarezii* and the yellowish brown

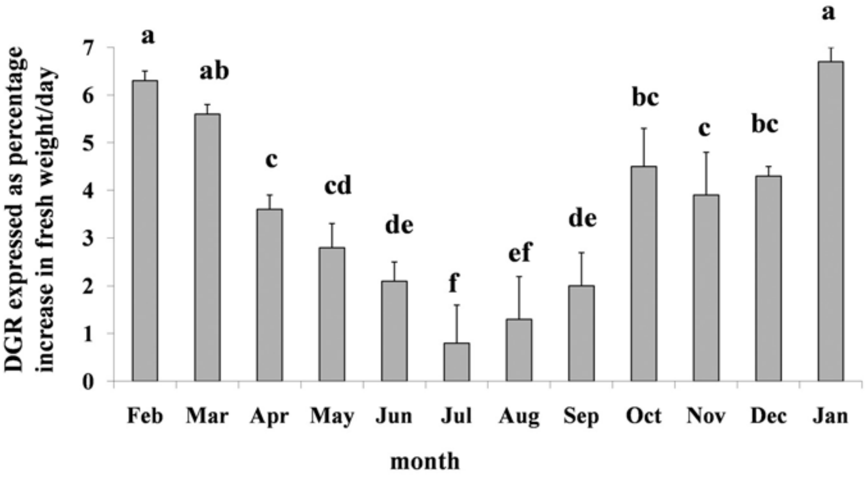


Figure 15. Average (± SD) daily growth grate of *K. alvarezii* in a commercial nursery in Zamboanga (Hurtado et al. 2012).

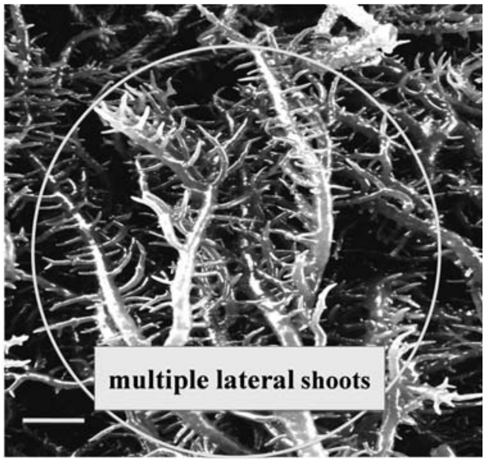


Figure 16. Multiple shoots in *K. alvarezii* treated with AMPEP (Photo courtesy of A.Q. Hurtado).

Color image of this figure appears in the color plate section at the end of the book.

K. alvarezii, grown in a commercial nursery, after 10–14 days of field growth could be attributed to the presence of growth elicitors which stimulated auxin-like activity in the seaweed. This further promoted shoot growth and paralleled the results of the study of Rayorath et al. (2008b), wherein the components of the commercial *A. nodosum* extract modulated the concentration and localization of auxins, which could account, at least in part, for the enhanced plant growth of the model plant *Arabidopsis thaliana*. Hurtado et al. (2012) further stated that though it was beyond the scope of the their study to speculate on the active compounds or mode of action of AMPEP, the enhanced plant growth effects of the extract treatment on the color morphotypes of *K. alvarezii* could be correlated with 'auxin-like', 'gibberellin-like' elicitors and primers (Rayorath et al. 2008), precursors of ethylene and betaine (Mckinnon et al. 2010) and cytokinin-like effects which are potentially involved in enhancing plant growth responses (Crouch and van Staden 1992, Wally et al. 2013).

The use of AMPEP in commercial nurseries prevented the worst of the deleterious effects of macro-epiphytism. Though *K. alvarezii* was enveloped with the deleterious *Ulva*, in the trials, no traces of pits or cavities were observed on the surface of the cultured seaweed (Hurtado et al. 2012), in fact the surface of the seaweed remained smooth and clear and the whole thallus appeared to be brittle. Severe macro-epiphytism, especially during periods of intermittent rains and sunny days, is a serious problem for the seaweed farmers. Sometimes when macro-epiphytes are dense, they are not readily removed, and therefore can remain attached to *K. alvarezii* for several days; fragmentation of the cultured seaweed was seen to be the ultimate result. Such phenomena were observed with *K. alvarezii* seedlings which had not been dipped in AMPEP. At this time, only few seaweed farmers use AMPEP in seaweed farming, but the number is expected to grow. In experiments using AMPEP in Brazilian commercial cultivation, it was observed that the rafts near the samples treated with AMPEP had less biofouling (R. Marroig, personal communication).

In the commercial mariculture of *K. striatum* (barako) in Panabulon, Guimaras Is., Philippines, the use of 1 mg/L AMPEP was sufficient to increase the monthly biomass by 2.5–3.5x the original weight (Data not shown). Further, consistent monthly dipping of the seaweed into a solution of 1 mg/L AMPEP increased the resistance to 'ice-ice' and *Neosiphonia* infestation for almost 18 months, without any change in the source of propagules (E. Ferrer, personal communication). The usual practice of seaweed farmers in the Philippines is to use only the material from the same propagules over 2–3 cycles of production. After such time the plant was deemed to be no longer viable due to aging, 'ice-ice' and/or *Neosiphonia* infestations and new sources of seedlings had be obtained. However, the erratic weather conditions experienced in 2011 in the Philippines, in the western Visayas in particular and the continuous vegetative cutting of the same seaweed stock perhaps contributed to weakening and loss of vigor of the commercial seaweed, which after 18 months became susceptible to *Neosiphonia* infestation brought on by environmental stresses.

The report of Borolongan et al. (2011) showed that dipping the cultivars of *Kappaphycus* in AMPEP induced more vigor and increased resistance to 'ice-ice' malaise and *Neosiphonia* infestation when the seedlings were experimentally cultivated at four different depths. The authors claimed that the use of AMPEP significantly increased ($P<0.05$) the growth rate of TUNG (Tungawan) and GTAM (Giant Tambalang) *Kappaphycus* varieties tested, but also decreased the percent occurrence of *Neosiphonia* sp. The percent occurrence of *Neosiphonia* sp. infection (6–50 percent at all depths) of both the *Kappaphycus* varieties tested (Figs. 17a-b) with AMPEP treatment, was significantly lower than the controls (i.e., 10–75 percent at all depths). Both the growth rate of the cultivated seaweed and the percent occurrence of the epiphytes decreased as the cultivation depth increased. Plants dipped in AMPEP, and suspended at the surface, had the highest growth rates (i.e., 4.1 percent, TUNG; 3.1 percent, GTAM) after 45 days. Those which were not dipped in AMPEP had the highest percentage occurrence of *Neosiphonia* infection (viz. 70–75 percent). The occurrence of the *Neosiphonia* infestation was found to correlate with changes in irradiance and salinity at the depths observed (Table 5). These results suggested that both varieties of *K. alvarezii*, used in this study, had the fastest growth rate when grown immediately at the surface. However, in order to minimize damage caused by the occurrence of epiphytic *Neosiphonia*, it was recommended that *K. alvarezii* should be grown within a depth range of 50–100 cm. These observations are important for the improved management of *Kappaphycus* for commercial farming. Furthermore, the use of AMPEP treatments for enhanced growth and reduction of *Neosiphonia* sp. infections parallels the results obtained in agricultural crops such as spinach (Fan et al. 2011), carrot (Jayaraj et al. 2008) and greenhouse cucumber (Jayaraj et al. 2011).

The administration of AMPEP extract to *K. alvarezii* under laboratory conditions reduced the effects of the oxidative burst (production of hydrogen peroxide) which may be extremely aggressive for an individual and its epiphytes (Loureiro et al. 2012). The bleaching of the non-corticated portions of *Polysiphonia subtilissima* thalli, which were co-cultivated as simulated epiphytes with AMPEP treatment, confirmed that the reaction was from hydrogen peroxide effects. The use of AMPEP acted as "seaweed vaccine", eliciting activation of the natural defenses of *K. alvarezii* against pathogens and thereby ameliorated the negative effects of long term exposure to oxidative bursts. The effectiveness of the commercial *Ascophyllum* extract was also confirmed on *K. alvarezii* thalli that were protected from bleaching, as compared with their control. Also *in situ*, when *K. alvarezii* was submitted

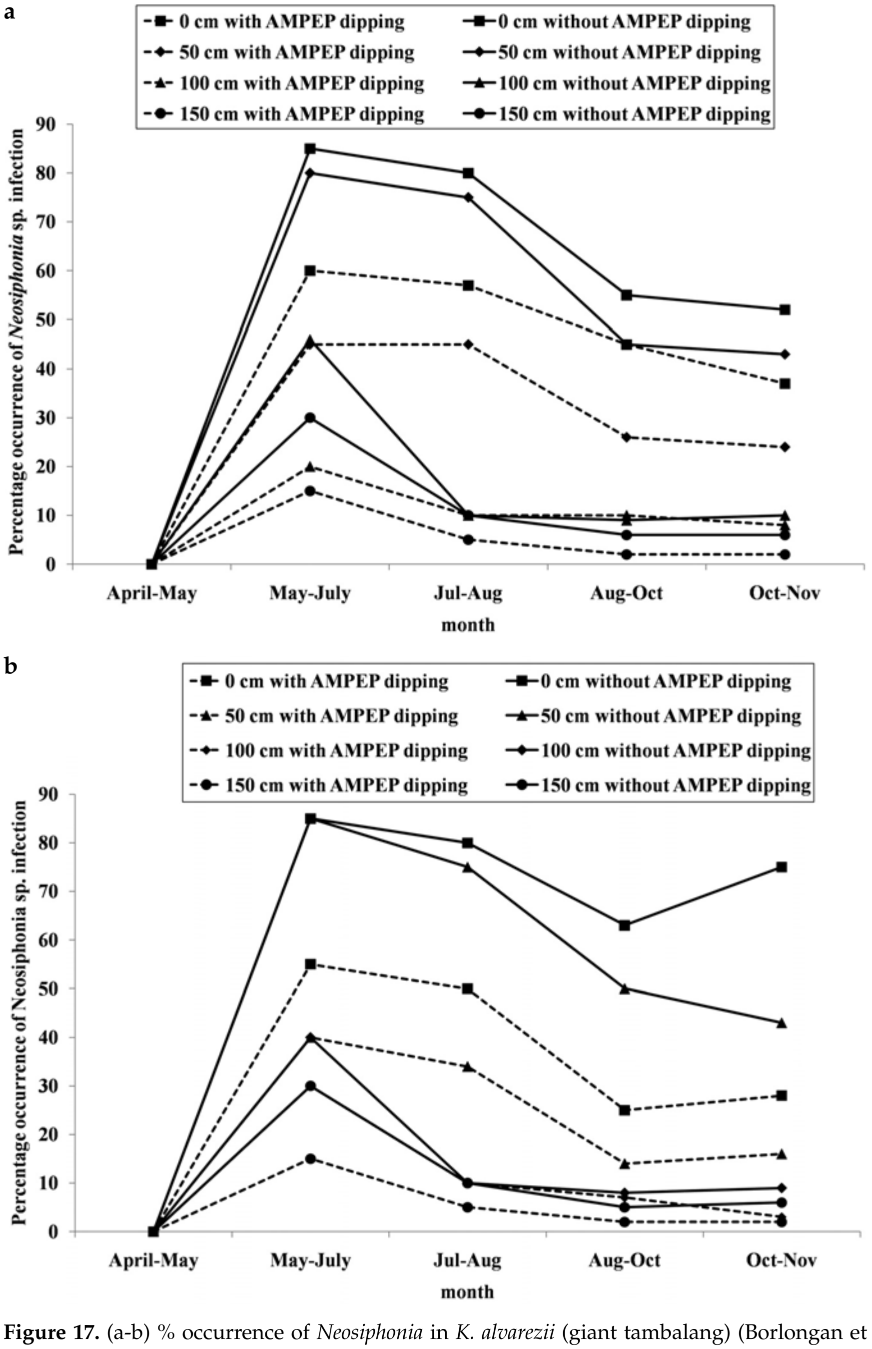


Figure 17. (a-b) % occurrence of *Neosiphonia* in *K. alvarezii* (giant tambalang) (Borlongan et al. 2011).

Table 5. Correlation coefficient between percent occurrence of *Neosiphonia* sp. and some environmental parameters in two varieties of *K. alvarezii* dipped and not-dipped in AMPEP (Borlongan et al. 2011).

Varieties of *K. alvarezii*	**Irradiance µmol photons $m^{-2}s^{-1}$**	**Salinity ppt**	**Temperature °C**	**Turbidity NTU**
Tungawan with AMPEP dipping	1.00	–0.99	0.21	–0.03
Tungawan without AMPEP dipping (control)	0.94	–0.95	0.46	–0.21
Giant tambalang with AMPEP dipping	0.97	–0.98	0.36	–0.14
Giant tambalang without AMPEP dipping (control)	0.94	–0.96	0.47	–0.25

to lethal temperatures that occur in some countries (16±1°C) where this species was introduced, the seedlings treated with AMPEP can be used as a preventive action for the cultivation of the seedlings in tanks and in the sea in periods of low temperatures since higher growth rates and carrageenan yield were obtained (Loureiro et al. 2013). They suggested that the better growth was due to the presence of betaines (that promote protection of the disruption of electron transport by the photosystem II and degradation of pigments) and cytokinin (an elicitor activity in the AMPEP extract and its antioxidant properties). Probably, the proline acted as mediator of osmotic adjustments, stabilizer of proteins and membranes, inducer of osmotic stress related genes, and source of reduction equivalents during stress recovery. Moreover, the elevated presence of LPCs significantly increased the concentration of soluble sugars in the cytosol in response to freezing stress, showing the mode of action of LPCs in protecting the tested samples at low temperatures and their effects in addition to priming cold response genes. Also the exudation of H_2O_2 (preventing effective action of catalase—CAT and the induction of ascorbate peroxidase gene—APX) may be responsible for the delay in responses appropriate to CAT and APX which guarantees an acclimatization in the long term, permitting *K. alvarezii* (Barros et al. 2006) to increase its growth rate under adverse environmental conditions combined with favorable compounds present in AMPEP and its antioxidant action.

In relation to the increased carrageenan yield on AMPEP treated samples, it is known that AMPEP act as defense-compound of macroalgae in the elicitation and priming in response to abiotic stresses. Since their daily growth rate was unaffected, it seems that the chlorophyll content was less affected. Nair et al. (2012) attest that AMPEP has a positive effect on reducing the expression of chlorophyllase under chilling stress.

6 Socio-economic Impact of Seaweed Diseases in *Kappaphycus* Cultivation

The socio-economic impacts of seaweed farming on coastal communities have been overwhelmingly positive (Alih 1990, Firdausy and Tisdell 1991, Samonte et al. 1993, Hurtado-Ponce et al. 1996, Crawford 1999, Hurtado et al. 2001, Hurtado and Agbayani 2002, Sievanen et al. 2005, Msuya et al. 2007, Hurtado et al. 2008, Hurtado 2013). Family-type operations are far more advantageous than the older style, initially corporate-owner-operator style farms, especially in remote areas where coastal communities are faced with a limited number of economic alternatives. The seaweed industry in Zamboanga Peninsula and elsewhere served as an alternative activity to farmers in their quest for survival, security and development (Jain 2006). Whether the industry can ultimately liberate the farmers from the trap of poverty depends largely on the support and attention that can be provided to the poor farmers by the government, local and international non-government organizations, big export traders, and also the local and multi-national corporations engaged in carrageenan processing. Seaweed farming provides an income that can help alleviate the hardship faced by this vulnerable group. The income from seaweed farming also reduces the impact of lack of food and malnutrition. The concept that the seaweed industry is the farmer's alternative to poverty holds true, depending on the adequacy of income achieved to allow the farmers access to the basic and prime necessities for survival security and empowerment (Jain 2006).

Prior to engaging in seaweed farming, many of the community or island people were marginal fishermen living below the poverty level. With sheer diligence and hard work, the life of the seaweed farmers became more meaningful. Incomes derived from the sale of seaweeds, allowed many farmers to experience substantial improvements in their standard of living, such as education for their children to college level, introduced improvements to their dwellings (from light materials to concrete houses), enhanced diets, increased purchasing power of material goods (e.g., appliances, vehicles, communications devices (mobile)) phones, and also access to political leadership in the community, improved banking credibility and social acceptance in the community (Cooke 2004, Hurtado 2005a). Women in seaweed farming are doubly respected in their communities, being engaged in an income-earning activity and at the same time efficient housewives (Aming 2004, Hurtado 2005a,b).

The most recent report on the socio-economic dimensions of seaweed aquaculture (Hishamunda and Valderrama 2013) is a collection of six contributions from six countries: Indonesia (Neish 2013), the Philippines (Hurtado 2013), Tanzania (Msuya et al. 2013), India (Krishnan et al. 2013), the Solomon Islands (Kronen et al. 2013) and Mexico (Robledo et al. 2013).

Each contributor emphasized that there is durability even under internal-chain stress; stability in the face of internal value-chain shocks, robustness under external value-chain stresses, resilience in the face of external value-chain shocks, and sustainability of value-chain functions over time and generations of farmers. Though the profitability of seaweed farming during 'ice-ice' outbreaks and *Neosiphonia* infestations seemed to reduce significantly since the carrageenophyte crops are damaged, amidst these circumstances, the seaweed farmers are resilient enough to bounce back. The seaweed farmers are still in the business after more than 40 years of providing the raw material for the processing of carrageenan. They were able to cope with, and recovered from stresses and shocks, maintained and enhanced the capabilities and assets, while not undermining the natural resources as reflected from their many years of seaweed farming and improved family life, both socially and economically. Until the present day, seaweed aquaculture is a sustainable activity (Hurtado 2013).

7 Global Future of Seaweed Farming in Relation to Diseases Brought by Climate Change

A limited number of papers have shown the impact of 'ice-ice' outbreaks and *Neosiphonia* infestations on seaweed farming. Surface seawater temperature, salinity, pH, water movement and light intensity all act in combination to play significant roles in *Kappaphycus* health and growth. It is not conclusively established if these changes are in fact related to global climate change in surface seawater temperature and/or other environmental/biological variables. There is an imperative need to pursue further research studies on the physiological and ecological processes involved between *K. alvarezii* and its environment, especially in relation to its productivity. The initial move to use the commercial extract from *A. nodosum* as biostimulant for growth, health and vigor is very encouraging. However, a thorough investigation to elucidate the mechanism(s) by which AMPEP exerts effects on enhancing disease resistance through the induction of defense genes or enzymes in *K. alvarezii* needs to be undertaken. When this aspect is fully understood, then possibly, the production of *K. alvarezii* will increase. On the other hand, the quest for faster growing and disease resistant strain(s), with ever high yielding carrageenan *K. alvarezii* must continue through natural selection, not through genetic modification.

The economic impact of the decline of *Kappaphycus* cultivation in some areas, especially the Philippines, requires more intensive and regional investigation due to its economic importance. There is an important need to sustain carrageenophyte farming due to the innumerable economic and coastal development benefits which one derives, if practiced and maintained in a sustainable manner. The key players along the value chain must work in harmony, providing increased and sustained effort to continue to provide the industry with this high-value natural colloid. If this is not the case, industry could switch to other sources of hydrocolloid with similar or competing rheological properties.

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