CULTURE TECHNOLOGIES FOR SOME ECONOMICALLY IMPORTANT SEAWEEDS

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I. Values of Seaweeds

Seaweeds consist of algae species that have monocular and multicellular structure, which are distributed from the brackishwater tidal areas to the remote seacoast areas. The uses of seaweeds include the following:

A. Seaweeds as human food

A number of seaweed species used as food include *Ulva, Monostroma, Caulerpa, Enteromorpha, Laminar, Porphyra, Hypnca, Gigartina, Cantenella, Gracilaria, Kappaphycus, Eucheuma*, etc. Seaweeds are also processed into instant foods such as salad, agar-agar, etc. Seaweeds are used as food in many countries, consuming approximately 454,730 mt of dry seaweeds (Mehugh, 1991). South Korea, Japan and China have the highest production of seaweeds in the world, and every year South Korea and China used over 200,000 mt of dry seaweeds as food.

B. Seaweeds as food for domestic animals

In many countries, seaweeds are also used as main food for animals. A large amount of food for animals is produced annually from seaweeds in America, Norway, and Denmark. In general, the nutritive value of seaweeds is good. In fact, the food produced from seaweeds for animals, are known to prevent diseases in the domestic animals. The very common species of seaweeds used as food for hogs include *Gracilaria* and *Ceratophyllum*.

C. Seaweeds as fertilizer

Seaweeds are also good sources of fertilizer. Seaweeds fertilizer are known to promote the process of sprouting and assimilation, and also in making plants disease- and cold-resistant. In many coastal areas, *Sargassum* is used as fertilizers in sugar-cane fields, coffee plantation, tomato gardens, watermelon fields, etc.

D. Seaweeds for phycocolloids

There are three types of phycocolloids: agar, carrageenan and alginate. Colloids agar and carrageenan are extracted from red algae (*Rhodophyta*), while colloid alginate is from brown algae (*Phacophyta*).

Every year, about 900,000 mt (wet weight) seaweeds are used for processing phycocolloid in the world, accounting for 1/4 of the global production of harvested seaweeds (Luning, 1990). About 50,000 mt of phycocolloid are processed in the world (Richards-Rajadurai), of which the production of alginates is 22,000-25,000 mt/year, carrageenan 15,000 mt/year, and agar 7000 mt/year, respectively (James, 1990). All these phycocolloids, have utilizable values in the industries as foodstuffs, cosmetics, medicines, textile, agriculture and biotechnology.

II. Seaweeds Production in the World

In 1998, the production of economically important seaweeds in the world was about 4 million mt (fresh seaweeds), which has been maintained since 1983 (Richards-Rajadurai, 1990). According to Luning (1990), the total volume of dry seaweeds in the world was approximately 3 million t. According to the statistics from FAO, the production of red seaweeds of the world was 1,256,981 mt in 1992.

The following countries have the highest yields of red seaweeds in 1992:

South Korea	383,773 mt
Philippines	350,554 mt
Indonesia	188,218 mt
China	158,990 mt
Chile	69,145 mt

A. *Production and utilization of phycocolloids*

Alginate colloids are mainly processed in the U.S.A., Norway and France. Alginates have varied uses in industries, but their most important use is in textiles (50%) and foodstuffs (30%). Japan and Korea have the most requirements of alginates both for export and domestic utilization.

Carrageenan colloid is mainly processed from *Eucheuma* spp. which are cultured in the Philippines. Indonesia and recently, in Tanzania. The U.S.A. and some European countries have high requirements of this product. On the other hand, agar colloid is processed in Japan, Spain. Chile. China. and South Korea. with Japan having the biggest need of this product, which is about 2000 mt agar per year. The U.S.A. and EC countries need 1000 mt/year and 1300 mt/year. respectively.

III. Production of Seaweeds in Vietnam

Since 1995 (Anonymous), a study was carried out on *Gracilaria* and some various commercial seaweeds in Quang Binh. In 1929, Petelot recorded some production of *Gracilaria* at Cua Viet, Quang Tri. Before 1975, Luong Cong Kinh (1964) mentioned the results of the investigation on seaweed resources for processing to agar in the coastal provinces of South Vietnam.

In North Vietnam, the investigation of seaweed resources was given more attention by the government since 1960. The Research Institute of Marine Products was the first institution to investigate the seaweed resources of Vietnam. Results from the investigation recorded some 653 seaweed species in the coastal regions of Vietnam, of which about 90 are economically important, including the species *Gracilaria*, *Sargassum*, and *Hypnea*.

In recent years, *Gracilaria* has been exploited and cultivated in many coastal areas in Vietnam with a production of about 3000 mt/year (dry weight). Specifically, the species being exploited and cultured are *Gracilaria asiatica* Chang et Xia (G. verrucosa Hudss. Papenf), G. tenuistipitata Chang et Xia, G. blodgetti Harv.

Studies on biology, culture techniques and processing of seaweeds were given attention for nearly 30 years. Many researches were conducted at the national and branch levels, in accordance with the above objectives. The technological processes of *Gracilaria* cultivation with high productivity (2-4 dried mt/ha/year) and agar processing from *Gracilaria* have already been adopted in Vietnam. However, *G. asiatica* cultivation and agar processing are still developing in many provinces of Vietnam. Hundreds of mt of agar are used domestically in the country as well as for export every year.

Moreover, culture of the red algae was introduced to Vietnam only in 1993. *Kappaphycus alvarezii* (Doty), which comes from Philippines, has been tried for culture in the coastal regions of the country.

IV. Biological Characteristics of Seaweed

A. Morphology and structure

Seaweed is a thalliform vegetation, where all thalli have the same functions as the autophyte. Some seaweeds belonging to the order of brown algae (Fucales), have main thallus looks like higher plants. with "stem". "blade", "holdfast", air bladder, and reproductive bodies. But the blade, stem, and holdfast have no specific functions as in higher plants.

Seaweeds come in varied sizes, the monocellular structure are small sized, which could not be seen with a naked eye. But the genus of brown algae (Laminaria), is ≥ 10 m while the size of brown algae (Macrocystis) could reach 100 m. The structure of the seaweeds is from simple to complex. Red alga which is more developed than the other species (order of Gelidiales, Gigartinales), has inter-structure with divisions such as columnar cell, pinned columnar cell, histiomonocyte, adventitial cell.

B. Growth of seaweeds

The growth of seaweeds involves direct segmentation of the cells in the thallophyte, increasing the mass and size of the thallus. The growth of seaweeds depends on species medium, and culture conditions. If the ecological condition is good, some species can reach a maximum growth of 20-30% /day. The average growth is 5-8% /day.

C. Reproduction of seaweeds

Reproduction of seaweeds is vegetative, asexual, and sexual. The organ of asexual reproduction is sporangium and its offsprings are spores. For the sexual reproduction are the male and female gametophytes. Some of the evolved red algae has male gametophytes called spermatocyst and female gametophytes called carpospore.

V. Relationship Between the Environment and Seaweeds

The main marine ecological factors effecting the life histories of seaweeds include physical, chemical, and dynamic factors. There are also factors that relate to other organisms in the environment.

A. Physical factors

1. Bed form

The bed form is not significant to floating alga this is very important for the distribution and growth of the attached alga. For the higher plants, the substrate is a direct medium that supplies minerals, nutritive salt, and water. For seaweeds, the substrate's function is for the adhesion of spores and gametes to form new algal thalli. In the growth process, the thalli do not absorb nutrients from the substratum, but directly from the water medium.

The living medium of seaweeds in the littoral regions is normally the bottom such as gravel, reef. rock fragment, coral, sand, mud, and shells. Some seaweeds only grow on solid substrates such as rock bottom because of the molluscs which it can attach to. The adhesion of the spores in seaweeds is suitable for the rocky bottom because of its rough surface. The other seaweeds however, can grow on gravel bed with a sandy layer covering.

The most general character of seaweeds is their distribution being scattered on the reefs of the littoral regions with developmental haptera. Some species which are distributed in brackishwater ponds belong to the bottom living form which can hardly have a haptera structure. There are many seaweed species that directly settle by adhesion in the substrate.

B. Temperature

Temperature affects the life history of algae, especially their distribution, growth, and reproduction.

1. Effect of temperature on distribution

Kjellman and Sberkoj divided seaweed flora by temperature scale. These are from 0°C to 5°C which is known as the cold seaweed flora; from 5°C to 15°C (average 10°C) as the subcold seaweed flora; from 10°C to 20°C (average 15°C) as the sub-tropical seaweed flora, and about 25°C as the tropical seaweed flora.

Some eurythermal algae have a world-wide distribution such as the *Cladophora*, while some stenothermal algae, which are suitable for the cold climate are distributed in the cold sea regions such as: *Macrocystis, Laminaria, Jaonica, and Porphyra tenera*. Their size is big, the longest being *Macrocystis* which can reach 100 m, while *Laminaria* can grow upto 10-15 m.

The seaweeds in Vietnam belong to the sub-tropical and tropical seaweed flora. The tropical seaweed flora are becoming increasingly popular from the north to the south of the country.

2. <u>Influence of temperature on growth</u>

The growth of algae distributed in different geographical regions, depends on the suitable temperature. *Porphyra tenera* can grow well at the temperature $\approx 10^{\circ}$ C, *Padina* in 20°C-25°C, *Gracilaria asiatica* in 25°C attained at 6-8%/day and at the limited temperature $< 10^{\circ}$ C and $> 32^{\circ}$ C, at 1-3%/day. Some green algae can grow within water temperature of 50-70°C.

When algae are distributed in the same region, the temperature obviously affects their growth. In north Vietnam, *G asiatica* can grow best at the end of spring and beginning of summer with seawater temperature of 20°C to 32°C.

3. <u>Influence of temperature on reproduction</u>

Temperature affects the reproduction of algae. G. asiatica in the north formed carposporangia, which often bursts in April until June every year. Porphyra tenera, distributed in China is the big species of Porphyra which can grow in low temperature and in winter. Paraspore can be formed within the temperature range of 17°C to 20°C in summer. The small Porphyra tenera can also survive in autumn temperature. The process of projection and adhering of spores in many algae also depends on the condition of the medium.

C. Light

Light also affects the vertical distribution of algae. The energical solar radiation has the smallest wavelength known as violet ray (380-430 millicrometer), while the largest is red ray (600-780 millicrometer). Infrared ray could not be seen with the naked eye, its biggest wavelength is from 780-340,000 millicrometer. The rays, which are light violet, green, blue, yellow, and red, can influence the photosynthesis of the algae.

The distribution depth of light could also be influenced by geography, turbidity, time of day, and silence of the water body. If the light has the smallest wavelength, the distribution is deeper. The red light is distributed at the surface, while the green, blue and yellow lights are distributed at the middle layer, and violet is distributed at the bottom layer. The distribution ability of light in the water bodies can reach the highest depth of 1700 m. If light is abundant, algae can grow fast.

1. Relationship between seaweed, light and depth

Green algae (*Chlorophyta*) are adaptable to red light and its distribution is at the surface. Brown algae (*Phacophyta*) has a lot of sub-pigment such as *Phycophein* and *Fucoxanthyl*, which are adaptable to orange and yellow light and their distribution is at the middle stratum.

Red algae (*Rhodophyta*) have sub-pigment such as *Phycoerythirin* and *Phycocyanin*, with light adaptation to green and blue, and their distribution is at the deepest water stratum, which can reach over 100 m depth. In sea tropical region, seawater is normally clear. The light intensity is wide so that seaweeds can distribute within 300 m depth.

However, a few algae are not distributed following the abovementioned rule. For example, Caulerpa spp. is adaptable to weak light and their distribution is at the deeper layer (the sub-littoral regions). Red algae (Bostrychia), are adaptable to strong light and their distribution is at the supralittoral area. The color of the algae is different so their pigmental elements are different too. The light which could not be absorbed by the algae, is reflected in the light color of the algae.

2. Effect of light on growth and reproduction

Different algae have different growth ability in the various lighting conditions, as shown in the following table.

<u>Species</u>	lighting time	Adaptable limited light to
	(morning/night)	the growth
Gracillaria verrucosa	16/8	40-50 W.m ⁻²
G. chorda	14/10	25W.m ⁻²
G. tenuistipitata	16/8	340 MEM ⁻² .S ⁻¹
G. edulis	16/8	4000 lux
Porphyra tenera	14/10	100-1000 lux

Light intensity is one of the most important factors influencing the growth of the spores. In the intensity from 100 to 1200 lux, filamentous alga (*Porphyra tenera*) can grow well. If the light intensity reduces from 6 to 16 lux, the algae will be light in color and the filament is also smaller.

VI. Chemical Factors

A. Salinity

1 Efect of salinity on distribution

The adaptation of algae with the changes in salinity varies. Approximately 90% of the species, belong to the family of brown and red algae distributed in the sea, while the green and blue algae species occupy only 10%. The green and blue algae are mainly distributed in brackishwater and freshwater areas.

The adaptation of seaweeds with the changes of wide salinity range is also varied. Alga is divided into two groups: euryhaline alga and stenosalinity alga. The euryhaline alga can exist and grow in the change condition of euryhaline waters. For example, *Cladophora* species can grow in freshwater as well as in brackishwater.

The stenosalinity group can grow and exist in limited salinity. The group of stenosalinity alga can be divided into those that are adaptive to high salinity and those that are adaptative to low salinity.

2. Effect of salinity on growth

Salinity influences the process of photosynthetic respiration thus, affecting the growth of the seaweeds. For example, *Laminaria japonica* grow well within the salinity range of 30-31% while *G. asiatica* can grow in salinity 16-25 %.

3. <u>Effect of salinity on reproduction</u>

The process of spore release, attachment and growth of seaweeds, are influenced directly by the salinity. For example, the specific weight suitable for the attached and projectile process of spores of G. asiatica is 1.010 - 1.015. The species is suitable for growth in salinity range of 15-22 ‰.

B. Nutritive Salt

The nutritive salt in the life history of seaweeds, is the mineral salt which comprises the N and P elements. The nutritive salt comprising Si is necessary for diatoms. In addition, some mineral elements needed for the growth of some seaweeds are Ca, K, Na, Mg, Bo, Co, Cu, etc. The mineral nutritive salts affect directly the growth and reproduction of seaweeds.

In general, the required mineral nutrient P/N/C in the ratio 1/10/100 is necessary for the growth of seaweeds. The suitable method of fertilization is affected by the following factors: culturing mass (productivity, estimated harvested production), time, nutrient requirements, environment, etc., as well as the estimated rational time and volume of fertilization.

C. pH

Seaweeds can grow and develop well within the medium range pH of 7.0 -8.5. Except for the *Dermarestia* species which can grow in low medium pH of 1-3. Even in alga, there are many species which can adapt to a wide range of pH.

D. Dissolved oxygen

The air in the water medium such as CO_2 , O_2 , NO_2 , NH_3 , H_2S , CH_4 and those of the air medium such as CO_2 , O_2 directly affect the photosynthetic and respiratory processes of the seaweeds. However the influence of CO_2 , O_3 in the seawater medium is not very critical.

The medium in brackishwater ponds especially in aquaculture lagoons fertilized with NO₂, NH₃, H₂S, CH₄ can badly affect the cultured seaweeds and fishes. Based on this characteristics, some authors opined that fertilizing the seaweed culture ponds in high amount of fertilizer to make H₂S, NO₂, limits the growth of harmful seagrasses. Seaweeds can tolerate higher levels of H₂S, NO₂ than the tolerable level for harmful seagrasses.

VII. Dynamic Factors

Dynamic factors such as tide, sea current, wave, and wind, also affect the life of seaweeds. Specifically, the phenomena of high and low tide have a relationship with the vertical distribution of the seaweeds while the natural currents facilitate changing of the water needed in the growth processes of seaweeds.

Sea current, and high and low tide enhance the propagation ability of the reproductive media and increase the horizontal distribution of seaweeds. Wave and wind actions also affect the distribution of the attached algae, and influence the cultivating area as well as the culture structure, such as in floating methods and pond culture. However, some seaweeds are also cultivated in areas where there is no wave nor wind movements.

VIII. Factors Related to Organisms

Seaweed also serves as food for some marine fishes: i.e Haliotis eats Gracilaria and Sargassum. Rapana eats Porphyra while Actinia eats Ulva, Ostrea eats Chlorella, and Chanos chanos eats green alga.

Moreover, in some attached seaweeds, the spores release out maternal thallus and then adhere to shells or thalli of other algae. Thus some green alga attaches on *Hippopus*, while *Polysiphonia sertulariodes* attaches on the thalli of *Gracilaria* in brackishwater ponds and *Fucus* attaches on *Undaria*.

The competition in the medium of distribution, light, and nutritive salt between *Gracilaria* and some harmful seagrasses in brackishwater pond, is very clear. The growth of some blue algae can produce toxicogenic substance for the medium used in the culture of *Gracilaria* and also for the culture of shrimps, crab, and fishes.

IX. The Relationship Between Seeweeds and Biotic Community of Mangroves

The biotic community in brackishwater lagoon consists of plants such as seaweeds, seagrasses, timber-trees (mangroves, etc.), and animals such as fishes, crustaceans, molluscs, benthos, etc. There is therefore a distinct competition for nutrition and distributive medium between seaweeds with seagrass and timber trees. However, seaweeds serve as food for some fishes such as *Chanos chanos* and breams.

When shrimps are cultured in a closed system, seaweeds (*Gracilaria*) play an important part in making the pond water clean for the shrimps. *Gracilaria* cultivation in a shrimp closed system increases the possibility of the controlled environment to be disease-free. Consequently, the income from such cultivation system also increases.

X. Techniques of Seaweed Culture (Gracilaria)

A. Importance and biological characteristic of Gracilaria

1. Economic significant of *Gracilaria*

Gracilaria. which has high commercial value, is a good material for agar extraction. Agar is used widely in industrial fields such as in foodstuff, textile, printed fabrics, in medicine, agriculture, etc. Gracilaria is also a good source of agarose, a product similar to agar but of higher quality than agar. Moreover, Phycoerythirin pigment which can be extracted from Gracilaria. has a great significance in biotechnology. Gracilaria can also be used as food for hogs, and for the abalone Haliotis. About 25-30,000 mt of dry weight seaweeds are used to extract about 5000 mt of agar per year (Mclachlan and Bird, 1986). In recent years, the world market demand for seaweeds and their products has significantly increased. The agar production of the world has also increased, about 9,000 mt/year.

However, the volume of agar which the world needs, is higher than the harvested production. The countries which have have high production of seaweeds are Chile, Argentina, Japan, and China. While the countries which have high production of agar are Japan, Spain, Chile, South Korea, Portugal, Taiwan, and Argentina.

In Vietnam, the production of seaweeds through natural exploitation and culture is about 3000 mt/year. There are agar processing factories and small enterprises (normally, household scale) in Vietnam, where hundreds of tons of agar are processed every year mainly for domestic consumption. Seaweed cultivation and agar processing have socio-economic significance for Vietnam in terms of job opportunities and increased income especially to the farmers living near the coastal areas.

2. Taxonomy of Gracilaria

The genus *Gracilaria* belongs to the Family *Gracilariaceae*, Order *Gigartinales*, Class *Florideae*, and Phylum *Rhodophyta*. There are more than 150 species of *Gracilaria* in the world, of which about 20 species have been identified in Vietnam.

The most common species of *Gracilaria* which are being exploited and cultivated in Vietnam include *Gracilaria asiatica* Chang et Xia, G. blodgettii Harv., G. tenuistipitata Chang et Xia, and G. heteroclada Zhang et Xia.

3. Formation and structure

a. Formation

In general, the thalli of almost all species of *Gracilaria* are cylindrical and filariform. A few have thalli which are blade-shaped such as *G. textori* and *G. eucheumoidex*. The style of the apex is thamniun alternate or irregular and dichotomous. A few species have holdfast which is somewhat constricted such as in *G. cacallina* (J.Ag) Dawson. The color of the thallus is pinkish, light pink or brownyellow and a little bit green, depending on the medium and growth stage of each species.

Thalli are erect and the base of the thallus has small discoid holdfasts. The holdfast is composed of a ramified "root-like" structure of haptera which is also solid. When thalli are attached to the adhesion, the haptera develops. In brackishwater ponds. *Gracilaria* grows in vegetative reproductive form, thus the haptera may or may not develop at all.

b. Structure

Thallus is divided into three portions of transection. The medulla part is a row of central cell and 4-5 rows of pericentral cell. This layer comprises thin, small cell walls without color and contains reserve substance. The inner layer consists of multiangulous cells. The wall is thick with few pigments making it darker and in tight order.

The external layer consists of a lot of small, thick cell walls that are tightly ordered and pigmented. This layer is the main anabolic layer of the body. There are 4-6 rows of oval cells. The inner cells are bigger than the external ones. The size and number of medullary cells and the change of cells which form cortex to the medulla are used for species identification.

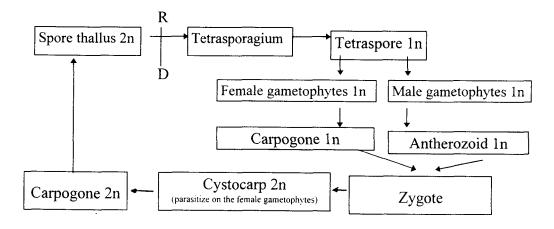
4. Reproduction

The life cycle of *Gracilaria* comprises three alternative stages: asexual, haploid, and diploid. In asexual reproduction, the cells have 2n chromosomes (diploid thallus) while in monoploid form (gametophyte) the cells have 1n chromosome. Diploid is a zygote, which has 2n chromosome and parasitic female gametothallus, and the gametophytes are the female and male thalli.

The male thalli produce small gametes (antherozoid) by cutting off the spermatangial cells from the surface cortical cells, producing antheridium, which has antherozoid. The female thallus produces carpogonial branches which have one or antherozoid and one of the thalli. The zygote develops into cytocarp which is a sphere coming out to the surface of the thalli.

Through the process of asexual reproduction, tetrasporagium is formed which divides into claviform or cruiciate form. One tetrasporagium has four spores. The process of sexual reproduction forms carposporagium by fertilization, which is a common form for some species of *Gracilaria*, growing in brackishwater areas.

5. Reproductive cycle of Gracilaria



6. <u>Ecological characteristics of Gracilaria</u>

a. Distribution

Geographically, the genus *Gracilaria* is widely distributed, especially in regions with a temperature range of 5°C-35°C. In the Asia Pacific region, production of *Gracilaria* is much higher than in other regions. *Gracilaria* is distributed from high tidal areas, but can also grow well at the middle of tidal areas.

b. Substratum

The attached population is suitable in areas with solid bottom such as rock, coral, shell, etc. On the other hand, the "free living" population is found in sandy-mud and muddy-sand bottoms.

c. Turbidity

Gracilaria can grow well in the ponds where the water is clear with limited light of 8 - 10,000 lux.

d. Temperature

Gracilaria can grow in warm weather, within the temperature range of 5-38°C. But its optimum growth can be obtained in areas with a temperature range of 20-25°C.

e. Salinity

Gracilaria can grow even in areas with the limited salinity of 3-25 ppt. However, optimum growth is obtained within the salinity range of 12-24 ppt.

f. pH

Gracilaria can grow rapidly in brackishwater ponds where the pH of the bottom substratum is less than 6.0 and water pH range is 7.5-8.5.

g. Fertilization

G.~asiatica can grow well with supplementary fertilizer such as NaH₂PO₄, KH₂PO₄, KNO₃, NH₄NO₃, (NH₄)₂SO₄, urea, and manure from pig, chicken, cow, etc. The requirement for the nutrients NP is the ratio 6/1-10/1, and the use of NP depends on the culture density. The growth is suitable under certain ecological conditions such as cultivating time, temperature, etc. The mineral contents N=2-4mg/1 and P=0.2-0.4 mg/1 are suitable for the growth of *Gracilaria*.

h. Relation with community

The other plants in the community also influence the growth of *Gracilaria*, because they compete for the distributed medium, light, and nutrients. The more dangerous plants are some algae that attached on *Gracilaria* such as *Polysiphonis*, whose growth could cause rapid death of the *Gracilaria*.

Seaweeds are however, useful for the medium for mud crab, *P. monodon*, and tilapia culture. Seaweed makes water in the medium clean for the cultured aquatic animals.

i. Toxic elements in seaweed culture

The elements that make the medium of seaweed culture toxic are: H_2S , NO_2 , AL^{+3} , Fe^{+3} , etc. These elements should therefore be avoided in the culture medium.

B. Cultivation methods

1. Monoculture

a. Site selection

Site selection is one of the important factors to be considered in seaweed culture. Infact, this could be the primary determining factor for the success or failure of any farming venture. The selected area should have a natural distribution of seaweeds.

If culture is to be done in an area where no support natural stock of the species exists, then this should not be selected even if it has comparable ecological conditions as the sites where natural stocks are found. The local geographical and sea conditions should also be considered and suitable measures should be taken accordingly (such as means of transport, social security, etc.). Specifically, the site must be protected from the direct effects of strong wave and wind; it must be near the sources of freshwater and seawater. The height of bottom pond must be placed in the best suitable position in order to promote early exchange of water during the local tidal regime. The best substratum is sandy-mud, but muddy or muddy-sand substrata could also be selected.

b. Ponds

The area of a pond may be equal or smaller than 1.0 ha, having a coffer-dam with gate (inlet) and drain (outlet) so that the flow of seawater can be controlled. The width of the gate should be 0.5 - 0.8 m, while the depth of the pond should be 0.6 -0.8 m. Bottom ponds should slope into the outletgate for water exchange. For this purpose, the seawater should first be drained and the ponds dried in the sun for days. The bottom pond should be ploughed and dried before manure is applied. Lime may be applied to improve the bottom pond and making it rich, but this depends on the pH of the bottom soil.

c. Method of cultivation

Before planting, the pond should be drained and dried for some days and then allow new water to enter the ponds. The best thalli are then selected as seedlings. Transport of the seedlings from the source to the pond should be carried out at dawn or when it is not sunny. If the distance of transporting the seedlings is far, water should be applied on the seedlings. Upon arrival at the site, seedlings should be immediately planted. Before planting, the thalli of seedlings must be cut into small portions by pruning them. These are then spread regularly on the bottom pond at a density of 500-600g/m².

d. Management

Changing water is very important for the growth of the seaweeds. It is therefore necessary to change water regularly according to the tide. The level of water in the pond should 30 - 40 cm deep. In summer, the water level in the pond may be increased to 60-80 cm. In winter, the level of water can be increased in order to limit the reducing temperature in the ponds. About 50-70% of water volume should be changed at each time, from 12-16 times/month since there are two tidal cycles in a month.

When there is no water exchange and a lot of harmful seaweeds appear, fertilizer maybe be applied, such as KNO_3 , NH_4NO_3 , $(NH_4)_2SO_4$, KH_2PO_4 , NaH_2PO_4 , urea, also organic and inorganic fertilizers. The dose of fertilizer depends on the growth and density but should take into consideration the content of nutritive salt at N=2-4 mg/l and P=0.2-0.4 mg/l which is very suitable for the growth of seaweeds.

Some species of green algae often grow in ponds such as *Cladophora*, *Chactomorpha*, *Enterromorpha*, *Polysiphonia*, and some weeds such as *Rupia*, *Myriophylum*, etc. Before planting, the ponds and lagoons should be cleared of the harmful seaweeds and weeds by ploughing and drying the bottom ponds and applying lime. Organic chemicals in high dosage to make H₂S and NO₂ in ordewr to get rid of the harmful weeds.

Seedlings should be selected carefully making sure that harmful seaweeds are eliminated. However, during the culture if harmful seaweeds appear, these should be taken out, and at the same time limit the inorganic matter by applying more water. If the harmful seaweeds grow well, the best measure is to improve the ponds and lagoons again, and replant with new seedlings.

e. Harvest and primary treatment

The first harvest can be carried out after a culture period of two months. Then after 20-40 days, a second harvest may be done, depending on the environmental conditions and culturing season. Boats, rake and some other simple equipment may be used to collect the seaweeds.

Harvested seaweeds should be washed with water from the pond to take out debris and mud. This will then be dried on bricks or cement ground or on the lawn. The dried seaweeds is tightly placed in plastic sacks and stored in a dry place.

2. <u>Integrated seaweeds culture with shrimp, fish, crab</u>

Seaweed culture can be combined with shrimp, fish, and crab in brackishwater ponds. A culture model from Taiwan integrates the culture of *P. monodon* and *Scylla serrata* in seaweed ponds. The volume of stocks for 1.0 ha is usually 400-5000 kg of seaweeds, 10,000 - 20,000 fry of shrimps and 500 - 10,000 fry of crab. The food of crab is fish waste and ground snail. After three months, crab may be harvested while shrimps will be harvested 4-7 months later. The rate of survival of crab is expected to be 80% while that of shrimps is 80-90%. This aquaculture model has attained the best harvest in Taiwan, where the profit has been reported to increase 2-3 times compared with monoculture.

In Vietnam, many ponds and lagoons used for shrimp, fish and crab culture, are also used for the integrated culture of seaweeds. For the inetgrated system, selecting ponds with the suitable environment for the growth of seaweeds should be the very first step. This is followed by the improvement of the pond by ploughing and supplementing with 1.0-1.5 mt of manure and 100-200 kg of $\text{NaH}_2\text{PO}_4/\text{ha}$. Then the best seaweed stocks are selected and spread at regular intervals on the pond bottom at a density of $200\text{-}300\text{g/m}^2$. Water should be changed regularly according to the tide. When necessary, fertilizers may be supplemented on the harvest and cost, taking into consideration the mineral content of N=1 mg/l and P=0.1-0.3 mg/l, which is appropriate for the growth of seaweeds.

3. Seaweeds culture on the littoral areas

There are two forms of littoral areas: the first have high depth with high turbidity, where the bottom is rocky and sandy, and not too soft. During low tide, the bottom of the pond is not exposed.

The other type is with low depth and with low turbidity, so that as the tide rise, the depth of the water is 50-100 cm. and at low tide, the bottom is exposed. The bottom is muddysand making it convenient to put stakes. Two different methods of seaweeds culture can be applied in these two littoral forms.

a) Raft method

This method (Fig. 1) is used in deep areas where the water depth should be 50 cm at low tide. The turbidity is high, but the water movement is not too strong, that is, the water currents should be moderate near the estuaries in order to receive the nutrients.

Raft is constructed using bamboo frame 2x4 m with six empty plastic containers used as floats (30 cm in diameter). Anchors which are attached to the stakes, may be rocks or concrete. The distance from raft to the anchor depends on the water depth of the area. Bunches of the seedlings are attached to nylon lines, spaced at 30-40 cm intervals.

About 12-14 nylon lines are attached to stakes, which are set on the raft. The raft is 20-30 cm deep from the water surface. When the temperature is high, the depth of the raft may be \geq 30 cm. This method can also be applied not only to plant seedlings but also for the seeds of the spores.

b. Rope farming

This method is used in areas with low depth or in lagoons which is over 1.0 m deep. Bunches of seedlings are tied to the nylon lines at 30 cm intervals. The lines are stretched tightly and the other ends attached firmly to the opposite stake in the next row. The line and the bottom should be parallel where the distance from the line to the bottom depends on the water depth. When tide is lowest, the lines of *Gracilaroid* should not be exposed to sunlight.

For this method, the lines should be tied in bamboo, wood or concrete. All stakes are firmly driven into the bottom making a 45-50° level. Rows are spaced at 50 cm interval and bunches of *Gracilariod* are tied to the stakes. After 2-2.5 months, the seaweeds can be harvested.

This method is more efficient because of the absence of competition for nutrient by seagrasses and harmful seaweeds. However, seaweeds culture using this method, should be managed properly to avoid losing the seaweeds by the wave and wind actions.

XI. Culture Technology of Eucheuma, Kappaphycus

A. Economic Value

The genera Euchema and Kappaphycus are two important carrageenophytes, which are abundant in the Philippines, tropical Asia and the Western Pacific region. More recently however, some species of red algae (Acanthophora spicifera) have been reported to contain certain lambda carrageenan which is not produced by either Eucheuma or Kappaphycus.

Eucheuma or Kappaphycus are at present the main base of the seaweed industry in the Philippines and Indonesia. The development of modern culture technology has increased the production tremendously in the Philippines to about 60.000 in 1987.

Lambda carrageenan, extracted from *Eucheuma* and *Kappaphycus*, is used in the industries such as in foodstuff, textile, print, cosmetics, pharmacy and agriculture. Statistical record indicated that the whole world uses about 15.000 mt of carrageenan annually.

B. Classification

The genera Eucheuma and Kappaphycus belong to the Family: Solieriaccac, Order: Gigartinales, Class: Florideae, and Phylum: Rhodophyta. There are about 24 species of this genus in the world, 6-7 species are found in the Philippines. In Vietnam, some species of this genus are the E. okamurai, Kappaphycus alvarezii, and K. inorrme

In 1993, Kappaphycus alvarezii (Doty) was transported from Japan to Central Vietnam. and has proven to have good potentials for culture. At present, K. alvarezii culture has been tried in some waters as Cat Ba Island (Hai Phong) and Van Don area (Quang Ninh).

Eucheuma alvarezii, Kappaphycus alvarezii and E. denticulatus are produced by floating and rope methods (rope is stretched tightly between two wooden stakes), in the Philippines and Indonesia. Kappaphycus alvarezii and E. gelatinae are also cultured in Hai Nam Island. China. and in several other Western Pacific island countries.

C. Formation and structure

The thalli of *Eucheuma* and *Kappaphycus* consist of cylindrical or compressed-terete and rameous. They are cartilagenous and may be prostrate or erect in structure. Except in *K. procrusteanum*, the whole thallus has thick and flatten blade.

Gametophytic and sporophytic thalli have also been reported. The color of the thalli is yellowish green or dark green. The inner structure of the thallus consists of rows of cells: the central and cortical cells. The sizes of above-mentioned species are different.

D. Reproduction

The life cycle of *Eucheuma* and *Kappaphycus* consists of three alternating somatic stages: asexual. haploid and diploid. The asexual form has 2n chromosome (diploid tetrasporophyte): the haploid form has 1n chromosome (tetraspores) with the terasporophyte and tetraspores have the same size and form; and the diploid form (carposporophyte) which is the zygote with 2n chromosome and is very small parasitic on the female gamethallus.

E. Culture technology

1. Site selection

In an attempt to expand the area for seaweeds farming, the first thing that should be done is careful site selection, unless farming of *Eucheuma* and *Kappaphycus* is already being carried out in the area. Reconaisance survey of the reef areas should be made to identify potential good localities. *Eucheuma* and *Kappaphycus* should be evaluated in terms of good growth rate.

Reef areas far from the freshwater sources are preferred because *Eucheuma* and *Kappaphycus* are stenohaline species and salinity below 30 ppt may have adverse effects on the plants. Areas with coarse, sandy to corally bottom substrata, moderate water currents are good sites.

Water movement in general favors the growth of the seaweeds by facilitating the rapid nutrient entry. It also prevents the extreme fluctuation of the other ecological factors such as temperature, salinity, pH, dissolved gases, which can adversely affect the growth of seaweeds. Areas with sand, coral reef, are also factors that support the culture system and reflect the existence of good water movement in the area. Substratum of fine sand or mud is not suitable as farming sites of the seaweeds.

Water depth at low tide is also an important factor because it influences the cost of farming. Areas of 0.6-1.0 m deep at the low tide are good and ideal because in very deep areas, it will be difficult to construct the cultivation system, making it more costly in terms of labour and materials. Sandy reef coral areas where *Eucheuma* and *Kappaphycus* are naturally distributed, are good potential sites for culturing the seaweeds.

After the site has been selected, test-planting should be carried out with the desired species. Test-plots consisting of a few monolines, each planted with 50-100 g of test plants are constructed at different locations in the area. Growth of the test plants is monitored at weekly intervals and their daily growth rates are determined. The area supporting the daily growth rates of 2-5 % or higher is a potentially good site.

A period of 2-4 months monitoring may be sufficient to begin a small family seaweed farm, and can be used as basis to construct a larger farm. In general, the areas where the yield increase is doubled within 15-39 days or less, are productive and suitable cultivation sites for *Eucheuma* and *Kappaphycus*.

2. Cultivation method

The development of a seaweed farm starts with the clearing of the site of seagrasses. harmful seaweeds, sea urchins, big rocks, and corals. Then the area is divided into small sections of 0.25 ha or smaller. Some common implements used are mangrove stakes, nylon monofilament line (>10 m), soft plastic material (for tying), large net bags, large rattan baskets, knife, hammer, bolo, etc.

a. Fixed, off-bottom monoline methods

This method (Fig. 2) is presently the most commonly practised in many countries because of its many advantages over the others. This net method is cheaper and easier to install and maintain. Stakes are driven deep into the substratum, spaced at 10 m intervals, in rows 1.0 m apart. The end of the nylon monofilament line (about 10.5 m long) is tied to one stake in the opposite row.

Selected *Eucheuma* or *Kappaphycus* cuttings (50-100 g) are tied to the monolines at 25-30 cm intervals, using soft plastic straw as tying material. The plants are allowed to grow to 1.0 kg or more before they are harvested. Depending on the growth rate, the crop may be harvested after 2-3 months. The whole plant would be harvested and replaced by new cuttings. This method can also be applied for the culture of *Eucheuma* and *Kappaphycus* in ponds with high water salinity.

The construction of the support system starts with digging holes in the substratum using a pointed iron bar (3-4 cm in diameter) and a sledge-hammer. Mangrove stakes with pointed head (60-80 cm long) are then firmly driven into the holes using the sledge-hammer. The wooden stakes are arranged in rows at 1.0 m intervals with distance of 10 m between the rows. A loop is made at the end of the monofilament line (≥ 10 m long) and is attached to a stake then stretched tightly and its other end is attached firmly to the opposite stakes in the next row. The distance of the monofilament line from the ground is 0.3-0.5 m, depending on the depth of the water during low tide. Monolines may be positioned parallel or perpendicular to the direction of the current. In the areas where the current is relatively strong, the monolines are arranged parallel to the current and an extra stake placed midway between the original rows of stakes to provide additional support for the monoline. Site specific adjustment in the construction of the support system may be made to adapt to local conditions. One thousand lines (10 m long) will make one hectare of farm.

b. Floating method

In areas where space is limited and the fixed, off bottom monoline method is impossible to implement because of relative problems such as the existence of an animal community, diseases or changes in the degree of water movement brought about by typhoon, rain, etc., the floating method may be used. The advantages of this method include minimal or very limited grazing by bottom-associated animals; and the plants, being near the water column, are exposed to more moderate water movement caused by the waves. This method, however, is not recommended in sites exposed to strong wave action.

The same principles used in the fixed. off-bottom monoline method are employed. However, in this method, the cuttings are tied and attached to a raft system. Bamboo is used for constructing the floating structure or if wood is used as raft frame, floatation materials such as styrofoam or empty plastic boxes are used. In some areas, plastic bags filled with air are used as floats. The raft is anchored to the bottom by means of nylon lines. In order to maximize production per unit raft, good cuttings should be used. The distances between lines and cuttings attached to the monolines should be close enough.

The size of raft depends on the length of the frame materials. This method therefore maximizes the number of plants on a raft, while the adverse effects of intense sunlight near the surface of the water column is offset by the slightly crowded spacing of the plants.

On the other hand, long line floating raft may also be used where six nylon monofilament lines (10 m or longer) are attached to bamboos (2 m long) which are tied by nylon lines at 15 cm intervals. The four corners of the unit are anchored to wooden stakes. One hundred long lines can be accommodated in one-hectare farm. Recently, this method has been used in many areas where either the water current is weak or in protected areas, where water movement is mainly due to wave-action generated by wind. This method is also adapted in place where the water is deep and the bottom topography is very irregular.

The same principles applied to the fixed-off-bottom monoline method is used except that in the raft method the monolines are attached to the wooden frame (3x4 m) using bamboo as floats. A total of 15 lines 4.5 m long are spaced at 20 cm intervals and attached to the wooden frame. About 300-400 cuttings are planted on the rafts that are joined together and anchored to the bottom from the corner. Additional anchors may be used when necessary. A total of 100 rafts can be accommodated in one hectare farm.

In the floating method, 6 nylon monofilament line (≥ 10 m) are attached to bamboo (2 m long), which are set at 5 m intervals. Monolines are attached 30 cm apart to the bamboo. Plants are tied to the monolines at 15 cm intervals.

3. Preparation of seedlings

Seedlings of selected species or variety are obtained from the nearest source, transported to the farm site in the shortest possible time and protected from direct exposure to sunlight, rain and wind. If the seedlings are in transit for a longer period, these should be occasionally soaked in clean water. In this case, the use of styrofoam boxes with holes in the upper sides to facilitate aeration. is the most efficient transport container. Seedlings should be drained of excess seawater before these are placed in covered boxes, and must be placed in seawater immediately upon arrival at the farm site. The cuttings (the volume of each cutting is from 50-100 g) are tied at 20-25 cm intervals to the monolines (Fig. 3).

The maintenance and management of the farm are facilitated by planting on a per unit area basis, that is one farm unit (0.25 - 0.125 ha), should be planted before proceeding to the next one. The plants are ready to be harvested when they are about 1.0 kg or bigger. The time required to grow *Eucheuma* and *Kappaphycus* to harvestable size vary, depending on the growth rate of the plants, as affected by site-specific ecological conditions. In good farming areas, the seaweeds may be harvested after 6-8 weeks.

4. <u>Maintenance of the farm</u>

The management of seaweed farms comprises weeding-out of poorly growing plants. repairing the support system, replacing lost plants, and removing benthic grazers and other species of seaweeds which grow in close association with *Eucheuma* and *Kappaphycus* as epiphytes or on the monofilament lines and stakes. The epiphytes compete with *Eucheuma* and *Kappaphycus* for nutrients, light, space and also add to the "drag" on line in areas with a strong current which result in breakage and loss of the plants.

Maintenance is a necessary component of farming that may also significantly influence production. In areas characterized by strong currents, a retaining fence made of nylon net (approximately 10 cm mesh size) should be constructed on the leeward side of the farm to catch the thalli that may be washed out by the current.

5. Harvesting

When the thalli of *Eucheuma* and *Kappaphycus* have grown to reach the desired size and volume, they should be harvested and replaced by new cuttings (≈ 100 g) for the next cropping, using the best plants from the harvest are used for this purpose. This practice has replaced the pruning method, where the plants were pruned during harvest to approximately 100 g to serve as the seed for the next crop. This practice was found to be inefficient since branches left behind are the old portions of the thalli that grew slowly. In addition, the tying materials used usually last for only one growing season.

6. Drying of the produce

Drying is an important post-harvest activity that affects the quality of the product. The harvested crop is first cleaned of foreign materials such a old tying materials, weeds, attached animals, etc. and then spread on drying bamboo platforms under the sun to dry. The platforms may also be braided nylon nets. The product is regularly turned over to ensure complete sun drying. The drying crop must be protected from rain, so that before the onset of rain, the crop is piled into heaps by pulling the lining net to one side of the platform, then covered by a water proof sheet. During hot, sunny weather, the material may be dried for 2 or 3 days. Dry Eucheuma/Kappaphycus should not contain more than 40% moisture. The dried material is tightly packed in plastic sacks and stored in dry places.

Eucheuma/Kappaphycus are exported in four forms: as dried raw seaweed, as alkalitreated chip, as semi-processed powder, and as pure carrageenan. The semi-processed product is preferred by big processors of pure carrageenan and by users in the food and canning industry.

XII. Culture Technology of Caulerpa

1. Economic value

The genus *Caulerpa* is a common component of seaweed communities in tropical and subtropical waters. Some species of this genus are utilized as food such as salad and some food uses such as those of the green alga. *Caulerpa lentillifera* is big sized, succulent, with soft thallus and of high quality, which is widely collected and used in the Philippines. At present, there are over 400 ha of ponds utilized in the cultivation of *C. lentillifera* in the Philippines.

Since 1986. C. lentillifera has been cultured using the cage method in Okinawa, Japan. In recent years, the requirement for the use C. lentilliferra has increased rapidly. The cost of 1.0 kg Caulerpa is 28-30 pesos in Philippine markets.

B. Classification

The genus Caulerpa Lanx, 1809 belongs to the Family: Caulerpaceae Grev, 1930, Order Siphonales (Endl) Blockm et Tansl., Class: Chlorophyta Kuetz, and Phylum: Chlorophyta (green algae). Some species of Caulerpa are cultured in Vietnam such as: C. pelltata, C. racemosa, C. verticillata, C. ashmedii, C. taxifolia, C. scalpelliformis, C. racemosa var corynophora Vietnamensis.

C. Form and structure of thalli

The thallus is decumbent, cylindrical, and ramiform. The under-face of the thallus has holdfasts, which attached to rocks or into the sand and mud. Some erect branches grow from the upper-surface, they are cylindrical, stripe or compressed-terete. Some species have ramified "grape-like" branches.

The erect branches may be ramified ramuli or not, or they may be stalks or not. In the sinus of the stem, there are many cross strings from the cell walls. The size of the pigment is small and is not pyrenoid. In cortical cell, Xelluloza, which is inside of the cell cortex is replaced by Callosa with cytoplast.

D. Reproduction

The reproductive form of *Caulerpa* is mainly vegetative. One new cutting from old thallus can develop into a new plant. If vegetative reproduction does not occur, the thalli of *Caulerpa* will become gametes that are flagellate cells, or heterogamia in last blades by sexual reproduction.

The gamete escapes into the water through fuced-like hemispherical lumps that develop in the surface of the erect blades, sometimes in the apex of the prostare blades. Then they combine with zygote, which attach into the zygote segment and germinate to new thallus.

E. Technology of cultivation

1. Culture in ponds and open lagoons

Caulerpa lentillifera is cultivated in ponds and open lagoons in the Philippines (Trono and Ganzon-Fortes 1988, Trono, 1990). The aquaculture of Caulerpa consists of major activities, such as site selection, pond construction, planting, management and fertilization of the ponds, and post-harvest activities.

a. Selection of farm-size

Successful farming of Caulerpa depends partly on the location of the farm. The important factors to be considered in the evaluation of the potential areas for cultivation include: proximity to a source of unpolluted seawater supply; the site must be far from freshwater sources such as rivers, creeks etc.; the level of the pond bottom should be at or just a little above the low-tide level; the site must be protected from the direct effects of wind-driven waves that can easily erode and destroy the dikes; and the substratum must be muddy-loam. Soft bottom or muddy substrata should be avoided. Ponds which were once used for fish culture and are being abandoned or has become inefficient, may be selected for Caulerpa cultivation.

b. Pond Construction

The maintenance of good water quality, achieved through proper pond management, depends on the appropriate design and construction of the ponds. The pond should be divided into 0.5 -1.0 ha area with two gates for supplying and draining seawater, respectively. The gates are arranged appropriately to change water easily. A canal system, which is suitable to supply and drain water easily in dry and rainy seasons, surrounds the pond.

c. Seaweed cultivation

The ponds are first drained of water to a depth of 30 m to facilitate planting. The initial stocking rate should be 100g/m^2 or 1000 kg/ha. The seedstock is planted on the pond bottom uniformly by burying a handful of seedstock at approximately one-meter interval. Another method is to scatter the seedlings from boats, but this not suitable because *Caulerpa* maybe washed out by the waves and will result in poor growth.

After planting, the depth of the water in the ponds should be adjusted to about 50-80 m. Flooding should be done slowly so that newly planted seedstock is not uprooted by the water currents.

d. Management of the pond

Water management in the pond is one of the key factors in the successful culture of *Caulerpa*. Ideally the pond water must be changed at least every other day to maintain adequate level of nutrients thus ensuring good growth and development of the plants.

In the *Caulerpa* culture ponds, usually some weeds and harmful seaweeds grow, which compete with *Caulerpa* for the medium, space and nutrients and badly affecting the growth of *Caulerpa*. Weeds and seagrasses should therefore be cleared by hand or by means of bamboo rakes. The dike system, bank pond, and sluice-gate system should be checked and repaired regularly to avoid breaking and eroding the pond.

The light green or slightly yellow color of *Caulerpa* means that it is growing slowly. Fertilizer may therefore, be added at 1.0 kg per ha (nitrogen). On the other hand, inorganic manure (urea or NH4)2SO4) if applied, should be dissolved first before scattering it in the pond.

e. Culture in lagoons

The following conditions should be taken into consideration, when culturing *Caulerpa* in the lagoons: water depth of 0.3 - 0.5 m at the low tide; seawater coming in and out easily; substratum is clay-mud; protected from strong wind and waves; and less weeds and harmful seaweeds.

Lagoons should be cleaned before cultivation. The cuttings of *Caulerpa* (30-50 cm) are cultivated in the bottom at uniform intervals. When *Caulerpa* is of high quality and glossy, it can be harvested but leaving some seedlings for the next crop.

f. Harvesting of the crop

Depending on the growth rate, the crop may be harvested after two months when seaweed dense in the pond bottom is green, glossy, succulent, and of high quality. The next harvest will be after 2 weeks. *Caulerpa* is usually harvested by hand when about 20-25% mass is observed in the pond. The seaweed is washed and then transferred into floating barrels or boats. Then the *Caulerpa* is washed again, classified and packed. At this stage, it can already be used as vegetable salad.

2. Cage Method

Cage culture method of *Caulerpa* is practiced in some warm areas in Yonaha Gulf, Miyako, Okinawa, Japan. The shape of the cage is cylindrical with some segments. The cuttings of *Caulerpa* (10 cm long) are tied into the bottom segments. Then, the cage is put into the sea where the current is moderate, and waves are not too strong. The cage should be cleaned regularly.

In the rainy season, the salinity at the surface layer should be lower than the depth layer. In this case, the cage should be put lower, about 50 cm from the surface water. Salinity suitable for growth of *Caulerpa* is approximately 25 ppt. *Caulerpa* can be harvested after one month if the growth is good. Harvesting makes use of knife to cut the thalli. Portions are left behind to serve as future plants.

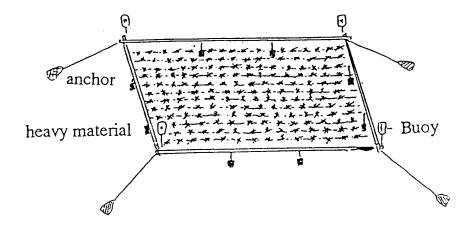


Figure 1. Raft method

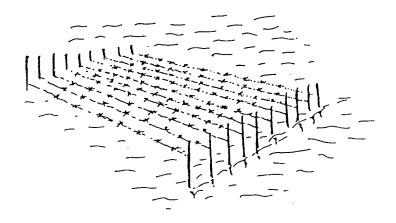


Figure 2. Fixed, off-bottom monoline method

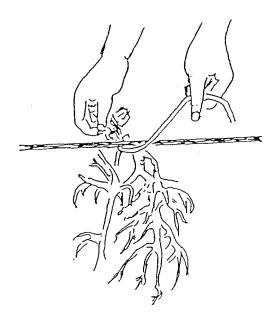


Figure 3. Tie cuttings of Eucheuma/ Kappaphycus

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