Research on Molluscs and Seaweeds

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Abstract

This paper reviews the progress of mollusc and seaweed research at SEAFDEC/AQD from 1995 to 1999. Because of the pressing need for seedstocks for stocking, research on the donkey's ear abalone, *Haliotis asinina* focused on the development of seed production and culture techniques. To improve the spawning performance and egg/larvae production of captive broodstock studies on reproductive biology, management of broodstock and development of diet were conducted. Studies to refine techniques for post-larval settlement and metamorphosis and development of nursery rearing techniques were carried out to increase production of abalone juveniles. An artificial diet has been developed to enhance growth rates of juveniles during nursery as well as grow-out. However, since long-term use of artificial feeds did not favor the growth and survival of abalone during grow-out culture in tanks due to difficulties in maintaining water quality, sequential feeding with artificial diets and then seaweed *Gracilariopsis bailinae* seemed more practical.

Broodstock development and seed production were the major research areas for the window-pane oyster *Placuna placenta.* Gonad development was enhanced by feeding a mixture of high densities of *Isochrysis galbana* and *Tetraselmis tetrahele* at a 3:1 ratio. *P. placenta* larvae reared with *Isochrysis* as feed showed the best growth and survival. Settling stage was reached after 14 days of rearing. A salinity of 34 ppt was optimal for larval survival. Poor growth and survival of larvae was observed at low (10 ppt) and high salinity (40 ppt) levels. Re-stocking of immature adults and juveniles was conducted in a depleted coastal bed to evaluate the potentials for recruitment of the window-pane oyster. After 91 days, a survival rate of 51% was observed among immature adults. No juveniles survived after re-stocking.

Studies on seaweeds focused on three economically important genera of red algae: (1) *Gracilaria,* (2) *Gracilariopsis,* and (3) *Kappaphycus.* These studies are in recognition of *Gracilaria* and *Gracilariopsis* as agarophytes and *Kappaphycus* as carrageenophyte having significant roles in the seaweed industry. Research studies therefore aimed to optimize culture techniques for and to develop environment-friendly aquaculture of these seaweeds. Optimization of biomass production was attempted by manipulating the nutrient environment, biomass density, the proportion of harvested biomass, and crop quality for conversion to agar and carrageenan. The use of *G. bailinae* as a bio-filter focused on the capacity of the seaweed and its agar to sequester heavy metals like cadmium copper, lead and zinc after exposure to various concentrations of these metals. Likewise, excess levels of nitrogen and phosphorus in a finfish broodstock tank with re-circulating water were reduced, demonstrating the efficiency of the seaweed as a bio-filter in aquaculture. Eco-physiological studies of *Gracilaria changii*, *G. coronopifolia*, *G. firma*, and *G. bailinae* involved the mass production of spores *in vitro* as a possible source of seedlings for outplanting. A socioeconomic survey of *Kappaphycus* culture in the Philippines revealed that, although expensive, deep-sea farming of *K. alvarezii* using the multiple raft long-line technique was more productive and profitable than the traditional mono-line or the popularly practiced hanging long-line technique.

Introduction

Research on the donkey's ear abalone *Haliotis asinina,* window-pane oyster *Placuna placenta*, oyster *Crassostrea* spp., and green mussel *Perna viridis* was recommended during the Seminar-Workshop on Aquaculture Development in Southeast Asia (ADSEA) in 1994. However, during the last five years, mollusc research focused mostly on abalone. Abalone hatchery production is still limited by low post-larval settlement. Therefore, research studies centered on the refinement of hatchery and nursery techniques to increase the production of juveniles for grow-out culture. Since abalone are known to be slow-growers, probably due to the relatively low protein content (ca. 15-17 %) of the seaweed (*Gracilaria bailinae*) they eat, development of artificial diets aimed to improve growth rates and to replace seaweeds as a major food source. Refinement of hatchery techniques and rehabilitation of depleted beds by searanching or re-stocking are major concerns for the window-pane shell, *P. placenta* research.

Seaweed research focused on *Gracilaria* and *Gracilariopsis* spp., and *Kappaphycus alvarezii.* Studies optimized culture techniques for biomass production and developed environment-friendly culture protocols for these seaweeds. Eco-physiological studies of *Gracilaria* spp. involved the mass production of spores *in vitro* as a possible source of seedlings for out-planting. A socioeconomic survey compared the cost-effectiveness of the different culture techniques and determined the most applicable and most profitable culture method for seaweed farmers.

Molluscs

Donkey's Ear Abalone Haliotis asinina

Breeding

Histological observations of gonad sections from wild abalone adults showed that mature individuals were present only from January to April and from July to December (Capinpin et al., 1998). During the other months of the year (April, May, June), animals of both sexes have immature gonads or at a pre-proliferative stage. Maximum ripeness in both sexes was observed during October. The smallest wild-caught individual with mature gonad measured 41 mm in shell length (SL) while hatchery-bred adults attained first sexual maturity at 35 mm SL.

Abalones have an asynchronous spawning pattern based on their wide range of gonad bulk index (GBI), a measure of the degree of reproductive synchrony within a given population (Capinpin *et al*., 1998). Attempts to artificially induce spawning of captive *H. asinina* broodstock by desiccation, thermal shock treatment, ultraviolet-irradiation of seawater, and hydrogen peroxide immersion have not been successful (Capinpin and Hosoya, 1995). However, spontaneous group-spawning of tank-held broodstock occurred year-round (Fig. 1, Fermin et al., in press a). With the end-view of controlling

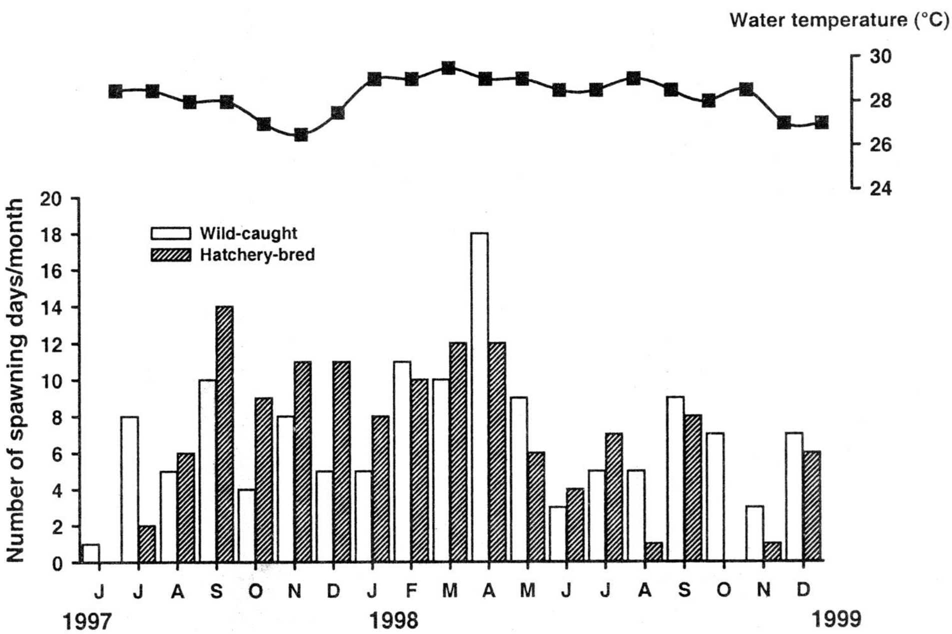

Fig. 1. Frequency of spontaneous spawning in *Haliotis asinina* held in tanks (Data from Fermin *et al.*, In press)

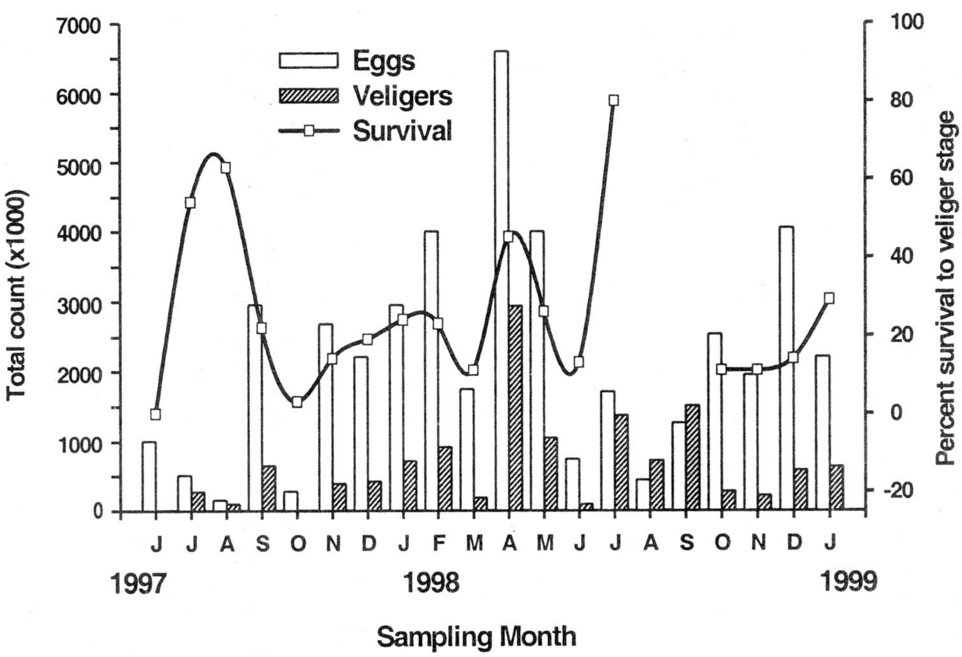

Fig. 2. Composite numbers of veliger from spontaneously spawned eggs of wild-caught and hatchery-bred abalone broodstock held in tanks. Asterisk (*) above bar means percent survival was not determined as veliger mixed with eggs during collection (Data from Fermin *et al.*, In press)

hatchery operations and maximizing larval production, optimal sperm concentration is important. For *H. asinina,* from $5x10^3$ to $1x10^5$ sperm per ml is required for maximal egg fertilization and normal trochophore development (Encena *et al*., 1999).

Potential fecundity or the total number of ripe oocytes of hatchery-bred females (size range: 56-71 mm SL) ranged from 6,000 to 12,000 oocytes per g body weight and it increased with body size (Fermin *et al*., in press a). In contrast, instantaneous fecundity or the total number of eggs spawned was higher (8,000 eggs per g body weight) in smaller females than in larger individuals (2,500 eggs per g body weight; Fig. 2). These results indicate the efficiency, in terms of lesser food consumption, of using younger (and smaller) females in the hatchery.

Hatchery-bred abalone have short spawning intervals that ranged between 15 and 30 days (Capinpin *et al*., 1998, Fermin *et al*., in press a). Younger female broodstock spawn more frequently than older females (Fermin et al., in press a). The short re-maturation period was attributed to sufficiency of food and optimal rearing conditions in captivity. Over a 20-month period, spontaneous mass spawning in tanks showed similar spawning frequency of 6 and 7 times per month for wild-caught and hatchery-bred broodstock, respectively (Fermin et al., in press a).

Evaluation of artificial feeds to replace partly or completely the seaweed *G. bailinae* as major food source for abalone was conducted with the end-view of improving the reproductive performance of captive broodstock. Spawning frequency of abalone fed artificial diets did not differ significantly with abalone fed seaweeds (Bautista-Teruel, in press). However, fertilization rate and spontaneous fecundity were higher in abalone fed a combination of artificial feeds and seaweeds than those singly fed either of the feeds.

Seed production

Studies to refine existing techniques of post-larval settlement and metamorphosis were conducted. Abalone settlement refers to the permanent attachment of larvae to a suitable substrate after shedding the velum to complete metamorphosis. Diatoms, preferably *Navicula* and *Nitzschia,* were cultured on settlement plates several days prior to stocking. "Hardiflex" boards (made of combined fiberglass and cement) as substrate materials for diatom attachment harbored the highest diatom (*Navicula* sp.) population (RSJ Gapasin, personal communication). Lowest counts were obtained from corrugated sheets, which is commonly used as a plate substrate for post-larval settlement. For *Nitzschia,* plexiglass boards had the highest cell counts while canvas the lowest. In terms of cell density (per cm^2), *Navicula* was higher than *Nitzschia*

A 24-h photoperiod significantly induced higher survival (12%) of metamorphosed larvae than did larvae at different light-and-dark periods (Fermin *et al*., in press b). Post-larvae held at 24-h darkness had the lowest or zero survival after 10 days of culture (Fig. 3). Oxygen depletion caused by diatom respiration and larval consumption may have caused heavy mortalities in larvae held in total darkness. Survival of metamorphosed larvae was inversely related to stocking density. A 12% survival rate was obtained at a stocking density of 100 larvae per l and was significantly higher than larvae stocked from 200 to 1000 larvae per l (Fermin *et al*., in press b).

The type of settlement substrate affected the survival of metamorphosed larvae. Corrugated polyvinyl chloride (pvc) plates filmed with a combination of epiphytic diatoms and mucus secreted by grazing abalone juveniles induced higher settlement and survival rates among metamorphosed larvae than ordinary diatom-filmed or clean pvc plates (Fermin *et al*., in press b).

After 60 days on pvc plates coated with epiphytic diatoms and mucus, early juveniles attained

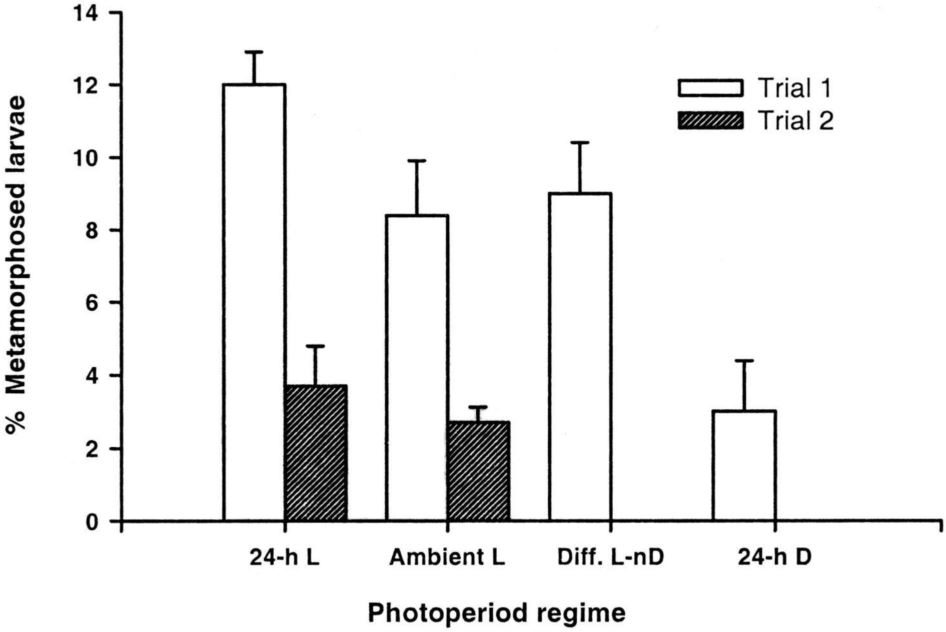

Fig. 3. Composite numbers of veliger from spontaneously spawned eggs of wild-caught and hatchery-bred abalone broodstock held in tanks. Asterisk (*) above bar means percent survival was not determined as veliger mixed with eggs during collection (Data from Fermin *et al.*, In press)

sizes ranging from 5 to 10 mm SL. At this size, weaning of juveniles on seaweed *G. bailinae* did not hamper their growth and survival, which appeared similar to those on an extended feeding on diatoms for 15 days (Fermin et al., in press b). Growth and survival of juveniles reared at varying stocking densities ranging between 183 and 687 individuals per m^2 in perforated plastic baskets did not vary significantly. Juveniles grew at daily growth rates of 170-181 μm and 54-64 mg to attain a final body size of 32-33 mm SL and 6.7-7.9 g body weight with a 96-99 % survival rates during 120 days of culture (Fermin *et al.*, in press b).

Abalone nursery culture in outdoor tanks proved better than indoor. Outdoor tank-reared juveniles had faster growth rate at 170 mm and 65 mg per day compared with juveniles reared indoors at daily growth rates of 111 mm and 35 mg per day (Fermin *et al.*, in press b). Higher growth rates of juveniles reared outdoors were due to higher feeding rates (26-33% per day) than juveniles reared indoors (14-18%) during the first 44 days of culture. After 65 days, juveniles reared in outdoor tank attained a final body size of 28 mm SL and 5 g body weight, which were significantly higher than abalone reared in indoor tanks with 24 mm SL and 3 g body weight. However, survival rates of 98-100% did not differ between groups.

Grow-out culture

Culture of juvenile abalone (25-30 mm SL) to marketable-size (50-60 mm SL, 40-55 g body weight) is carried out within 180-290 days in tanks or in sea cages using seaweed as feed. Although artificial diets promoted the best abalone growth rates during the first 90 days, its long-term use did not sustain culture up to marketable size (Capinpin and Corre, 1996). Abalone fed seaweed *G. bailinae* in excess maintained growth over an extended period until 360 days. The red alga *Kappaphycus alvarezii* was not suitable as feed for abalone.

Table 1. **Daily feeding rates of various sizes of abalone *H. asinina* on seaweed *G bailinae*[a] (Capinpin *et al.*, 1999)**

Shell length (mm)	Body weight (g)	Feeding rate (% body weight per day)[b]
16-20	0.8-1.7	35-40
21-25	1.7-3	30-35
26-30	3.1-6	25-30
31-35	6.2-9	20-25
36-40	10-15	15-20
41-45	16.5-21	12-17
46-50	22.5-30	10-15
51-55	35-45	8-12
56-60	50-60	6-10
>61	>65	5-9

[a]Fresh weight basis.
[b]Calculated as (amount of feed consumed/biomass)/time in days, usually every 7 days.

Density-dependent growth of abalone reared in sea cages (Capinpin *et al.*, 1999) and in tanks has been demonstrated (Fermin and Buen, submitted manuscript). During the first 150 days of culture in sea cages, abalone juveniles of 20 mm initial SL and stocked at 100 per m^2 grew to a size of 45 mm SL (Capinpin *et al.*, 1999). Stocking density was reduced to 40 per m^2 during final rearing to 60 mm SL within 180 days. Generally, smaller juveniles had higher daily feeding rates (DFR) than larger individuals so that a 16-20 mm-juvenile had a DFR of 35-40% while a 41-45 mm- individual can feed only as much as 12-17% of their body weight (Table 1, Capinpin *et al*. 1999).

Under tank conditions, abalone stocked at 25-50 per m^2 grew faster than those held at 100 per m^2 (Fermin and Buen, submitted manuscript). From an initial shell length of 32 mm, abalone stocked at 25 and 50 per m^2 grew to a final size of 60 mm SL and 59 g BW after 290 days of culture.

Feed development

To improve the growth rate of juvenile abalones, practical diets were formulated to contain graded levels (22, 27, and 32%) of animal and plant proteins and compared with natural food using seaweed *G. bailinae* having 17% crude protein as control feed (Bautista-Teruel and Millamena, 1999). Abalone fed diets with graded levels of animal and plant proteins had higher weight gain, daily growth rates, and specific growth rates compared to those fed seaweed. A feed conversion ratio (FCR) of 1.5-2.3 was obtained among animals fed artificial diets, which were higher among seaweed-fed abalone. The optimum protein level for juvenile abalone was 27% with an energy level of 3,150 kcal per kg metabolizable energy. In addition, preliminary results of artificial feeding to improve the reproductive performance of abalone broodstock indicated no significant difference in the spawning frequency of abalone fed the diets or seaweed (M Bautista-Teruel, personal communications). However, instantaneous fecundity and fertilization were higher in the diet-fed abalone than the seaweed-fed group.

Leaf meals from terrestrial plants and freshwater aquatic fern containing 27% crude protein and 5% lipid were evaluated as major protein sources in the diets for grow-out culture of abalone (O Reyes, personal communication). Abalone fed "malungay" (*Moringa oleifera*)-based diets showed a 90% weight gain compared with seaweed (*G. bailinae*)-fed abalone, which had a 75% weight gain. However, highest crude protein (70.3%) in the carcass was obtained from abalone fed papaya (*Carica papaya*) leaf meal-based diet while seaweed fed-abalone had the lowest carcass protein level (60%).

Window-pane oyster **Placuna placenta**

Broodstock development

The effects of feeding algae given at high density on gonadal maturation of *Placuna placenta* were investigated. Gonad development was rapid in broodstock fed a combination of *Isochrysis galbana* and *Tetraselmis tetrahele* at a 3:1 ratio with cell densities of 2 x 10^5 cells per ml (Madrones-Ladja, in press a). Broodstock fed high-density algae showed a higher gonad index (GI) of 330 compared with animals fed low-density algae (GI: 250).

Male and female window-pane oyster were induced to spawn by intra-gonadal injection of 0.5 ml of a 2-mM serotonin or by UV light (925-1395 mW h/l) irradiated-seawater (Madrones-Ladja, 1997). Spawning occurred 15-30 min after serotonin injection, and 30-60 min after exposure to UV light irradiated seawater. The latter method seemed more practical for spawning individuals as well as for groups of broodstock.

Seed production

Studies on feeding various algal species, i.e. *I. galbana, T. tetrahele,* and *Chaetoceros calcitrans* singly or in combination, to oyster larvae were conducted. Larvae fed *I. galbana* showed the best growth with 12.5% survival, but larvae reared on *Chaetoceros calcitrans* exhibited lowest growth (Madrones-Ladja, in press a). Survival rates were not different among larvae fed *C. calcitrans, T. tetrahele* either alone or in combination with each other or with *I. galbana.* Generally, setting stage was reached at day 14 (Madrones-Ladja, 1997).

In other experiments, the effects of various salinities on survival of oyster larvae were examined. Larvae reared at 34 ppt showed the highest survival of 13% whereas, larvae held at varying salinities ranging from 10 to 40 ppt had low survival rates (range: 4.5-7%). Larvae reared at the lowest (10 ppt) and highest (40 ppt) salinity tested had the lowest growth rate. Settling stage of larvae held at 34 ppt salinity was attained at day 14 while those at 16 ppt on day 19.

Stock enhancement

To rehabilitate depleted natural beds of the window-pane oyster, immature *P. placenta* adults (72 mm SL and 14.5 g body weight) were stocked at 75 individuals per m^2 in a 40 m^2 muddy area along the Tigbauan coastline in Iloilo, Philippines (Madrones-Ladja, in press b). Juveniles measuring 40 mm SL were also stocked. One month after stocking, veliger larvae were observed in plankton samples. An assessment of broodstock survival showed a 51% recovery 91 days after stocking. SL and body weight increments were 15 mm and 13 g, respectively. About 40% of recovered broodstock were induced to spawn by UV light irradiation of surrounding seawater.

Seaweeds

Seaweed research at SEAFDEC/AQD for the past several years focused on three economically important genera, namely: *Gracilaria, Gracilariopsis,* and *Kappaphycus.* Areas of studies for these genera were mainly on life history, optimization of biomass production, bio-remediation, and socio-economic.

Life history

To determine the viability of sporelings grown under laboratory conditions for possible outplanting, the life history of various species of *Gracilaria* (*Gracilaria changii*, *G. coronopifolia*, *G. firma*) and *Gracilariopsis bailinae* was examined. Mass production of sporelings from fertile cystocarpic plants grown at 12L:12D photoperiod and 25°C was conducted by using a dry-immersion method using Grund's medium. Sporeling development of all species were of the 'immediate discal type' like the other species of *Gracilaria* (Guimarães *et al*. 1999). Young plants (80-100 mm long) were obtained after 6 months of culture. Large scale mass production of sporelings will soon be attempted.

Optimization of biomass production

The amount of seaweed biomass produced in a given area at a particular time is significant since it determines the technical, environmental, and economic viability of the culture activity. Approaches to meet this objective were made on *Gracilaria, Gracilariopsis* and *Kappaphycus* on the following areas: (1) nutrient environment, (2) biomass density, (3) harvested biomass, and (4) crop quality for the conversion to agarose and carrageenan.

Nutrient environment

Apical segments (5 cm) of *K. alvarezii* were tested in the laboratory for growth performance in various levels of ammonium phosphate and indole-acetic acid (IAA), applied either singly or in combination for 8 weeks. Plants grown in 1 mg per l IAA alone resulted in the highest specific growth rate (SGR, 2.1% increase in wet weight per day); however, it was not significantly different from those grown in 5 mg per l ammonium phosphate and the control. Dawes and Koch (1991) reported healthy explants of *K. alvarezii* when treated with 1, 5 and 10 mg per l of IAA under laboratory conditions. When the same plant was outplanted in cages using the bamboo raft method of culture, further addition of nutrients to the plants was not required. After 8 weeks of culture, plants previously grown in a single dose (5 and 10 mg per l) of ammonium phosphate had comparable growth (6.7-8.0%) with those grown in IAA (1 and 5 mg per 1; 5.7-8.3%). Growth of *K. alvarezii* was slightly better (7.1-7.5%) when grown in higher combination levels of ammonium phosphate/IAA (10/l and 10/5 mg per l) than in lower combination levels (5/1 and 5/5 mg per l; 6.8-6.5%). The addition of ammonium phosphate appears more practical than the addition of IAA, a plant growth regulator, because the latter is expensive.

Experiments in production ecology were conducted to establish an appropriate tank culture method for *Gracilaria.* Salinity tolerance, nitrogen source (ammonium-nitrogen and nitrate-nitrogen), and concentration were determined in relation to growth rate. Optimum growth was observed at 25 ppt. Growth was found to be more efficient when the seaweed was grown in a medium enriched with ammonium-nitrogen rather than nitrate-nitrogen. The addition of phosphate as di-sodium phosphate at 1 ppm did not significantly affect growth.

Biomass density

Stocking density in a culture system may result in the acceleration or stunting of growth of the organism, which in turn is dependent on several environmental factors. The culture of *Gracilariopsis bailinae* in tanks at various stocking densities (2, 4, 8, 12 kg per ton) was examined. A low stocking density (2 kg per ton) promoted growth even if water was not changed for 2 weeks. Higher stocking densities resulted in lower growth rates and harvested biomass (Chavoso and Hurtado-Ponce, 1995).

Harvested biomass

Vegetative thalli of brown and green *Kappaphycus alvarezii* were cultivated in Panagatan Cays in Caluya, Antique, Philippines for 60 and 90 days using hanging-long line (HL), fixed off-bottom (FB), and hanging long line-fixed off-bottom (HL-FB) method to determine daily growth rate and yield. After a 60-day culture period, daily growth rates and yields in all cultivation methods were lowest in July-August and highest in January-February. High growth rate (2.3-4.2% per day) and fresh wet yield (3.6-15.8 kg per m per line) were obtained from September to February. Significant differences in growth rate and yield were also determined between culture months. In a 90-day culture period, no significant differences in growth rate and yield were observed among culture months; however, significant differences were observed among culture methods. Higher estimated production (dry weight) was obtained from the brown strain (6.2-10.2 t per ha per yr) than the green strain (4.9-9.6 t per ha per yr) in all culture methods. The use of HL and HL-FB methods resulted in faster growth rate and higher biomass production than the FB method.

Crop quality for conversion to agarose and carrageenan

Harvested biomass either from a culture system or from a natural population determines the quality of the extracted phycocolloid. Such quality is influenced by the characteristic of the strain, environmental conditions, and the culture system used.

The quality of agarose extracted from wild populations of *Gracilariopsis bailinae* showed significant seasonality in yield and gel strength; however, gel strength was inversely proportional to carrageenan yield. Highest gel strength (296 g per cm) was recorded in April and lowest in December (108 g per cm). A slightly positive correlation existed between agarose yield and salinity. Water temperature, turbidity, and pH exhibited no significant correlation with gel properties.

Likewise, carrageenan gel strength of the brown and green strains of *Kappaphycus alvarezii* varied seasonally when grown by fixed off-bottom long line, hanging long-line, and a combination of the two cultivation methods. Seaweeds grown by a HL-FB method had higher gel strength compared with those grown by the two other methods.

Environment-friendly aquaculture

Earlier studies have shown that *Gracilaria* and *Gracilariopsis bailinae* have the capacity to absorb and store nutrients and to sequester heavy metals, making them useful in intensive aquaculture and in polluted coastal areas. However, heavy metals are retained in the tissues and in the extracted colloid. *G. bailinae* was grown in various concentrations (50, 100, 150, 200 and 250 µg per l) of copper, zinc, cadmium and lead for various periods (12, 24, 36, and 48 h). Copper was easily sequestered followed by zinc, cadmium, and lead similar to the report of Murugadas *et al* (1995). The same heavy metals were still detectable in the extracted agar. For example, unlike lead, copper showed greater affinity to the agar of the macroalga. The results demonstrate that gathering of seaweed like *Gracilariopsis bailinae* from heavy metal-polluted coastal areas should be discouraged.

When *G. bailinae* was integrated in the culture system of finfish broodstock, the seaweed recorded a specific growth rate of 10% per day. Ammonia concentration in tanks with seaweed was lower than in tanks without seaweed. Nitrogen in the tissue of *G. bailinae* became saturated after five days of culture, suggesting that the saturation of nitrogen pools in the plant tissue may stimulate growth.

Socioeconomics

The commercial farming of *Kappahycus alvarezii* in Sacol Island near Zamboanga City, which was originally called *Eucheuma cottonni*, started in the early '70s. The traditional fixed off-bottom line method was used in shallow waters until it was modified when cultivation was made in deeper waters.

The multiple raft long-line method, commonly called 'alu', was introduced in the mid-80s. A survey was conducted last May-June among seaweed planters of *K. alvarezii* in the three major production areas (Tictauan Island, Taluksangay, and Maasin) of Zamboanga City. A total of 30 respondents were interviewed based on: (1) personal background, (2) farming practices, (3) marketing system (4) economic impact, and (5) problems.

Seaweed farming in these areas shared common characteristics like: (1) family entrepreneurship, (2) labor hiring, and (3) capital sharing. Although farming of *K. alvarezii* by multiple raft long line method is expensive, return of investment is still high (59-226%) and payback period is comparable (0.4-1 yr) with the single bamboo raft method (0.4-0.9 yr) reported by Samonte *et al* (1993). Projected production of 21-38 t (dry weight) per ha per crop was higher in the multiple raft long line compared with single bamboo raft reported by Samonte *et al.* (1993). Problems like high moisture content (40-60%) and impurities, seasonal occurrence of 'ice-ice' (Alih, 1990) and poaching are common.

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