MACROALGAE IN AQUACULTURE: AN OVERVIEW AND THEIR POSSIBLE ROLES IN SHRIMP CULTURE.

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1. Culture: In terms of the quantity produced, macroalgae are the second most important group of cultured aquatic organisms behind finfish. FAO figures for 1991 show that 3.9 million mt (wet weight) of aquatic plants were produced in 1991. This figure accounts for 24 % of the total aquaculture production and was worth \$US 3.88 billion (at about \$ US 1 kg⁻¹) or 14 % of the value of the worlds' total aquaculture production (Anon., 1993). Of this total harvest by aquaculture, China produced over 2.3 million mt in 1991. Other important producers included Taiwan, South America, Southeast Asia and South Africa (Anon., 1993).

Seaweeds have been harvested from the wild since 3,000 BC, but have become increasingly popular for culture due to the overexploitation of wild stocks, leading to a shortage and high demand on the world market. Seaweeds have a number of benefits over the culture of fish and shellfish. These include the fact that they require relatively simple, low technology systems with low inputs and operating costs (i.e. no feeding) and are thus suitable for small-scale production systems; they can provide women with one of their few sources of income; they are energy efficient due to their position low on the food chain; they can produce high value products after processing; and they are environmentally friendly, cleaning up, rather than creating environmental pollution, and not requiring energy-wasteful inputs. Due to these factors they may provide direct economic benefit to a greater number of people (Uusitalo, 1987; Ricohermoso, 1988).

- 2. Species: There are many important species of algae used or potentially useful in aquaculture. The macroalgae, or multicellular "seaweeds" include red algae such as Gracilaria, Porphyra, Eucheuma, Sargassam and Gelidium, brown algae such as Laminaria and Undaria and green algae including Enteromorpha and Ulva. However, most of this discussion will be concentrated on Gracilaria due to its high potential in aquaculture for reducing nutrient levels in farm effluents, its high value for agar production and its nutritional value.
- 3. Methods: Methods for culture of seaweeds such as *Gracilaria* are diverse, but are usually accomplished by seeding algal pieces, spores or by natural settlement onto ropes, nets or polythene tubes which are then either hung, suspended or staked or merely placed in bottom culture into specially-constructed ponds or impoundments, the sea, estuarine or other brackishwater habitats (Avila, 1989).
- 4. Yields: From a review of the literature, production of *Gracilaria sp.* whether in sea/estuary or ponds on ropes or nets yielded typically 2-6 % of wet wt. d⁻¹ (mean 5 %, range 1-18 %) (Bird et al., 1977; Nelson et al., 1980; Ryther, 1982; Chennubhotla, 1986; Chennubhotla et al., 1986; Lignell et al., 1987; Largo et al., 1989; Wongwai, 1989; Chandrkrachang, 1990; Rincones-Leon, 1990; Orosco & Ohno, 1992). Under controlled conditions in tanks with submersible lights however, yields of up to 47 % wet wt. d⁻¹ have been achieved (Lignell et al., 1987). In terms of culture density, typical yields have ranged from 2-12 g dry wt. m⁻²d⁻¹ (mean 8 g m⁻²d⁻¹, equating to nearly 30 t ha⁻¹yr⁻¹) (Chiang, 1981; Ryther, 1982; De Araujo et al., 1986; Subbaramaiah, 1986; Guerin & Bird, 1987; Tachanavarong, 1988; Tra, 1989; Subbaramaiah & Thomas, 1990; Chaiyakom & Tunvilai, 1992; Westermeier et al., 1993). Although yields of up to 22-44 g m⁻²d⁻¹ were achieved under controlled conditions in tanks (Lapointe & Ryther, 1978, 1979; Ryther, 1980).

From a review of the literature, Agar yield is typically 33 %, ranging from 10-50 % of the dry weight of *Gracilaria sp.* (Drago *et al.*, 1980; Durairatanam, 1987; Hurtado-Ponce & Umezaki, 1988; Tra, 1989; Machiavello, 1990; Matsuhiro & Urzua, 1990; Engledow & Bolton, 1992).

5. Growth conditions: The yield of both the seaweed itself and the quantity and quality of agar (its most valuable constituent) it produces is influenced greatly by the conditions under which it is cultured. Among the most important influences are the ambient nutrient concentration, aeration, flow rate, light and hence depth,

temperature and salinity levels under which it is cultured.

The growth rate of Gracilaria has been found to increase with increasing nutrient concentration of the culture medium. These concentrations can be provided successfully either continuously or by pulsed flow since such macroalgae have the capacity to store sufficient nutrients for two weeks growth at maximum rates in only six hours exposure to high nutrient concentrations (Ryther et al., 1981; Ryther, 1982; Friedlander et al., 1991). Growth and photosynthetic rate, protein content and agar gel strength and melting point (due to the larger agar polymers) of *Gracilaria* have all been found to increase with the nutrient concentration and/or with the frequency of pulses of nutrient rich water at all concentrations (Bird et al., 1981; Bird, 1984, 1988; Dawes et al., 1984; Lapointe, 1985; Rotem et al., 1986; Christeller & Liang, 1989; Friedlander et al., 1991). Agar yield, however, may decrease with increasing nitrogen concentration (Bird et al., 1981; Rotem et al., 1986; Sivalingam, 1989, Luhan, 1992), but increase in the presence of phosphate (Sivalingam, 1989). Normally, nitrogen in the form of ammonium (NH₄-N) has been shown to promote better growth rates than nitrate (NO₃-N) nitrogen (De Boer et al., 1978; D'Elia & DeBoer, 1978; Lapointe & Ryther, 1979; Yoneshigue-Braga & Baeta-Neves, 1981; Fujita & Goldman, 1985; Friedlander et al., 1991). Phosphorous, however, is usually the most limiting nutrient in seawater and hence may sometimes be the primary growth stimulant (Lapointe, 1989). Both ammonium (> 3 mg Γ^1) and phosphorus (> 10 mg Γ^1), however, have been noticed to inhibit growth at very high concentrations, while nitrate concentrations of up to 20 mg l⁻¹ and nitrite levels between 2 and 20 mg l⁻¹ have not reduced growth rate (Lapointe & Ryther, 1979; Sivalingam, 1989; Friedlander & Ben-Amotz, 1991).

Since all algae require carbon dioxide for photosynthetic energy production, the level of aeration and/or current flow is of importance. The growth rate of *Gracilaria* has been found to increase significantly by increasing flow rate up to 7.5 cm sec⁻¹ (Lapointe & Ryther, 1978, 1979; Parker, 1982; Fujita & Goldman, 1985) or aeration levels (Ryther, 1982; Guerin & Bird, 1987; Friedlander & Ben-Amotz, 1991). The growth rate of *Gracilaria* has usually been shown to be positively correlated to light intensity (Peterson & DeBusk, 1985; Bird, 1988; Liang *et al.*, 1989; Ekman & Pederson, 1990). The best production figures yet achieved were obtained when *G. secundata* was grown in tanks with submerged light sources. The highest irradiance tested was still insufficient to light-saturate the algae. However, UV light exclusion, or irradiation with only blue and green light has been shown to enhance growth rates further (Friedlander & Ben-Amotz, 1991). Similarly, algal production is correlated negatively with depth due to light limitation, optimum production usually occurring in the top metre of water (Bird *et al.*, 1977; Rincones-Leon, 1990; Subbaramaiah & Thomas, 1990). The temperature and salinity optima for growth, agar gel strength and photosynthetic rate vary depending upon species, strain and location, but growth is generally best in the tropics at high and constant temperatures, 20-35 °C and salinities, 17-33 % (Dawes *et al.*, 1984; Bird, 1988; Daugherty & Bird, 1988).

6. Problems: One of the major problems facing algal culturists, particularly in pond or sea culture under variable environmental conditions such as high nutrient loadings is that of epiphyte competition. Epiphytes commonly comprise undesirable nuisance algae such as diatoms, Enteromorpha or Ulva. These plants compete for nutrients and represent contaminants in the harvested crop. Low growth rates and crop loss due to smothering are common problems caused by such epiphytes (Breden & Bird, 1988; Oliveira et al., 1990; Friedlander & Ben-Amotz, 1991). Partial control of epiphytes has been achieved with the use of pulse-fed media containing high concentrations of ammonium and relatively low irradiance, promoting the growth of G. conferta and inhibiting its epiphyte biomass, presenting a competitive relationship (Ryther, 1980; Freidlander et al., 1991). The technique of soaking algae in nutrient rich media (for example shrimp pond wastes) and then removing them for culture in areas of low nutrient concentrations and thereby limiting epiphyte infestation may thus serve a dual function and is worthy of future research. In an alternative technique, a herbivorous fish, the sailfin molly (Poecilia latipinna) has been used to graze on epiphytic algae and reduce their nuisance value (Breden & Bird, 1988).

One of the other main problems in algal culture is that of predation. An analysis of the fauna associated with the culture of *G. edulis* in India found that of the 26 fish species encountered, gut content analysis showed only fish of the genus *Siganus* (rabbit fish) fed to any extent directly on the seaweed (James *et al.*, 1986). The successful use of nets to prevent *Siganus* damage inside sheltered ponds or bays may, however, permit successful culture of Gracilaria (James *et al.*, 1986; Chaiyakom & Tunvilai, 1992). Significant, but occasional predation of seaweeds has also been attributed to animals including crabs, gastropod and bivalve molluscs and polychaetes. However, it was also reported that most of the damage to the cultured seaweed could be traced to wind and wave

action during rough weather (James *et al.*, 1986). This may therefore place the same restraints on open sea culture of seaweeds as are already in force for cage and pen fish farms. Research is also required in optimum management strategies for algal production taking into account the particular set of conditions encountered in any location.

7. Uses: The major industrial use for seaweeds such as Gracilaria is as a source of phycocolloids such as agar. The agar is then used mostly in the production of tissue culture media. Many countries such as Thailand and India in Southeast Asia have the potential to export agar from seaweed production, but are net importers in rapidly increasing amounts of seaweeds and their products (Edwards et al., 1982; Subbaramaiah, 1986; Uusitalo, 1987; Tachanavarong, 1988). Techniques are now available for the simple and cheap extraction of agar from Gracilaria. These techniques, together with the high value (from 20-60 Bt kg⁻¹ in Thailand currently), enhances further the potential of such seaweeds for mariculture (Santos & Doty, 1983; Chandrkrachang, 1990; Usov & Ivanova, 1990).

Another important quality of seaweeds such as Gracilaria is their ability to recover nutrients from seawater. This ability may be exploited further with the increasing demand for solutions to the water quality and environmental degradation problems imposed upon many third world countries as a result of intensive shrimp culture. Major problems of nitrogen and phosphorous buildup in both shrimp ponds and the environment after water exchange may be combatted by the use of Gracilaria either in tanks or the culture or settlement ponds. Trials in tanks have shown that Gracilaria can uptake nutrients, especially ammonium very quickly, particulary at high concentrations, suggesting the possibility that short term soaking of seaweed in effluents, followed by tank or sea culture may be both profitable and environmentally sound (Smith & Briggs, unpublished data; D'Elia & DeBoer, 1978; Nelson et al., 1980; Fujita, 1985; Ryther et al., 1981; Schramm, 1991). Similarly, pond and seabased trials under field conditions have shown that Gracilaria can remove nutrients effectively and grow rapidly under the right conditions (Fralick et al., 1981; Friedlander & Dawes, 1985). At an average Gracilaria production rate of 30 t hardyr and at 5 % of dry weight as nitrogen, potential nutrient removal is in the order of 4.1 kg N ha⁻¹d⁻¹. This compares with the average nutrient discharge of about 1.2 kg N ha⁻¹d⁻¹ from intensive shrimp ponds in Thailand (Briggs & Smith, unpubl. data). If seaweed was therefore not cultured in the ponds directly, 30 % of the pond area would have to be used for seaweed culture/settlement ponds to absorb all of the nitrogen wastes produced. Macroalgae may also have potential in limiting blooms and crashes of microalgae in shrimp ponds due to their capacity to rapidly absorb nutrients and sunlight and survive over periods of low nutrient density (Smith & Briggs, unpublished data; Camara-Neto, 1978).

Seaweeds also have great potential as a source of nutrition for humans, animals and plants. Seaweed is already a source of nutrition for poor coastal peoples (Edwards et al., 1982) and is being investigated as an ingredient for a low fat burger by Macdonalds (Anon., 1991). They have also been used as plant fertilisers from Western Europe to Asia (Subbaramaiah, 1986). Macroalgae such as *Gracilaria* has potential for use in grow-out diets for fish and shrimp. This potential is shown in many areas: Seaweeds containing phycocolloids have been used as binders for pelleted feeds (Hashim & Mat-Saat, 1992); Gracilaria contains from 7-13 % protein, increasing with the nutrient concentration of the culture medium (Smith & Briggs, unpublished data; Nelson et al., 1980; Peterson & DeBusk, 1985); Gracilaria contains small quantities of marine lipids including the nutritionally-valuable cholesterol (Gaydou et al., 1986; Dhar & Tiwari, 1991; Levy et al., 1992); Gracilaria contains carotenoid pigments which are important in shrimp and fish diets (Pollesello et al., 1992); and seaweeds are also a valuable source of both vitamins and minerals which may be incorporated into aquaculture diets.

Seaweeds also have other important uses as sources of energy (anaerobic fermentation to methane gas)(Hanisak, 1981; Ryther, 1982); additives in medicines and pharmacies (Kauser & Ali, 1986; Subbaramaiah, 1986; Sjafrie, 1990; Santos, 1990); as superconductive ceramic fibres (Anon., 1989); as alternatives for cosmetic paper (Chandrkrachang, pers. comm.), in the chemical industry (Subbaramaiah, 1986) and so on.

8. Research: Research work on this British government-funded programme has concentrated to date on quantifying the amount of nutrients removed from seawater spiked with various levels of ammonium (NH₄-N) and phosphorus (PO₄-P). The results of these trials performed both in small-scale tank and large-scale concrete pond trials have shown that a) the *Gracilaria* algae itself, rather than the bacteria naturally colonising the algal fronds is responsible for nutrient removal; b) up to 3 mg Γ^1 d⁻¹ (81 %) of NH₄-N and 0.5 mg Γ^1 d⁻¹ (68 %) of PO₄-

P can be removed from seawater by 2 g Γ^1 of *Gracilaria* depending upon initial nutrient concentration; c) nitrite (NO₂-N) and nitrate (NO₃-N) nitrogen levels were similarly (though more slowly) removed by *Gracilaria*; d) increasing the levels of PO₄-P in the seawater increased (but not significantly) the removal rate of NH₄-N. Future research will be aimed at examining the short and long term ability of *Gracilaria* to reduce nutrient loadings from shrimp pond waste water under field conditions, and the value of *Gracilaria* meal in shrimp diets.

Research work still required includes more large-scale field testing of the contribution of seaweeds (perhaps in combination with bivalves) in nutrient reduction in both waste and recirculating water of shrimp farms, such as is being carried out by the National Institute of Coastal Aquaculture (NICA) in Songkhla and the Biopolymer Research unit (BRU) of Srinakarinwirot University in Bangkok (Chandrkrachang *et al.*, 1991). Also necessary is an investigation of the feasibility and techniques of culturing seaweeds within intensive shrimp ponds to discover the extent to which macroalgae can replace microalgae in pond water quality maintenance and be produced as a bycrop (Camara-Neto, 1978). Other fundamental research required in the algal culture industry is an examination of species and strain potential and further refinement of culture techniques for optimum production of agar and algal biomass. In addition, alternative uses for algae and their byproducts are required for market diversification and to enhance the economics of algal production.

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Table 1. Growth rate of Gracilaria sp. under various conditions.

Species	Production (g dry wt. m ⁻² d ⁻¹)	Production (% wet wt. d ⁻¹)	Conditions	Author
G. arcuata G. edulis		2.0 5.1	Tank in laboratory Tank in laboratory	Nelson, Tsutsui & Best, 1980
G. sp.		7.2	Basket in estuary	Bird et al., 1977
G. tikvahiae	35.5	7.1-14.3	Ropes in sea	Ryther, 1982
G. tikvahiae	21.4		Tanks outside	Ryther, 1980
G. foliifera	44		Tanks outside	Lapointe & Ryther, 1979
G. tikvahiae	34.8		Tanks outside	Lapointe & Ryther, 1978
G. sp.		2-4	Net cages in bay	Orosco & Ohno, 1992
G. cornea		1.4-2.5	Ropes in sea	Rincones-Leon, 1990
G. verrucosa		10 4.3	intertidal area semi-enclosed pond	Largo, Bacolod, Cusi, Orosco & Ohno, 1989
G. edulis	11		Ropes in sea	Subbaramaiah & Thomas, 1990
G. edulis		5	Ropes in sea	Chennubhotla, 1986
G. edulis		18.3	Ropes in sea	Chennubhotla, Kalimuthu & Selvaraj, 1986
G. sp.	1.8 8.6		Ponds Nets in lagoon	Tachanavarong, 1988
G. sp. (G-16)	12-22			Guerin & Bird, 1987
G. secundata		47 6 3	Tanks with lights spray system Tanks	Lignell, Ekman & Pederson, 1987
G. verrucosa	12.9-17		Ropes in ponds	De Araujo et al., 1986
G. sp.	1.9-3.3		Ponds	Shang, 1976
G. sp.	4.4-11.8		Ponds	Chiang, 1981
G. verrucosa	2.7-9.6		Ponds	Tra, 1989
G. tenuistipitata		2.4-3.3	Ponds	Renzhi et al., 1989
G. chilensis	1.5-3.4		Ropes in estuary	Westermeier et al., 1993
G. fisheri	2.9		Nets in lagoon	Chaiyakom & Tunvilvi, 1992
G. sp.		6.7 66.7	Lines in lagoon Ponds	Wongwai, 1989
G. edulis	2.7		Ropes in sea	Subbaramaiah, 1986
G. sp.		0.7-1.3	Nets in lagoon	Chandrkrachang, 1990

Table 2. The yield of agar from various species of Gracilaria.

Species	Agar (% dry weight)	Author
Gracilaria verrucosa	32-34	Engledow & Bolton, 1992
Gracilaria sp.	8-15	Machiavello, 1990
Gracilaria arcuata Gracilaria adulis Gracilaria sp.	49 24 24-49	Hurtado-Ponce & Umezaki, 1988
Gracilaria chinensis	43	Matsuhiro & Urzua, 1990
Gracilaria verrucosa	50-60	Drago, Genchi & Sortino, 1980
Gracilaria verrucosa	15-26	Tra, 1989
Gracilaria edulis	20-25	Durairatnam, 1987