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MINIREVIEW

IMPACT OF ALGAL RESEARCH IN AQUACULTURE[1]

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Algal aquaculture worldwide is estimated to be a $5–6 billion U.S. per year industry. The largest portion of this industry is represented by macroalgal production for human food in Asia, with increasing activity in South America and Africa. The technical foundation for a shift in the last half century from wild harvest to farming of seaweeds lies in scientific research elucidating life histories and growth characteristics of seaweeds with economic interest. In several notable cases, scientific breakthroughs enabling seaweed-aquaculture advances were not motivated by aquaculture needs but rather by fundamental biological or ecological questions. After scientific breakthroughs, development of practical cultivation methods has been accomplished by both scientific and commercial-cultivation interests. Microalgal aquaculture is much smaller in economic impact than seaweed cultivation but is the subject of much research. Microalgae are cultured for direct human consumption and for extractable chemicals, but current use and development of cultured microalgae is increasingly related to their use as feeds in marine animal aquaculture. The history of microalgal culture has followed two main paths, one focused on engineering of culture systems to respond to physical and physiological needs for growing microalgae and the other directed toward understanding the nutritional needs of animals—chiefly invertebrates such as mollusks and crustaceans—that feed upon microalgae. The challenge being addressed in current research on microalgae in aquaculture food chains is to combine engineering and nutritional principles so that effective and economical production of microalgal feed cultures can be accomplished to support an expanding marine animal aquaculture industry.

Key index words: **cultivation; culture; human food; macroalgae; mariculture; microalgae; seaweeds**

Abbreviations: **DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid**

The algae, as a group, represent the third-largest aquacultured crop (after freshwater fishes and mollusks) in the world today (Hanisak 1998, Anon 2000). Total harvest in 1998 was estimated to be approximately 9.5 million metric tons worth US$5.4 billion per year. By far, most of this production is located in Asia, where brown and red seaweeds dominate production. Macroscopic marine algae—or seaweeds—for human consumption, especially nori (*Porphyra* spp.), wakame (*Undaria pinnatifida*), and kombu *(Laminaria japonica)*, are widely cultivated algal crops, but the list of taxa used as human foods is relatively diverse (Table 1). Use of cultivated seaweeds in Europe and the United States is less direct, although phycocolloids (carrageenan, agar, alginates) are used widely in processed foods (Zemke-White and Ohno 1999). Carrageenan is extracted from a number of taxa (*Kappaphycus, Chondrus, Eucheuma, Gigartina, Iridaae*), agar from *Gracilaria* and *Gelidium*, and alginates from *Lessonia, Macrocysytis*, and *Laminaria*. Algal biomass from which phycocolloids are extracted may be from wild-harvest populations or from areas cultivated to some extent. Most carrageenan is produced from cultivated materials (Canada still supports a modest carrageenan industry from wild harvest), and a significant amount of alginate is extracted from *Laminaria* cultivated in China. Edible seaweeds and seaweed farming is big business on a worldwide scale with appreciable economic impact. *Chondrus crispus* is also aquacultured as an edible crop using land-based tank farms (50,000 m^{-2} in Nova Scotia; Craigie et al. 1999).

Human use of single-celled microalgae is much less developed than for macrophytes. Few examples of human foods from microalgae can be cited, although the cyanobacterium *Spirulina* has a limited "health-food" market. Similarly, cultured *Chlorella* has a limited but lucrative (US$500 million per year) health-food market in Japan. Some microalgae are cultured for extractable compounds (e.g. the pigment beta-carotene, xanthophyll pigments such as astaxanthin, and the fatty acid docosahexaenoic acid [DHA]), nutritional supplements, or food additives. Potential applications of microalgal culture to such advanced concepts as synthetic fuel production from solar energy and oxy-

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TABLE 1. List of the main algal genera used in commercial aquaculture (compiled from Critchley and Ohno 1998 and Wikfors 2000).

Class	Genus	Uses
Cyanophyceae	*Spirulina*	Human food, component of mariculture feeds coloration for gold fish in Japan and China
Eustigmatophyceae	*Nannochloropsis*	Live feed in marine fish food chain
Prasinophyceae	*Tetraselmis*	Live feeds for marine mollusks and crustaceans
	Pyramimonas	Live feeds for marine mollusks and crustaceans
Chlorophyceae	*Chlorella*	Live feed in marine fish food chain, health-food supplement for humans in Japan
	Dunaliella	Extracted pigments, beta-carotene
	Haematococcus	Extracted pigments, astaxanthin
Cryptophyceae	*Rhodomonas*	Live feeds for marine mollusks and crustaceans
	Cryptomonas	Live feeds for marine mollusks and crustaceans
Dinophyceae	*Crypthecodinium*	Extracted lipids, DHA, human food
Prymnesiophyceae	*Isochrysis*	Live feeds for marine invertebrates, fish food chain
	Pavlova	Live feeds for marine invertebrates, fish food chain
Bacillariophyceae	*Chaetoceros*	Live feeds for marine mollusks and crustaceans
	Thalassiosira	Live feeds for marine mollusks and crustaceans
	Nitzschia	Live feeds for marine mollusks and crustaceans
Chorophyta	*Monostroma*	Edible seaweed, human food
	Enteromorpha	Edible seaweed, human food
Phaeophyceae	*Laminaria*	Alginates, edible seaweed, human food
	Undaria	Edible seaweeds, human food
	Cladosiphon	Edible seaweeds, human food
Rhodophyceae	*Gelidiella*	Agar, human food, and medical
	Gelidiopsis	Agar, human food, and medical
	Gelidium	Agar, human food, and medical
	Gracilaria	Agar, human food, and medical
	Pterocladia	Agar, human food, and medical
	Chondrus	Carrageenan, human food
	Eucheuma	Carrageenan, human food
	Kappaphycus	Carrageenan, human food
	Gigartina	Carrageenan, human food
	Hypnea	Carrageenan, human food
	Iridaea	Carrageenan, human food
	Porphyra	Edible seaweed, human food

gen regeneration during long-term space travel have been investigated without subsequent commercialization (F. R. Trainor, University of Connecticut, personal communication). By far, the most widespread application of microalgal culture has been in artificial food chains supporting husbandry of marine animals, including finfish, crustaceans, and mollusks. Farming of marine animals is the most rapidly growing sector of the human food supply (Anon 2000); therefore, microalgal feeds for marine animals is a focus of current research and commercial attention.

Production statistics for cultivated (in the sea) and cultured (in tanks) algae have been compiled with much greater precision and specificity elsewhere (Anon 2000). The intent of this brief review is to assess the role of scientific research in past, current, and future human uses of cultured algae. If we assess how knowledge gained through scientific inquiry has been applied to practical problems of fundamental human importance (i.e. food security for an exploding human world population and economic activity that is environmentally sustainable), we may be better able to direct future research to solve remaining problems. We may also better recognize the value of knowledge acquired during pure scientific pursuits. Examples of both directed and serendipitous discovery are found in this assessment.

SEAWEEDS FOR DIRECT HUMAN CONSUMPTION

Domestication of a living organism implies human control of reproduction. Although this concept has been applied practically to land plants (and animals) for millennia, explicit knowledge, and thus control, of aquatic macrophyte life cycles has been acquired only within the past 50 years. Reliance on natural reproduction risks crop failures from climatological variance, ecological regime shifts, and other environmental factors not controlled by the farmer. Until the processes leading to the presence of "seeds" in coastal waters were understood, seaweeds could not be domesticated. Previous to technical descriptions and methods to control reproductive cycles, farmers/harvesters in many Asian regions developed highly successful methods—best described, perhaps, as "traditional" methods—that responded to natural seasonal cycles (Korringa 1976). For example, cultivation of *Porphyra* by traditional methods has occurred in Tokyo Bay since the 1670s. Chance observation likely taught farmers that "ripe" seaweeds dried in the shade for several hours and then reimmersed in seawater could be induced to release conchospores. Control of this process was developed commercially in Japan during the 1930s (Kafuku and Ikenoue 1983). By contrast, *Laminaria* cultivation in China was made possible only by clear life cycle control since the 1950s

(Tseng 1981). Similarly, *Undaria* cultivation in Japan was made possible by life-cycle control around 1970, and *Laminaria* cultivation of Japan was started in 1975 (Critchley and Ohno 1998). At present, seeding of *Porphyra*, *Laminaria*, and *Undaria* cultivation systems commonly involves selecting and hybridizing among cultivated strains and wild strains. Research to produce improved strains of cultivated macroalgae is the most active pursuit at research laboratories in Japan, Korea, and China (Tseng 1986, 1987, 1989, 1990, 1993, Ohno and Critchley 1993, Zeng 1994).

Perhaps the clearest example of a scientific discovery providing enabling knowledge for a paradigm shift in algal aquaculture was the discovery, by K.M. Drew (Drew 1949), of the conchocelis stage of *Porphyra*. Identification of a previously described species (*Conchocelis* sp.) of filamentous red algae, found within the shells of bivalve mollusks, as an alternate life-history stage of the foliose rhodophyte *Porphyra umbilicas* solved not only taxonomic and ecological questions, but also set the stage for farmers to close the life cycle in nori cultivation. By the mid-1950s, practical methods for cultivation of conchocelis-stage *Porphyra* within oyster or scallop shells, induction of conchospore release, and "seeding" of nets used for grow out of the foliose stage intertidally were sufficiently developed for a practical manual to be published (Ueda 1958, as cited in Kafuku and Ikenoue 1983.). Technology developed within a decade of the breakthrough scientific discovery of the life history of *Porphyra*, by a partnership of scientists and farmers, is being used successfully today in Japan, China, and Korea (Ohno and Critchley 1993).

In addition to direct consumption, agars and carrageenans extracted from red seaweeds and alginates from brown seaweeds have been included in a remarkable array of prepared food products, serving mostly to modify viscosity or texture. *Kappaphycus* and *Eucheuma* (*Kappaphycus alvarezii* has replaced *Eucheuma cottonii* recently, so both names appear in the literature) are cultivated in tropical countries thereby being the source of most of the raw materials for carrageenan (Critchley and Ohno 1998). Methods for the cultivation of *Kappaphycus/Eucheuma* were developed by M.S. Doty at the University of Hawaii and by researchers in the Philippines during the 1960s (Doty 1970). Before this directed research and development effort, managed harvest of natural populations of *Chondrus crispus* (De Oliviera and Alveal 1990, Moseley 1990) and traditional cultivation methods (Critchley and Ohno 1998) produced most of the raw material for carrageenan extraction. World seaweed cultivation has been increased very dramatically by the research efforts of Doty et al. since the 1970s, expanding into Africa and South America (Ohno and Critchley 1993, Riconnes and Rubio 1999). Accordingly, commercial use of carrageenan has expanded significantly from preparation of simple jellies to use as a stabilizer in many prepared food products (Critchley and Ohno 1998). More recently, cultivation of the agarophyte *Gracilaria* has become important in Chile and several Southeast Asian countries (Critchley and Ohno 1998). *Gracilaria* cultivation borrows much from methods used for *Kappapycus* and *Eucheuma* (Critchley and Ohno 1998).

MICROALGAE

Farming of marine animals, including both finfish and invertebrates—chiefly crustaceans (shrimps) and mollusks—requires microalgae as a feed at some point in the life cycle (Jeffrey et al. 1994). Bivalve mollusks, such as oysters, clams, and scallops, feed on microalgae throughout life. Shrimp may feed directly on microalgae in early larval stages; however, as with finfish, microalgae mainly are used in the production and "conditioning" of zooplankton feeds consumed by larval stages. Rotifers (*Brachionus* spp.) and brine shrimp (*Artemia* spp.) are the zooplankters most commonly cultured as live foods for shrimp and finfish larvae. Populations of these live feeds may be produced using microalgae, yeasts, or other heterotrophic microorganisms or artificial diets formulated from agricultural and refined chemical components. Cultured microalgae, however, are used widely to improve the nutritional content of zooplankton live feeds by allowing the zooplankton to fill their digestive systems with microalgae before subsequently being fed to the fish or shrimp larvae. In this "conditioning" strategy, the zooplankton serve as "bags" of appropriate size that partially digest the algae and stimulate feeding behavior in the larvae, thereby transferring algal nutritional compounds to the larvae. Although microalgal feed production often is considered a bottleneck in marine aquaculture, development of artificial diets has met with only limited success; therefore, microalgal culture remains an integral part of marine animal aquaculture (Robert and Trintignac 1997).

The technical foundation for microalgal culture is built upon the research of such pioneers as Pringsheim, Fogg, and Provasoli. These researchers elucidated the basic physical and chemical conditions required for population growth of diverse microalgal taxa and established protocols to accomplish sustained culture of selected strains. The fundamental knowledge required to isolate and propagate microalgae is summarized in the first volume of the *Handbook of Phycological Methods* (Stein 1973). Application of fundamental knowledge about the physical and chemical requirements for microalgal population growth to commercial production has, however, not been a direct process, possibly because uses of cultured microalgae have not been direct (human consumption). Further, widely divergent applications (e.g. extractable chemicals versus aquaculture feeds) have led to divergent approaches to microalgal culture.

One of the most remarkable sustained scientific investigations of microalgal culture was conducted by the Carnegie Institution of Washington from the late 1940s through the 1960s (Burlew 1953). Seduced by the high protein content of microalgae, especially the

chlorophyte *Chlorella*, government and private interests envisioned a cheap efficient source of human food in microalgal culture. Early proponents of *Chlorella* farming seemed to take it on faith that the abundant proteins produced by *Chlorella* could be made nutritionally available somehow and focused directly on the biological and engineering challenges of optimizing biomass production. At these challenges, the Carnegie team excelled. Fundamental knowledge of chemical and energy stoichiometry and rates of photosynthesis in *Chlorella* was accumulated with a thoroughness unmatched in more recent history. Unfortunately, *Chlorella* proved to be refractory as a bulk human food (Powell and Nevels 1958), and no alternate large-scale commercial applications of this alga have been identified. Recently, in Japan, *Chlorella* has been used as a "health food" supplement in tablet or pill form. Despite the limited commercial development, the highly engineered approach to microalgal culture pioneered by the Carnegie team has provided the foundation for extractable-chemical production from microalgal biomass.

EXTRACTABLE CHEMICALS FROM MICROALGAE

At present, commercial activity in the microalgal extractable chemicals sector is limited to two main products: carotenoid pigments as human nutritional supplements from *Dunaliella* and the pigment astaxanthin as a coloring agent from *Hematococcus* (Wikfors 2000). In Japan, *Spirulina* also is used in the coloring of gold fish, but this is limited in scale. Development of *Dunaliella* farms was through modification of "trough" or "raceway" designs investigated in the Carnegie experience. In the case of *Dunaliella*, however, a market opportunity for the product, chiefly beta-carotene, drove development. Recognition that members of the genus *Dunaliella* produce abundant carotenoid pigments under certain conditions (mainly high light, with a photoprotective function) was the scientific finding enabling the commercial application (Ben-Amotz and Avron 1980, 1990). Open raceway culture of *Dunaliella* is possible with minimal protistan contamination because this alga is able to tolerate challenging conditions—hypersaline, hot, and very high pH—that are lethal to most potential contaminants. Raceway ponds of *Dunaliella*, generally located in coastal desert areas (Israel, Australia, Hawaii), may be several hectares in area but only several centimeters in depth and usually are stirred by a paddle wheel to ensure even light availability to the cultures (Becker 1994). Continuous culture management is used, with hydraulic residence time engineered to maximize product (pigment) yield, rather than biomass yield per unit time. Essentially the entire world market for "natural" beta-carotene is being met by *Dunaliella* culture, although this market is smaller than the volume of synthetic beta-carotene produced and sold.

Haematococcus culture for astaxanthin production is far smaller in economic value than is the case for *Dunaliella*; scale of production is much smaller, though also based on Carnegie technology (closed photobioreactors), and is mainly limited to the United States. The main use of astaxanthin is as a coloring agent in feeds for farmed salmonids; astaxanthin is the reason salmon flesh is pink (Torrissen 1989, Torrissen et al. 1989, Choubert and Storebakken 1989). In Europe, astaxanthin synthesized from petrochemicals has replaced pigment extracted from cultured algae; however, synthetic pigment is not permitted in aquaculture feeds in the United States. Thus, the future of *Hematococcus* culture is limited and somewhat uncertain.

A more recent development in culture of "algae" for extractable chemicals is heterotrophic production of the colorless dinoflagellate *Crypthecodinium cohnii* for DHA, which is included in infant formula (Behrens 1998, Apt and Behrens 1999). Aquaculture-feed products using heterotrophically cultured *Schizochytrium* (by some systematic schemes, an alga) are being marketed as well. Technology used in these processes owes much more to the brewery/fermentation research history than to phycologists. Nevertheless, the recognition of "heterotrophic" nutrition in both pigmented and nonpigmented protists attributable to algal and protozoan researchers (Hall 1965, Droop 1974) most likely played some part in this development. Furthermore, application of heterotrophic and mixotrophic culture strategies to aquaculture feeds development shows some promise of expanding uses of "microalgae" cultured nonphotosynthetically.

MICROALGAE AS AQUACULTURE FEEDS

Perhaps the most circuitous development of a microalgal aquaculture application is the use of live algal cultures as feeds for invertebrates. Difficulty arose from the need for new research information on two fronts: one needed to know what the "livestock" species required nutritionally and then efficient and effective methods were needed for culturing microalgae that could be followed successfully by nontechnical farm personnel. Focusing on bivalve mollusks as an example, there was debate ongoing into the 1950s as to whether microalgae (or phytoplankton) alone could provide a sufficient diet for bivalve mollusks (Loosanoff and Davis 1963). Bruce et al. (1940) were the first to observe that larvae of a bivalve (oyster) would eat and grow on cultured microalgae but the algal culture was not free of bacteria and perhaps other microbial contaminants. Observations that the most commonly cultured microalgae (e.g. the ubiquitous *Chlorella* and *Phaeodactylum/Nitzschia*) would not support molluscan growth (Ukeles and Wikfors 1982) further lent credence to theories that bacteria or particulate and dissolved organic compounds were essential foods for mollusks. One breakthrough report that demonstrated wide variation in the nutritional value of different microalgal strains was contributed by Davis and Guillard in 1958. This report led to a sort of "prospecting" research agenda wherein dozens of microalgal strains were tested in feeding trials with a variety of bivalve

mollusks. Much of this research was done at the Milford, Connecticut Shellfish Aquaculture Laboratory (Ukeles 1976) and at the MAFF Conwy Laboratory in the United Kingdom (Walne 1970). From a practical standpoint, the effort to identify nutritionally useful microalgal diets for mollusks was successful, and many of the algal strains used widely to this day as aquaculture feeds (various strains of *Isochrysis, Pavlova, Nannaochloropsis, Tetraselmis, Thalassiosira*, and *Chaetoceros*) were found. Nevertheless, an obvious question to ask about observed differences in nutritional value of different algae was "Why are they different?"

The research community's pursuit of fundamental knowledge about the nutritional requirements of bivalve mollusks has been hampered from the start by the inability to construct a complete "artificial" diet from defined chemical components (Langdon et al. 1985, Langdon and Newell 1996, Robert and Trintignac 1997). The main problems with microparticulate diets are that they either are not recognized as food in the mechanics and physiology of feeding, they are indigestible once ingested, or they do not retain both lipid- and water-soluble components when suspended in water. Thus, correlation analysis of chemical composition of good and bad algal diets was attempted. One report of this type stands out as a signpost missed by many who read and subsequently cited it as evidence that interspecific differences in algal biochemical composition could not account for nutritional differences. An article published by Parsons et al. (1961), if read carefully, does not refute the importance of gross biochemical composition in nutritional value (as implied by many citations) but rather concludes that protein, carbohydrate, and fat levels vary more *within* a strain depending on culture conditions than *between* strains. Thus, these authors reasoned if one alga is always better than another nutritionally, then there must be some other characteristic involved. The two brilliant observations made in this report—that algal chemical composition varied predictably with culture conditions and that nutritional factors other than protein, carbohydrate, and fat differentiated between good and poor strains—set the stage for advances that did not begin until 25 years later.

If gross biochemical composition alone did not account for differences in the nutritional value of different algal strains, then what "other factors" did? Hypotheses included trace elements, specific amino acids, vitamins, and specific lipid compounds. One landmark report identifying certain fatty acids (eicosapentaenoic acid or EPA 20:5n3 and docosahexaenoic acid or DHA 22:6n3) as being conserved in the phytoplankton–oyster food chains (Watanabe and Ackman 1974) soon led to a recognition that these important membrane-lipid components had a dietary rather than physiological source in crustaceans as well (Kanazawa et al. 1979). A follow-up study in which a diet of *Dunaliella*, which contains no fatty acids longer than 18 carbons, was supplemented with microparticles containing EPA or DHA and fed to oysters (Langdon and Waldock 1981) underscored the importance of the "essential" fatty acids EPA or DHA in molluscan diets as well. This recognition eliminated entire classes of algae (e.g. Chlorophytes) from consideration as single-species diets for mollusks. A similar line of investigation with sterols (Teshima 1981, Holden and Patterson 1991) revealed certain of these lipid compounds to be essential in diets for aquacultured mollusks as well. One interesting sidebar to the sterol story is the fact that research conducted to identify sterol/sterane biomarkers for petroleum prospecting (Peters and Moldowan 1993) or chemical taxonomy applications (Patterson 1992) remains the best source of technical information on sterol compositions of microalgae used as aquaculture feeds. Thus, the other factors hypothesized by Parsons et al. (1961) appear to be essential fatty acids and certain sterols. The main contribution of this article's senior author's laboratory to this effort has been the identification and commercialization of single-strain (*Tetraselmis*) diets with high levels of both essential fatty acids and sterols (Wikfors et al. 1996).

Practical use of selected strains of microalgae, especially *Tetraselmis*, in finfish and crustacean food chains had been established empirically before the biochemical basis for benefits was known (Okauchi 1988, Leber and Pruder 1988). Research, both in laboratories and at production facilities, continues to refine uses of various microalgal taxa, including the cyanobacterium *Spirulina*, for nutritional or otherwise health-promoting benefits (Hanson 1990).

The use of microalgal cultures as invertebrate aquaculture feeds requires simple, dependable, and robust culture practices. This requirement can only be described as "in progress." An early success was the development of a nutrient enrichment for seawater that is widely applicable to marine phytoplankton; this formulation, f/2, is found in one of the most-cited articles in the algal-aquaculture literature, "Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt and *Detonula confervaceae* (Cleve) Gran" (Guillard and Ryther 1962). This title is reproduced in full here to underscore the firm connection between fundamental research and algal aquaculture. Dr. Guillard fully intended the wide research applications of f/2; published in the article it was because of a combination of temporal coincidence and academic necessity to document methods. However, we do not believe even Dr. Guillard could have foreseen the current commercial availability of several competing "brands" of f/2 nutrient fertilizers for algal aquaculture. Widespread use of f/2 (and its variants and progeny) probably has done more to advance algal aquaculture—both micro and macro—than any other single research accomplishment.

Methods developed by research scientists for producing algal cultures of sufficient quantity and quality for the feeding trials described above (Ukeles 1973, Matthiessen and Toner 1966), although extremely successful in the intended application, generally are too technically demanding and expensive for use on the

farm. Until recently, there has been very little communication between researchers involved in the highly engineered systems used in algal mass culture for extractable chemicals and the aquaculture-feed sector. Recent efforts to address dependability and economics of the relatively small-scale, hatchery-based, algal feed culture efforts (Smith and Wikfors 1998, Rusch and Malone 1998) hold promise for the future and provide a focus for ongoing research and development efforts.

CONCLUSION

Three main aspects of the relationship between scientific research and aquaculture uses of algae came to mind during the preparation of this review. The first is a clear demonstration that basic fundamental research on biological questions concerning life cycles, biochemical physiology, systematics, and so on can revolutionize practical applications in ways never imagined by the researchers. The second insight is that many small incremental findings and applications follow "breakthrough" discoveries to fully assimilate and optimize the new knowledge. Finally, the contributions of commercial producers and "consumers," in terms of practical application of scientific knowledge to production and creation of demand for products, should not be underestimated. Technology must be embraced by producers and consumers of algal products to be successful. We in the research community will do well to keep the needs of our "customers" in mind as we work toward the most fundamental imperatives of human culture—food security and sustainability.

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