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Microalgae - A boon for larviculture of aquatic organisms

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Abstract
Microalgae are utilized diversely in aquaculture, as a live feed for culture of marine fin fish larvae. They are rich in nutrition and used as a component or as a food additive to supply basic nutrients and for the larvae. The larvae of molluscs, echinoderms and crustaceans as well as the live prey of some fish larvae feed on microalgae. The most frequently used species are *Chlorella* sp., *Tetraselmis* sp., *Isochrysis* sp., *Pavlova* sp., *Phaeodactylum* sp., *Chaetoceros* sp., *Nannochloropsis* sp., *Skeletonema* sp. and *Thalassiosira* sp. Combination of different algal species provides better balanced nutrition and improves animal growth better than a diet composed of only one algal species This study provides a background on the usage of microalgae in aquaculture, focusing on their nutritional value and their applications.

Keywords: Microalgae, immobilization, nutrition, by-products and larviculture

1. Introduction
Microalgae are cultured commercially, as a health food and as a source of valuable chemicals such as beta-carotene (Belay *et al*., 1994; Borowitzka, 1994) [4, 7], while macroalgae are farmed for their hydrocolloids as well as food (Abbott, 1996; Bixler, 1996). Microalgae are also an important live feed in the nursery rearing of many aquatic animals, especially the larvae and spat of bivalve molluscs, penaeid prawn larvae, besides being important in rearing of other live food organisms such as rotifers and *Artemia* sp. (Nell *et al*., 1996) [31]. Microalgae is the best preferred food source for increased production in aquaculture, while also improving overall economic management, water management, better feeding strategies, more environmentally friendly feeds, genetically fit stocks, health management and integration with agriculture (New and Wagner, 2000) [32].
In aquaculture, mass production of larvae and juveniles in good nutritional and health conditions is one of the most important factors to assure success. Larvae of aquatic organisms generally feed on different species of microalgae, and the amount and quality of food are some of the most critical factors affecting successful larval development in any aquaculture hatchery. Hence, the future of aquaculture depends on developing algal culture technologies to meet with the growing demand for newly domesticated breeds of fishes. There is immense scope for spearheading the blue revolution in the century to match the great leaps achieved in green revolution.

2. Nutritional profile of microalgae
Live diets includes three groups of organisms namely phytoplankton such as microalgae (2 - 20 µm), zooplankton such as rotifers (50 - 200 µm) and brine shrimp, *Artemia* sp. (200 - 300µm) (Annon, 2000) [2]. Moreover, they also can be used as biovehicles by which the nutritional components can be administered to the fish and shrimp larvae. This phenomenon is known as bio encapsulation of live food organisms (Tamaru *et al*., 2000). In nature, most fishes and shrimp feed on varied types of natural phytoplankton and zooplankton. There does not appear to be a strong correlation between the proximate composition of microalgae and nutritional value. However, algal diets with high levels of carbohydrate are reported to produce the best growth for juvenile oysters (*Ostrea edulis*) (Enright *et al.*, 1986) [21], and larval scallops (*Patinopecten yessoensis*) (Whyte *et al.*, 1989) [44] provided polyunsaturated fatty acids (PUFAs) are present in adequate proportions.

When cultured through to stationary phase, the proximate composition of microalgae can change significantly; for example, when nitrate is limiting; carbohydrate levels can double at the expense of protein (Harrison *et al.,* 1990; Brown *et al.,* 1993) [23, 9].

In recent years extensive studies have been undertaken to determine the nutritional requirements of the target species and the biochemical composition of algae which possibly can be used as a food source (de Roeck Holtzhauer *et al*., 1993; Dunstan *et al*., 1994; Brown and Farmer, 1994) [16, 19, 11] and these studies provided an excellent database for the selection of algal species for use in aquaculture. However, an understanding of the biochemical composition is not enough, and detailed studies on the digestibility of the target animal to feed on the algae, is essential. The identified algae must have rapid growth rates, be amenable to mass culture, and tolerate fluctuations in temperature, light and nutrients as may occur in hatchery systems. Finally, they must be free from toxins and have a good nutrient composition. Farming of marine finfish and invertebrates – especially crustaceans (shrimps) and molluscs requires microalgae as feed at some point in their life cycle (Jeffrey *et al*., 1994) [24]. Microalgae which are at the base of food chain, represent the third largest aquaculture crop in the world (Hanisak, 1998 and Annon, 2000) [22, 2].

Microalgae are also used widely to improve the nutritional content of zooplankton. By allowing the zooplankton to fill their digestive systems with microalgae prior to being fed to the fish or shrimp larvae has been observed to improve nutritional quality of live feed. The most frequently used species are *Chlorella* sp., *Tetraselmis* sp., *Isochrysis* sp., *Pavlova* sp., *Phaeodactylum* sp., *Chaetoceros* sp., *Nannochloropsis* sp., *Skeletonema* sp., *Scenedesmus* sp., *Picochlorum* sp., *Dunalliella* sp. *and Thalassiosira* sp. Microalgae grown to late-logarithmic growth phase typically contain 30 to 40% protein, 10 to 20% lipid and 5 to 15% carbohydrate (Brown *et al*., 1997; Renaud *et al.,* 1999) [10, 35]. High dietary protein provided the best growth for juvenile mussels (*Mytilus trossulus*) (Kreeger and Langdon, 1993) and Pacific oyster (*Crassostrea gigas*) (Knuckey *et al*., 2002) [25]. In order to be used in aquaculture, a microalgal strain has to meet various criteria, such as ease of culture, lack of toxicity, high nutritional value and correct cell size (Raja *et al*.,2004) [34]. Combination of different algal species provides better-balanced nutrition and improves animal growth better than a diet composed of only one algal species (Spolaore *et al.* 2006) [40]. A proper shape and a digestible cell wall is essential to make nutrients available (Patil *et al*., 2007) [33].

Microalgae can enhance the nutritional content of conventional food preparations and positively affect the health of the cultured animals. Protein and vitamin content is a significant factor in determining the nutritional value of microalgae. Besides, these polyunsaturated fatty acid (e.g. eicosapentaenoic acid (EPA), arachidonic acid (AA) and docosahexaenoic acid (DHA) content are also of significant importance. Different strategies are practised to improve the polyunsaturated fatty acid content in microalgae. Manipulation of processing conditions such as photoperiod, light intensity, nutrient status, and temperature and salinity variations allows the modulation of the lipid composition and consequent optimization of their overall yield and productivity.

Table 1: Co-Products from microalgae and their applications (Wan Loy Chu, 2012) [43]

Microalgae	**Products**	**Applications**
Pavlova sp., *Nannochloropsis* sp., *Monodus* sp., *Phaeodactylum* sp., *Crypthecodiuimu* sp., *Schizochytrium* sp., *Spirulina* sp., *Porphyridium* sp.	Polyunsaturated fatty acids (PUFA) Eicosapentaenoic acid (EPA) Docohexaenoic acid (DHA) G-linolenic acid (GLA) Arachidonic acid (AA)	Nutritional supplements and aquaculture feed
Spirulina platensis, *Porphyridium cruentum*, *Spirulina* sp., *Arthrospira* sp.	Phycobiliproteins Phycocyanin Phycoerythrin	Natural dye for health food and cosmetics (lipsticks and eyeliners) antioxidant Fluorescent agent, tool for biomedical research, Diagnostic tool.
Dunaliella salina, *Haematococcus pluvialis*, *Botryococcus braunii*, *Nannochloropsis oculata*, *Stichococcus bacillaris*, *Chlorella* sp., *Euglena gracilis*, *Chlorella protothecoides*, *Chlorococcum citriforme*, *Muriellopsis* sp., *Neospongiococcum gelatinosum*, *Chlorella zofingiensis*	Carotenoids ß-carotene, Astaxanthin, α-Tocopherol, Bixin, Fucoxanthin, Zeaxanthin	Food colourant; antioxidant; cancer-preventive properties Pigmenter for salmon, antioxidant
Aphanizomenon flosaquae	Mycosporine-like amino acids (MAA)	UV-screening agent; sunscreen
Porphyridium cruentum	Polysaccharides	Viscosifiers, lubricants and flocculants for industrial applications; antiviral agent
Dinoflagellates *(e.g. Amphidinium* sp.,	Phycotoxins – Okadaic acid, Gonyautoxins,	Experimental tools for investigations on neurodegenerative diseases

Prorocentrum sp *& Dinophysis* sp.	& Yessotoxins	
Chlorella protothecoides, Botryococcus braunii	Lipids – triglycerides and Hydrocarbons	Biofuels
All phototrophic oxygenic algae	Chlorophylls	Pharmaceutical and cosmetical (deodorant)

3. Microalgal production systems

Typical systems used indoors for microalgal mass culture include carboys (10 to 20 L), polythene bags (100 to 500 L) and tubs (1000 to 5000 L). These are usually operated in batch or continuous mode. For larger volumes, outdoor tanks or ponds are used, operated semi-continuously. Depending on their scale, hatcheries may produce between several hundred to thousands of litres of algae daily, with cell densities typically ranging from 10^5 to 10^7 cells/mL. There are clear economics of scale with algal production, hence production costs become significant for small hatcheries. High value nutritional and pharmaceutical products such as ω-3 fatty acids, antioxidants, vitamins, and proteins derived from these strains can justify the economics of commercial production and processing. In order to be able to produce 'clean' unialgal cultures closed culture systems are essential. Of the various types of closed bioreactor systems available, the tubular photobioreactor seems to be the most reliable, efficient and cost-effective. Simple types of tubular reactors were already proposed for aquaculture certain years ago (Canzonier and Brunetti, 1976) [12] and (Chaumont *et al.*, 1988) [13]. The helical tubular photobioreactor designed by Biotech Ltd (Robinson *et al.*, 1988) [38] has proven to be the most effective during long-term laboratory and pilot-scale studies. Millamena *et al.* (1990) observed flocculation with alum and lime could be used to concentrate *Chaetoceros calcitrans, Skeletonema costatum* and *Tetraselmis chuii.* When sundried these algae could be used to rear zoea and mysis of *Penaeus monodon*, further the flocculated algae frozen at 5 °C can be used later as inocula for fresh cultures. Lee and Low (1991) [26] have reported net biomass productivities of 3.64 g L^{-1} d^{-1} for *Chlorella pyrenoidosa* in a 1.2 cm diameter tubular reactor. Borowitzka, (1992) [6] observed that centrifugation, flocculation and floatation methods to harvest algae in hatcheries, makes the production process expensive in addition to damaging algal cells.

Studies using large outdoor pilot scale bio-coils photobioreactor with several microalgae viz., *Tetraselmis* sp., *Isochrysis* sp., *Chaetoceros* sp., *Pavlova* sp., *Nannochloropsis* sp., *Spirulina* sp., and *Chlorella* sp. etc. have been carried out in the UK and Australia (Chrismadha and Borowitzka, 1994 [7]; Borowitzka, 1996 [8]; Watanabe and Hall, 1996) [1]. This system not only provides a controlled, contamination-free environment, but it could be used for continuous culture at much higher cell densities than can be achieved with traditional systems. The higher cell densities are mainly a result of being able to work outdoors using natural sunlight. The ability to operate a reliable continuous culture system not only reduces production costs but provides algal biomass of consistent quality. Molina Grima *et al.* (1996) [27] have reported productivities of 2.7 g L^{-1} d^{-1} for *Phaeodactylum tricornutum* in a 3.0 cm diameter tubular reactor in Almeira, Spain. These high productivities can be attributed to the high incident irradiance at these sites, the ability to culture the algae at optimum temperatures and optimal turbulence conditions within the bioreactors. Microalgae such as *Spirulina* sp., *Chlorella* sp., *Dunaliella* sp., and *Haematoccocus* sp. are few of the commercially produced strains with significant production facilities in China, Japan, USA, India and Australia (Benemann, 2013) [5].

4. Immobilization of algae for culture

The culture of microalgae for the nutrition of juvenile abalone presents a unique problem. Abalone requires algae such as the diatoms *Nitzschia* sp. and *Navicula* sp., which grow on surfaces (Uki and Kikuchi, 1979; Ebert and Houk, 1984) [42, 20]. These algae cannot be grown efficiently in the more conventional systems and resulted in less biomass. With increasing interest in commercial abalone culture, there was an urgent need for some efficient largescale culture systems for these algae. In this circumstance, immobilization of microalgae was observed as a process for circumventing the harvest problem as well as retaining the high-value algal biomass for further processing (de la Noue and de Pauw, 1988) [15]. Application of immobilization technology provides more flexibility in the reactor design when compared with conventional suspension systems. The novel lab-scale Twin Layer Photobioreactor has been employed in successfully rectifying the harvesting related issues. The central concept of this technology is attachment based microalgae growth. This novel technology effectively separate microalgae from the bulk of their growth medium. With the Twin-Layer technology, there is no loss of cells by leaching and the harvested biomass has been successfully used for rearing of Marine copepod (*Oithona dissimilis*) and Zoea of *Penaeus vannamei* (Divya and Santhanam, 2019) [18].

5. The role of microalgae in nursery rearing

5.1 Molluscs

Molluscs are herbivorous and consume microalgae by filter the water through their branchia. However, this filtration is not selective and these animals are also suspension feeders, taking in living or dead, plant or animal particles which compose plankton. Those filtering molluscs are mainly oysters, clams, pectinids, and mussels. Live microalgae are the best feed, although new solutions like yeast, bacteria, micro-particles, slurry, paste, dried and frozen microalgae have been explored (Robert & Trintignac, 1997) [37]. None of these new products are sufficiently advanced to provide an alternative to live microalgae. Phytoplankton requirements differ, depending on whether they are for broodstock, larval or post-larval rearing. The larval stages require high bacteriological and biochemical quality, but in small amounts, for a short time. Post-larvae accept lower quality, but remain sensitive to the biochemical composition and require in large quantities, depending on the length of the nursery stage. The preparation of a broodstock for breeding requires both quality and quantity, but the number of animals is small. Typically, a commercial molluscan hatchery (*Crassostrea gigas*) operates about 8 to 10 months a year. Once the larval size exceeds 3 mm, the animals are generally transferred to an open medium or grown in outdoor nurseries. For outdoor cultivation, the microalgae

consumption rate is even higher, from 40 to 100 m^3 of 10^6 cells/ml in extensive culture per 10^6 juveniles (6 to 12 mm). Nearly one million of 0.2 to 3.0 mm post-larvae require about 14 kg of microalgae (DW) (Muller-Feuga, 1997). The species of microalgae commonly utilized are *Isochrysis galbana*, *Skeletonema costatum*, *Pavlova lutheri* and *Chaetoceros calcitrans.* Other uses of microalgae consist of refining the oysters before sale. In France, an intensive technique based on producing the diatom *S. costatum* in subterranean saltwater doubles the flesh content and triples the glycogen content in 30 days at temperatures ranging from 8 to 12°C, resulting in a substantial increase in the market price. Another technique called the 'greening' of oysters, which consists of their acquiring a blue-green colour on the gills and labial palps raises the product's market value by 40%. This refining process puts the oyster in contact with naturally or artificially grown algae (Barille *et al*., 1994) [3].

5.2 Shrimp

The microalgal feed has been used for shrimp larvae production systems in different countries. Microalgal feed are necessary from the second stage of larval development (zoea) and in combination with zooplankton from the third stage (mysis) in shrimp hatcheries. These larval stages require microalgae culture facilities which vary with the size of the hatchery and the level of control of medium parameters. Naturally occurring microalgae blooms are encouraged in large ponds with low water exchange, increased larval growth and development (Alam *et al*. 2009., Rosenberry 1991; Lopez *Elıas et al.,* 2003) [1, 39]. For e.g. A patented (PCT/IN05/00195 US patent application no. 0070275856) micronutrient ready-mix is available in the market with the trade name "Nualgi" at ~6.25 US$ kg^{-1}. In water, Nualgi causes diatom algae to bloom, although a pond, lake, estuary or coastal water may have many species of organisms, only diatoms require silica, and they consume Nualgi rapidly and cause a bloom. The optimal concentration of Nualgi was determined by setting up cultures in media with concentrations ranging from $0.5gL^{-1}$. This nano silica-based micronutrient mixture has nano silica as its major constituent along with iron and nine other trace metals (Suman *et al*., 2012). Sometimes fertilizers and bacteria are added to induce more favourable conditions. This production system, with poor control of microalgae, provides a better part of shrimp production. On the other hand, large-sized hatcheries require highly paid technicians, multimillion-dollar investments, and highly controlled medium conditions. They take about 1 m^3 of 3.106 cells mL^{-1} microalgal culture to produce 106 post-larvae, at the rate of about 65 g DW. This is only valid for clear water hatcheries. However, in green water hatcheries, since microalgae contribute to stabilizing and improving the quality of the rearing medium while providing food for the zooplankton, they are produced in far greater quantities than the strict needs of larvae feeding. The main microalgae genera used are *Skeletonema* sp., *Chaetoceros* sp., *Tetraselmis* sp., *Chlorella* sp. and *Isochrysis* sp. (Muller-Feuga, 2000) [30].

5.3 Fin-fish larvae

The use of microalgae in fish hatcheries is required for both production of live prey, and maintaining the quality of the larvae rearing medium. It could also be used in the formulation of dry fish food for on-growing (Spolaore *et al*. 2006) [40]. The use of small, live prey, *Brachionus plicatilis*, is still a prerequisite for success in hatcheries of marine finfish larvae like sea breams and flat fish. These preys can be raised on yeast-based artificial feeds; however it is less efficient than raising with microalgae. Microalgae present promising interests in three levels in rearing of rotifers; (i) quick recovery of rotifer populations after the collapse (ii) improved nutritional quality of rotifer and (iii) lower bacterial contamination, especially from *Vibrio* sp. For numerous fresh and marine animal species, the introduction of phytoplankton in rearing ponds leads to much better results in terms of the survival, growth and transformation index than when effected in clear water. Such plankton blooms have become a necessity, for sea bream, for success in culture. The decisive role of microalgae in the larval rearing ponds of fish, as well as shrimp, have not been completely elucidated (Richmond, 2004; Muller-Feuga, 2004) [36, 28]. There is no doubt that microalgae improve water quality and stabilize oxygen production, pH stabilization, etc. The excreted biochemical compounds is generally induces behavioural processes like initial prey catching of the larvae. Other positive impacts of microalgae as live feed are regulation of the bacterial population, probiotic effects and stimulating immunity (Dhert *et al*., 1998) [17]. The requirement of a 60 day old juvenile sea bream *Sparus aurata*, is 0.06 g DW individual during the nursery phase and the microalgae requirement for the rearing and enriching of rotifers is 6×10^9 cells. The microalgae are introduced in to the rearing tank from stock algal culture tank and has been reported to be more efficient in rearing fish larvae (Muller Feuga, 2000) [30].

6. Conclusion

Good selection of algal species is a prerequisite to support the aquaculture industry in order to improve nutritional quality, healthy growth and hatchery efficiency. Microalgal species such as *Spirulina* sp., *Chlorella* sp., *Dunaliella* sp., *Haematoccocus* sp., *Isochrysis* sp., *Pavlova lutheri*, *Tetraselmis* sp., *Picochlorum* sp., *Nannochloropsis* sp., *Chaetoceros calcitrana*, *Thalassiosira* sp., and *Skeletonema costatu*m are some of the commercialized live feed for larval rearing of aquatic organisms. Hence it could be concluded from the present study that live microalgae with high nutritional quality are essential for the rearing of all stages of marine bivalve molluscs (clams, oysters, scallops), and post-larval stages of some marine gastropods (e.g. abalone), as well as larvae of several marine fish species, penaeid shrimp and zooplankton.

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