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Application of Microalgae in Aquaculture

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Abstract

Microalgae play a crucial nutritional role with regard to marine animals in the open sea, and consequently in aquaculture. Most marine invertebrates depend on microalgae for their whole life cycle, so commercial and experimental mollusc or fish hatcheries have included a microalga production system in parallel to their animal production itself. Microalgae are utilized as live feed for all growth stages of bivalve mollusc, for larval juvenile stages of abalone, crustaceans and some fish species, and for zooplankton used in aquaculture food webs. Hatcheries typically cultivate microalgae indoor, with commercial concentrates now also being used widely. It should be emphasized that the productivity of any hatchery is directly related to the quantity and quality of the food source used. The difficulty of producing economically large quantities of microalgal feeds is currently one of the major impediments to the further development of the aquaculture industry.

Keywords: Aquaculture, Feeds, Hatcheries,Invertibrates,Microalgae, Zooplankton.

Introduction:

The main applications of microalgae for aquaculture are associated with nutrition as sole component or as food additive to basic nutrients and for inducing other biological activities (Jyothi, 2017). Microalgae are required for larval nutrition either for direct consumption in the case of mollusks and peneid shrimp or indirectly as food for the live prey fed to small fish larvae (Muller-Feuga 2000). The most frequently used species are *Chlorella, Tetraselmis, Isochrysis, Pavlova, Phaeodactylum, Chaetoceros, Nannochloropsis, Skeletonema* and *Thalassiosira*. Combination of different algal species provides balanced nutrition and improves animal growth better than a diet composed of only one algal species (Spolaore *et al*. 2006). In order to be used in aquaculture, a microalgal strain has to meet various criteria, such as ease of culturing, lack of toxicity, high nutritional value with correct cell size and shape and a digestible cell wall to make nutrients available (Raja *et al*. 2004; Patil *et al*. 2007). Cultivated microalgae have long been indispencible to the hatchery production of many commercially important aquaculture species. There is an extensive published literature on the suitability of different algal strains for use in aquaculture hatcheries, their cultivation techniques, methods of delivery and modes of operation (Guedes and Malcata, 2012; Tredici *et al*., 2009). The objective of the current article is to introduce the algae production and use in hatcheries, including recent industry trends and future outlook. In more extensive forms of aquaculture, fortuitous populations of microalgae are bloomed in ponds or tanks, in which the aquaculture species occupies the highest trophic level. By contrast, intensive aquaculture hatcheries cultivate individual strains of microalgae in separate reactors and supplied these regularly to the farmed species. The use of microalgae in fish hatcheries is required for both production of live prey and maintaining the quality of the larvae-rearing medium (Spolaore *et al*., 2006).

The significant role of microalgae in aquaculture hatcheries include cultivation of microalgal strains for broodstock conditioning, larval rearing and feeding of newly settled spat, as all developmental stages of bivalve molluscs are directly dependant on microalgae as a feed source. The planktonic larval stages of commercially important crustaceans are initially fed on microalgae. Farmed gastropod molluscs and sea urchins require a diet of benthic diatoms when they first settle out from the plankton, prior to transferring to their juvenile diet of macroalgae. The small larvae of most marine finfish species and some freshwater fish species also initially receive live prey in the presence of microalgae. These microalgae are allowed to bloom within the fish larval rearing tanks (green water) or are added from external cultures (pseudo-green water).

Microbial conditioning by microalgae may extend to prevent cell-to-cell signalling (quorum sensing) by bacterial pathogens (Natrah *et al*.,2011). In a laboratory screening study focused on microalgal strains commonly used in aquaculture, several of the tested strains interrupted signalling by pathogenic *Vibrio harveyii*, proving that such microalgae offer potential as aquaculture biocontrol agents.

Microalgal Strains Used in Aquaculture Hatcheries

Only a small number of microalgal strains are routinely cultured in aquaculture hatcheries depending on certain conditions such as availability of strain , method of culture, cell physical characteristics, nutritional composition, digestibility and absence of toxins (Guedes and Malcata 2012; Anon, 2010; Tredici *et al*., 2009; Muller-Fuega *et al*., 2004; Muller-Fuega *et al*., 2003a; Muller-Fuega *et al*., 2003b).

Table.1.Commonly used microalgae in a specific area of application in aquaculture.

Area of application in aquaculture	Commonly used microalgae
In formulated feed ingredient	*Arthrospira platensis (Cyanophyceae); Chlorella vulgaris, C.minutissima, C.virginica, Dunaliella tertiolecta, D. salina, Haematococcus pluvialis (Chlorophyceae)*
Feed for bivalve mollusks	*Thalassiosira pseudonana (Bacillariophyta); Pavlova lutheri (Haptophyta); Isochrysis galbana* *Chlorella minutissima, Gomphonema* sp*, Isochrysis galbana, Nitzschia* sp*, Phaeodactylum tricornutum, Tetraselmis subcordiformis.* *Tetraselmis suecica, T.chui (Chlorophyceae); Chaetoceros calcitrans,C. gracilis; Skeletonema costatum.*
Rotifer and Artemia live prey	*Crypthecodinium cohnii (dinoflagellates); Schizochytrium* sp*.; Ulkenia* sp*. Chlorella* sp*, Chlamydomonas* sp*, Nannochloris oculata, Tetraselmis tetrathele* and *T. chuii.*
Feed for crustacean larvae ((shrimps, lobst-ers))	*Tetraselmis suecica, T.chui (Chlorophyceae); Chaetoceros calcitrans, gracilis; Skeletonema costatum; Thalassiosira pseudonana (Bacillariophyta).*
Feed for gastropod molluscs and sea urchins	*Nitzschia* sp. ; *Navicula* sp.; *Amphora* sp.
Green water" for finfish larvae	*Isochrysis galbana; Nannochloropsis oculata*

A comprehensive literature exists on the nutritional composition of these and other microalgal strains and their efficacy as aquaculture hatchery feeds (Jyothi ,2017; Guedes and Malcata, 2012). Delivery of a balanced diet to the aquaculture species is generally achieved by supplying a mixture of different microalgal strains, guided by typical published nutritional profiles for these strains (Brown *et al*.,1997). The microscopic larvae are filter feeders, relying on microalgae throughout their planktonic phase (e.g., bivalve molluscs).They alternatively switching from filter feeding to predating on zooplankton during larval development (e.g., penaeid shrimps). These life history stage require supply of microalgae during some of the hatchery phase. A recent overview of the feeding strategies using typical microalgal strains for these groups of aquatic invertebrates was discussed by Tredici *et al.,* 2009. According to Helm *et al*., 2004 Bacillariophyte and Prymnesiophyte microalgal strains together are the most commonly used feed source for bivalves, both for nursery rearing and conditioning of broodstock. The microalgae continues to be provided into the nursery phase of bivalve mollusc production, since bivalves are obligate filter feeders throughout their life history. Bivalve hatcheries therefore tend to possess the highest microalgal production capacity, with particular attention being paid to sterilization status to avoid crashes or transfer of pathogens to the shellfish (Aji, 2011).

Microalgae for Zooplanktonic Live Prey

Microalgae have an important role in aquaculture as a means of enriching zooplankton for feeding to fish and larvae (Chakraborty *et al.*, 2007).The predatory finfish larvae and decapod crustacean larvae is to feed with zooplanktonic live prey rather than formulated inert diets.This reflects the technological challenge and high costs of providing nutritionally balanced feeds in the correct physical form for small planktonic larvae, whose digestive capacity is only partially developed.

Prior to 1960's aquaculture hatcheries use rotifers (*Brachionus* sp.) followed by brine shrimp (*Artemia* sp.) extensively as the key zooplanktonic live prey for larval finfish and decapods (Bengtson, 2003). These zooplankton have deficient nutritional composition are not the natural prey of the aquaculture species. Neverthless their rapid reproduction rates with high densities are more significant than their nutritional shortcomings in most cases (Lubzens and Zmora 2003; Dhont and Stappen, 2003). The nutritional quality of rotifer and brine shrimp were improved by manipulating their diet particularly to enhance n-3 highly unsaturated fatty acids (HUFA, e.g., docosahexaenoic acid and eicosapentaenoic acid) by microalgal strain selection or by incorporating dried microalgal biomass into formulated inert diets. Hatchery production of rotifers was initially based on feeding with live microalgae (*Nannochloropsis* sp., *Tetraselmis* sp., *Pavlova lutheri* and *Isochrysis galbana*) or baker's yeast (Conceicao *et al*., 2010). For instance rotifers fed with microalgae become rapidly enriched with ascorbic acid. After 24 h, rotifers fed on *Isochrysis* sp. and *Nannochloropsis oculata* contained 2.5 and 1.7 mg/g dry wt respectively, whereas rotifers fed on baker's yeast alone are deficient in ascorbate and contained only 0.6 mg/g dry wt (Brown, 2002).

Commercial feed formulations have been developed and are now widely used as alternatives to live microalgae and yeast. These products are intended to optimise growth and reproduction of the rotifers and to enhance their final nutritional composition before feeding to larvae. Hatcheries when adopted such artificial feeds for mass rotifer cultivation, it is easy to retain rotifer master cultures on live microalgae, and this simplifies hygiene maintenance. Live microalgae remain the preferred diet for planktonic groups (orders Calanoida and Cyclopoida), whereas benthic copepods (order Harpacticoida) are more manageable to cultivate on inert feeds (Stottrup, 2003). Among the products used as feed for aquaculture live prey are several marine microorganisms (Tredici *et al*., 2009). The dinoflagellate, *Crypthecodinium cohnii*, has been exploited due to its high docosahexaenoic acid (DHA) content.

Benthic Microalgae for Gastropod Molluscs and Echinoderms

The larvae of abalone (gastropoda) and some species of sea urchin (echinoidea), do not require microalgae during their planktonic phase, but they do initially graze on benthic microalgae (those living on surfaces) when they settle out from the plankton (Azad *et al*., 2010). Natural assemblages of benthic diatoms are encouraged to grow as a feed source, by pre-exposing artificial substrates to unfiltered seawater, upon which the microalgae grow (Heasman and Savva, 2007).This establishment process becomes limiting at higher abalone densities, where the rate of algal growth can be exceed by grazing (Dyck *et al*.,. 2011). The addition of cultured diatoms, such as *Navicula* sp., *Nitzschia* sp. and *Amphora* sp. offers greater control for intensive abalone nurseries (Vicose *et al.*, 2012). The publications exist on appropriate cultivation systems for diatoms were limited. The use of 20L polycarbonate carboys containing PVC filaments for culturing mixed benthic diatom strains was reported Araya *et al*., 2010, which were dispenced successfully to postlarval *Haliotis rufescens*. Silva-Aciares and Riquelme, 2008 described a Photobioreactor design for diatoms, based on an aerated acrylic cylinder containing a bottle brush-like array of PVC bristles.

Microalgae in Fish Larval Rearing Tanks

The practice of rearing marine finfish larvae in the presence of microalgae is typically, associated with higher growth rates than when larvae are reared in clear water (Muller-Feuga *et al*., 2003b; Conceicao *et al.*, 2010). In "green water" technique, microalgae and zooplankton are bloomed within large tanks, into which the fish larvae are stocked. This rearing method can be based on natural microalgal assemblages, which are encouraged to bloom by fertilizer addition (Shields, 2001). Alternatively, cultured microalgal strains can be inoculated into rearing tanks for this purpose provided the system water has been pre-treated to exclude competing microorganisms.The "pseudo-green water" rearing technique relies instead on regular addition of cultured microalgae to the fish larval rearing tanks, to replace that removed by live prey grazing and dilution.This approach is required to sustain the higher larval stocking densities that are typical in most commercial marine fish hatcheries. Commonly used microalgal strains for this purpose are *Nannochloropsis* sp., *Isochrysis* sp. and *Tetraselmis* sp.

Use of Microalgal Concentrates in Aquaculture Hatcheries

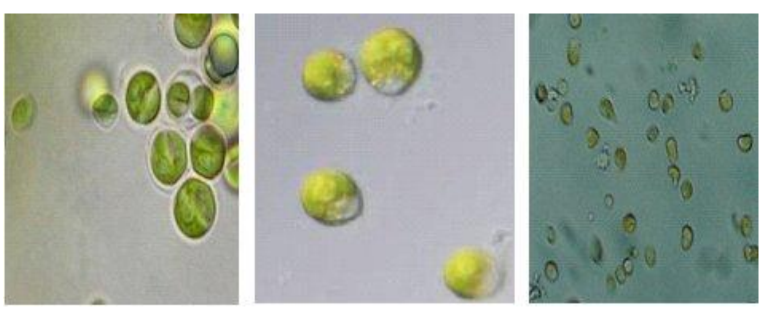
Tredici *et al*., 2009, reviewed on microalgal product development including the technologies involved in concentrating and stabilising microalgae and descriptions of a range of commercially available products which offer a convenient source of microalgae for aquaculture hatcheries. The practice of concentrating live microalgae originated for local use within individual hatcheries, typically using disk-stack centrifuges or membrane filters (Molina Grima *et al*., 2003). This practice is still used in some large hatcheries, although commercial concentrates have become widely adopted. From an aquaculture hatchery perspective, the key desired attributes for microalgal concentrates are high cell concentration without damage to cells, suitable nutritional composition; acceptable shelf life using standard cold storage methods; avoiding the use of preservatives that would be harmful to live prey; free from pathogens; avoidance of clumping and easy to suspend uniformly in water; regularly available and affordable.

The microalgal strains that are particularly suited for aquaculture and biotechnological industrial strains, such as heterotrophically produced *Chlorella* sp., that are available at higher volume with lower price have emerged .The performance discrepancy between concentrated and live microalgae is less marked in the area of live prey production, where industrially produced *Chlorella* is now routinely used for rotifer production, competing in the market with other forms of dry feed (Tredici *et al*., 2009).

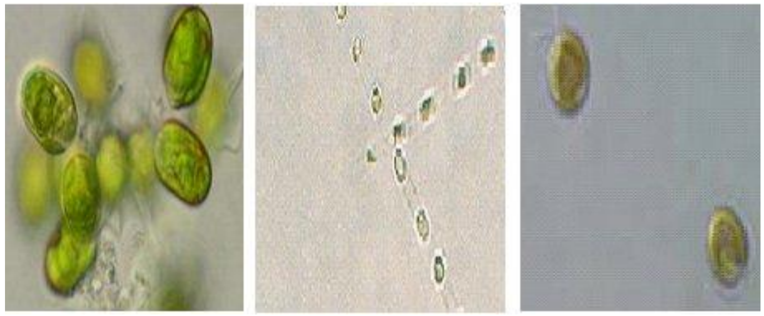
The Future

The high production cost of microalgae remains a limitation to many hatcheries. A good selection of microalgal species is also available to support the aquaculture industries (Lopez Elias *et al*.,.,2003). Apart from improvements in the cost-effectiveness of on-site algal production, an alternative is the centralization of algal production at specialized mass culture facilities using heterotrophic methods or photobioreactors to produce cheaper algal biomass. These technologies could be combined with post-harvest processing such as spray drying or algal concentration to develop off-the-shelf algal biomass for distribution to hatcheries (Lopez Elias *et al*., 2003). Although genetically engineered the microalgae has been studied in its application for biofuel production and bioremediation of heavy metals, there is a less research on its application in aquaculture. The insertion of genes determining the nutritional parameters into microalgae can increase the quality of fish in aquaculture (Sayre *et al*., 2001). A combined effort to standardize a genetically modified microalgae aided with a controlled bioprocess system will lead to an improvement in the status of aquaculture.

Photographs Of Some Microalgal Strains Used in Aquaculture Hatcheries



Chlorella vulgaris ***Dunaliella salina*** ***Isochrysis galbana***



Tetraselmis suecica ***Skeletonema costatum*** ***Pavlova lutheri***

Navicula* sp** ***Nitzschia* sp** ***Nannochloropsis oculata

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