World J Microbiol Biotechnol (2011) 27:1737–1746
DOI 10.1007/s11274-010-0632-z

REVIEW

Microalgae: a sustainable feed source for aquaculture

S. Hemaiswarya · R. Raja · R. Ravi Kumar · V. Ganesan · C. Anbazhagan

Received: 9 August 2010 / Accepted: 8 December 2010 / Published online: 24 December 2010

Abstract The need for nutritional sources safer than traditional animal products has renewed interest generally in plants and particularly in microalgae. Microalgae have diverse uses in aquaculture, their applications are mainly to provide nutrition and to enhance the colour of the flesh of salmonids. The larvae of molluscs, echinoderms and crustaceans as well as some fish larvae feed on microalgae. Several studies have confirmed that a live multi-specific, low bacterial and microalgal biomass remains essential for shellfish hatcheries. Major advances are expected from new production system, designs and operations from batch run open tanks to more sophisticated continuously-run and closed loop reactors. Currently, studies are underway to examine the cost-effectiveness of the on- and off-site microalgal production systems which can only be achieved by substantial scaling-up and improved quality control. In order to attain sustainability in the usage of microalgae, a systems-based approach is required which integrates different fields such as biotechnology, bioprocess and management procedures.

Keywords Aquaculture · Animal nutrition · Microalgae · Photo bioreactor · Zooplankton

S. Hemaiswarya · R. Raja (✉)
Department of Biotechnology, Indian Institute of Technology Madras, Chennai 600 036, India
e-mail: rraja307@yahoo.co.in

Present Address:
R. Raja · R. Ravi Kumar
Research and Development, Aquatic Energy LLC, Lake Charles, LA 70605, USA

V. Ganesan
Acme ProGen Biotech (India) Private Limited, Balaji Nagar, Salem 636 004, India

C. Anbazhagan
Department of Botany, Annamalai University, Chidambaram 608 002, India

Introduction

Aquaculture, the farming of aquatic organisms contributed 40% of the yield from wild fisheries in the year 2000 and is expected to surpass the yield of wild fisheries by 2020–2025 (Tacon 2003). India was one of the top ten producers of aquatic organisms within the Asian region in the year 2000 and contributed about 5% of the total aquaculture production (FAO 2002). Increased production in aquaculture is dependent on the adoption of an approach with overall economic management, improved water management, better feeding strategies, more environmentally friendly feeds, genetically fit stocks, improved health management and integration with agriculture. Although aquaculture dates back to the earliest parts of human history in Asia, Europe and the Pacific islands (New and Wagner 2000), it is only in the last few decades that aquaculture has begun to catch up with the science of feed manufacture and nutrition. Almost 40% of all aquaculture production is now firmly dependent on commercial feed. This is especially true of high value carnivorous species like shrimp, salmon and trout, whose feed contains large portions of marine inputs in the form of fish meal (Alvarez et al. 2007). The percentage of farms using commercial feeds varies from 100% for salmon and trout, 83% in marine shrimp to 38% in carp farms.

Algae are a diverse group of aquatic, photosynthetic organisms generally categorized as either macroalgae (i.e.

seaweed) or microalgae (unicellular). As aquatic relatives of plants, microalgae thrive in aerated, liquid cultures where the cells have sufficient access to light, carbon dioxide and other nutrients (Rosenberg et al. 2008). Algae are primarily photoautotrophic and few species are heterotrophic in nature. Unlike terrestrial plants, which require fertile land or irrigation, microalgae can grow in a wide range of habitats (Raja 2009). Successful commercial utilization of microalgae has been established in the production of nutritional supplements, antioxidants, cosmetics, natural dyes and polyunsaturated fatty acids (PUFA) (Spolaore et al. 2006). The worldwide annual production of algal biomass is estimated to be 5 million kg/year with a market value of about 330 US$/kg. Approximately one-fifth of this biomass is used to nourish the fish and shellfish that are cultivated in aquaculture hatcheries (Muller-Feuga 2004). In the year 1999, the production of microalgae for aquaculture reached 1,000 tonnes (62% for molluscs, 21% for shrimps and 16% for fish) (Spolaore et al. 2006; Gagneux-Moreaux et al. 2007).

The main applications of microalgae for aquaculture are associated with nutrition (as sole component or as food additive to basic nutrients) for coloring the flesh of salmonids and for inducing other biological activities. Microalgae are required for larval nutrition during a brief period, either for direct consumption in the case of molluscs 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 better 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. 2004b; Patil et al. 2007). Protein and vitamin content is a major factor determining the nutritional value of microalgae. In addition, polyunsaturated fatty acid (e.g. eicosapentaenoic acid [EPA], arachidonic acid [AA] and docosahexaenoic acid [DHA]) content is of major importance. Different strategies are practised to improve the polyunsaturated fatty acid content in microalgae. Manipulation of processing conditions such as light intensity, nutrient status or temperature allows the modulation of the lipid composition and consequent optimization of their overall yield and productivity. The influence of light intensity on the lipid profile of *Pavlova lutheri* showed that cultures grown under low light intensity (9 Wm^{-2}) had a higher fraction of EPA and DHA esterified in polar classes which had a favorable role in aquaculture (Catarina Guedes et al. 2010). Application of the genetic engineering concept, by using u.v.-light as mutagenic agent generated strains with EPA and DHA contents about 32.8 and 32.9% (in % dry biomass) higher than those of the control, native strain (Meireles et al. 2003). Use of process engineering, an on-line based controlled process in a model system has been employed to increase the production rates of both EPA and DHA by *P. lutheri* under various conditions of light and growth rate (Meireles et al. 2008). A multidisciplinary approach involving genetically modified microalgae and optimization of the production of the desired compound using process control would perform better than resorting to the classical methods. In this review, the current status of knowledge of the general attributes of microalgal species used in aquaculture, their nutritional properties, production systems, use of algae to enrich zooplankton and directions for future research is summarized and potential areas of research and industrial development are identified (Fig. 1).

Culture systems

Existing commercial microalgal systems range from 100 l to $>10^9$ l. Culture systems such as large open ponds, circular ponds and raceway ponds (Borowitzka 1997; Pulz 2001) exist. A common feature of most of the algal species commercially produced is that they grow in highly selective environments, which means that they can be grown in the open air and still remain relatively free of contamination by other algae and protozoa (Borowitzka 1997). For instance, *Chlorella* grows well in nutrient-rich media, while *Spirulina* requires a high pH of 10–11 with appropriate concentration of bicarbonate. Similarly *Dunaliella salina* grows at the very high salinity of 0.5–6 M (Raja 2003, 2004a, 2007b). On the other hand, those algae viz. *Chaetoceros*, *Isochrysis, Skeletonema*, *Thalassiosira*, *Tetraselmis* and *Crypthecodinium cohnii* which do not have these selective advantages must be grown in closed systems. Commercial large scale systems such as the cascade system were developed in Trebon, Czech Republic in the 1970's and heterotrophic fermenters have been used for the culture of *Chlorella* in Japan and Taiwan. Table 1 summarizes the commercial algal culture and their uses. Factors to be considered for production of microalgae include: the biology of the alga, cost of land, labor, energy, water, nutrients (also climate if the culture is outdoors) and the type of final product.

The various large scale systems also need to be compared with regard to such basic properties as light utilization efficiency, operation costs, ability to control temperature, gaseous transfer for carbon source, hydrodynamic stress placed on the algae, ability to maintain axenic culture and ease of scalability (Borowitzka 1997; Raja

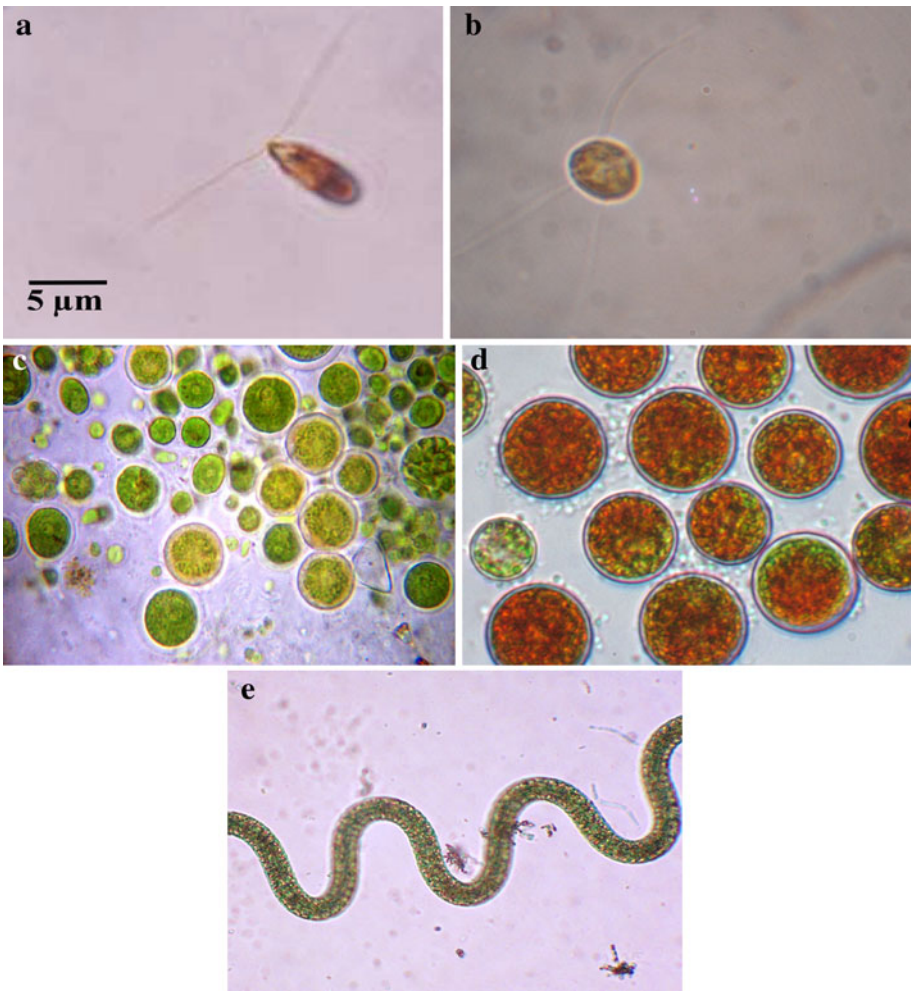

Fig. 1 Some of the microalgal cultures. **a** *Dunaliella* sp. 100x, **b** *Chaetoceros* sp. 100x, **c** *Chlorella* sp., **d** *Haematococcus* sp., **e** *Spirulina* sp.

Table 1 Commercial algal culture and its applications

Algae/Genus	Morphology	Purpose
Nannochloropsis	Small green algae	Growing rotifers and in fin fish hatcheries, used in reef tanks for feeding corals and other filter feeders, very high EPA level
Pavlova	Small golden-brown flagellate, very difficult to grow so it is not produced by many hatcheries	Used to increase the DHA/EPA levels in broodstock, oysters, clams, mussels and scallops, sterol composition so it is popular with cold water fish hatcheries (cod) for enriching rotifers
Isochrysis	Small golden-brown flagellate	Enrichment of zooplankton such as *Artemia*, used in shellfish hatcheries and used in some shrimp hatcheries, good size for feeding brine shrimp and copepods, oysters, clams, mussels, and scallops
Tetraselmis	Large green flagellate	Excellent feed for larval shrimps and contains natural amino acids that stimulate feeding in marine animals, used in conjunction with *Nannochloropsis* for producing rotifers, good size for feeding brine shrimp, standard feed for oysters, clams, mussels, and scallops, excellent feed for increasing growth rates and fighting zoea syndrome
Thalassiosira weissflogii	Large diatom	Used in the shrimp and shellfish larviculture, considered by several hatcheries to be the single best alga for larval shrimps, also good for feeding copepods and brine shrimps, post-set (200 μ and larger) oysters, clams, mussels, and scallops for broodstock conditioning
Dunaliella	Small green flagellate	Used to increase vitamin levels in some shrimp hatcheries and also for the coloration
Chaetoceros	Diatom	Used to increase vitamin levels in some shrimp hatcheries

et al. 2007a). The final choice is always a compromise between all of these considerations to achieve an economically acceptable outcome. Further development of the industry requires significant improvements in the design and construction of the photobioreactors as well as a better understanding of the physiology and physical properties of the microalgae to be grown. A review on microalgal reactors discusses the main parameters that determine its performance (Carvalho et al. 2006) such as contamination control, gaseous exchanges, mixing patterns, suitability of light supply (which comprises light quality and quantity), geometrical configuration and building material.

Replacement of live microalgae with other sources

Non-living diets generally give lower growth and higher mortalities compared to those fed with live microalgae (Ponis et al. 2003). Products other than live microalgae must be exempt from contamination and must be nontoxic. Bacteria can provide only a part of the metabolic requirements by supplying organic molecules and vitamins. Under conditions close to those found in rearing facilities, the bacterial input represents less than 15% of the microalgal contribution for mollusks larvae and juveniles of many species (Wikfors and Ohno 2001; Knuckey et al. 2006). The uses of bacteria as food source in hatcheries seems to be invalidated, since physical and chemical treatments are often used to limit the development of bacterial contaminations which are responsible for drastic larval mortalities. However, in live microalgal culture, the natural bacterial flora was proved to enhance the health of mollusks. Antibiotic suppression of microbial flora associated with juvenile oysters fed artificially reduced growth (Durmaz 2007). Oyster larvae fed with live microalgal diets showed improved growth with the addition of some bacterial isolates. Yeast was also investigated as an alternative food source but poor results were observed (Ponis et al. 2003). Therefore, these two alternatives are not suitable to replace live microalgae.

From a nutritional point of view, live microalgae have higher nutritive values and better digestibility compared to other substitutes. The nutritional quality of food sources mainly depends on many biochemical constituents such as polyunsaturated fatty acids, vitamins, sterols and carbohydrates (Dhont and Van Stappen 2003). Globally, the dried microalgae showed a low level or absence of ω-3 HUFA (highly unsaturated fatty acids) and low ingestion or digestion by bivalve larvae (Muller-Feuga et al. 2003). Several experiments indicated that these substitutes may be used as supplement when rations of live algae are insufficient. Spray-dried algae and algal paste were found to be useful to replace 50% of live algae. *Tetraselmis* seemed to be a good candidate for microalgal paste but its nutritional quality deteriorated quite rapidly (Robert et al. 2001). For example, drying microalgae can cause a loss by oxidation of highly unsaturated fatty acids, which are essential components for larval growth (Atalah et al. 2007). The poor performance reported by operators of dried microalgae was mostly associated with the difficulty of keeping cells in suspension without disintegrating them (Laing and Millican 1992). Moreover, when cell walls are broken, many water-soluble components cannot be ingested by the organism and may interfere with the water quality of cultures (Dhont and Van Stappen 2003). A possible pathogenic bacterial proliferation may occur and cause production losses. There are similar difficulties with algal paste as preparation procedures (centrifugation, flocculation or filtration) and preservation techniques (additives and freezing) must ensure that the cell wall integrity is preserved.

The artificial or non-living diets are rarely applied in the routine feeding process of bivalves and are mostly considered as a backup food source. The feasible alternative to live microalgae is the freeze-dried forms, since they maintain the original cell shape and texture. Air-dried or spray-dried microalgae shrink and shrivel due to high processing temperature and this decreases product quality. Freeze-dried products are easy to use, maintain and store and many research articles showed that there is no difference in using live algae or freeze-dried algae. For many applications freeze-dried microalgae give higher yields (Lubzen et al. 1995; Yamasaki etal. 1989). Larvae reared using freeze-dried microalgae had a survival of 100% rather than those reared with live microalgae (Pedro and Fernández-Díaz 2001).

An instant alga is another easy solution for replacing or supplementing live microalgae in commercial fish, shrimp, shellfish and ornamental hatcheries. A mixture of microalgae that have been demonstrated successfully with a variety of shellfishes including oysters, clams, mussels and scallops provides a much better nutritional profile, increasing both growth and survival rates (Brown 2002). The diet can be used with pre-set larvae all the way up through brood stock and will typically perform as live algae, so it can be used as a complete live algal replacement. One quart of diet will replace an equivalent of 1,800 l of dense algal culture. Instant algae are real microalgae grown on photobioreactors (Pulz and Scheibenbogen 1998) and this patented system consists of a number of outdoor and closed photobioreactors.

Health and nutrition

Microalgae can improve the production of larvae though the exact mechanism of action is unclear. Theories advanced include (a) light attenuation (shading effects), which has a beneficial effect; (b) maintenance of the

nutritional quality of the zooplankton; (c) growth-promoting substances such as vitamins being provided by the algae; (d) a probiotic effect of the algae. Most likely, the mechanism may be a combination of several of these possibilities. While microalgae provide food for zooplankton they also help to stabilize and improve the quality of the culture medium. Indeed, for numerous freshwater and seawater animal species, the introduction of phytoplankton to rearing ponds leads to much better results in terms of survival, growth and transformation index (Muller-Feuga 2000). The reasons for this are not entirely known, but may include water quality improvement and stabilization by algal oxygen production and pH stabilization, the action of some excreted biochemical compounds along with the induction of behavioural processes like initial prey catching and the regulation of bacterial population, probiotic effects and the stimulation of immunity (Hong et al. 2005; Raja and Hemaiswarya 2010).

Several factors can contribute to the nutritional value of a microalga (including its size and shape, digestibility, biochemical composition, enzymes, toxins and the requirements of animal feeding on the alga). Studies have attempted to correlate the nutritional value of microalgae with their biochemical profile (Richmond 2004; Durmaz 2007). However, results from feeding experiments that have tested microalgae differing in a specific nutrient are often difficult to interpret because of the confounding effects of other microalgal nutrients. Nevertheless, from examining the literature, including experiments where algal diets have been supplemented with compounded diets or emulsions, some general conclusions can be reached (Knauer and Southgate 1999). Microalgae grown to late logarithmic growth phase typically contain 30–40% protein, 10–20% lipid and 5–15% carbohydrate (Fujii et al. 2010). In the stationary phase, the proximate composition of microalgae can change significantly (e.g.) when nitrate is limiting, carbohydrate levels can double at the expense of protein (Liang et al. 2009). There does not appear to be a strong correlation between the proximate composition of microalgae and nutritional value, though algal diets with high levels of carbohydrate are reported to produce the best growth for juvenile oysters, *Ostrea edulis* (Ponis et al. 2006). Larval scallops, *Patinopecten yessoensis* provided polyunsaturated fatty acids in adequate proportions. In contrast, high dietary protein provided best growth for juvenile mussels, *Mytilus trossulus* and Pacific oysters, *Crassostrea gigas* (Knuckey et al. 2002).

Microalgal pigments transferred to zooplankton may contribute to nutritional value (Lorenz and Cysewski 2000; Gagneux-Moreaux et al. 2007; Raja et al. 2008) and it has been found that dominant pigments in the copepod *Temora* sp. are lutein and astaxanthin whereas in *Artemia* it was canthaxanthin (Kang and Sim 2008; Gentsch et al. 2009). When these prey items were fed to halibut larvae adequate amounts of vitamin A were found in halibut fed on copepods but not with halibut fed on *Artemia*. It was recommended that *Artemia* should routinely be enriched with astaxanthin and lutein to improve their nutritional value. Astaxanthin and canthaxanthin are the only pigments that can fix in the flesh of salmonids whose pinkening represents a 100 million US$, rapidly expanding market (Baker 2002; Raja et al. 2007c). This feed additive is produced by chemical synthesis and available at a price of 3000 US$/kg. Today, the biological supply sources for astaxanthin are the yeast, *Phaffia rhodozyma* (Sanderson and Jolly 1994) despite its low content (0.4%), and compared to the fresh water Chlorophycean, *Haematococcus pluvialis* containing 5% (Guerin et al. 2003; Kang and Sim 2008). Some companies like Algatec-Sweden, Norbio-Norway, Biotechna-UK, Aquasearch, Cyanotech, Maricultura, Danisco Biotechnology and Oceancolor-USA have entered the astaxanthin market. In fact, microalgal astaxanthin has been approved in Japan and Canada as a pigment in salmonid feeds (Spolaore et al. 2006). Feeds including 5–20% *Arthrospira* (rich in carotene pigments), enhance the red and yellow patterns in carp. This clarity and color definition increases their value. Another example is the traditional French technique called the greening of oysters. It consists of creating a blue-green color on the gills and labial palps of oysters using the diatom, *Haslea ostrearia*. This increases the product's market value by 40% (Gagneux-Moreaux et al. 2007).

Transgenic *Chlamydomonas* sp. has been used as a vaccine delivery system using the p57 antigen, the causative agent of bacterial kidney disease. This disease is caused by the intracellular bacterium *Reinbacterium salmoninarum* which affects wild and farmed salmonids. This is a huge concern from an economic point of view and the symptom of disease develops before it can be treated with antibiotics. Fish-fed algae (4% algal dry weight of feed) fed to juvenile trout produced immunoglobulin IgM expressed in different tissues such as skin epithelial or blood cells. In contrast, trout fed with wild type algae or no algae did not generate antibodies (Sayre et al. 2001).

Importance of PUFA/HUFA functions in Aquaculture

PUFAs derived from microalgae (e.g. docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), α-linoleic acid (ALA) and arachidonic acid (AA)) are known to be essential for various larvae (Sargent et al. 1997). A summary of the proportion of these important PUFAs in 46 strains of microalgae are clearly shown (Volkman et al. 1989; Dunstan et al. 1993). The fatty acid content showed systematic differences according to taxonomic group,

although there were examples of significant differences between microalgae from the same class. Most microalgal species have moderate to high percentages (7–34%) of EPA. Prymnesiophytes (*Pavlova* sp., *Isochrysis* sp. and cryptomonads are relatively rich in DHA (0.2–11%) while eustigmatophytes, *Nannochloropsis* and diatoms have the highest percentages of AA (0–4%). Chlorophytes, *Dunaliella* and *Chlorella* are deficient in both C_{20} and C_{22} PUFAs although some species have small amounts (3.2%) of EPA. Because of this PUFA deficiency, chlorophytes generally have low nutritional value and hence they are not suitable as a single species diet (Brown 2002). PUFA rich microalgae such as *Pavlova* sp. and *Isochrysis* sp. can be fed to zooplankton to enrich them in DHA. However, these do not provide the level of enrichment required. Instead commercial oil emulsions (DHA Selco from INVE) are often used. Recently, algae-like products such as AlgaMac 2000 and Docosa Gold which contains 5–15% of their dry weight as DHA have been utilized. These have produced similar levels of enrichment of DHA within the zooplankton compared to the commercial oils (Gara et al. 1998) and also produce DHA to EPA ratios of between 1 and 2, which are considered favorable for fish larval nutrition (Masuda 2003). EPA plays an important role in higher animals as a precursor of a group of eicosanoids which are crucial in regulating developmental and regulatory physiology. The eicosanoids are hormone-like substances including prostaglandins (PG), thromboxanes (TX) and leukotrienes (LT). Arachidonic acid (AA, 20:4 n-6) and EPA are precursors of eicosanoid compounds. However, the eicosanoids from these two fatty acids are different both structurally and functionally, and are sometimes even antagonistic in their effects. A balanced uptake of EPA/AA can prevent eicosanoid dysfunctions and may be effective in treating a number of illnesses and metabolic disorders (Gill and Valivety 1997).

EPA has been found in a wide variety of marine microalgae including Bacillariophyceae (diatoms), Chlorophyceae, Chrysophyceae, Cryptophyceae, Eustigamatophyceae and Prasinophyceae. The *Nannochloropsis* species are widely used as a food in aquaculture and have been proposed for the commercial production of EPA (Apt and Behrens 1999). A high proportion of EPA in *Porphyridium propureum* has also been reported (Wen and Chen 2003; Martínez-Fernández and Paul 2007). The diatoms *Phaeodactylum tricornutum* and *Nitzschia laevis* have been intensively investigated for their EPA potentials (Wen and Chen 2003). In contrast to a large number of EPA-containing microalgae, only a few microalgal species have demonstrated industrial production potentials (Raja et al. 2007c). This is mainly due to a low specific growth rate and low cell density of the microalgae grown under conventional photoautotrophic conditions.

The requirement for 22:6 n-3 (DHA) fatty acid in marine fish and shrimp nutrition has been established via feeding diets both rich and deficient in these lipids. The exact mechanism for this requirement has been well documented for fish and vertebrate animals. The most likely answer is that the biological membranes rich in di-22:6 (n-3) phosphoglycerides have a phase structure that is relatively constant in the face of changing environmental variables like temperature, pressure, salinity and also unchanging bilayer width. These considerations rest heavily on the facts that the double bonds in naturally occurring PUFA/HUFA are methylene interrupted in the *cis* orientation and contain more fatty acids with additional double bonds (Gara et al. 1998). This effect reaches a maximum in 22:6 (n-3). The requirement for PUFA/HUFA and 22:6 (n-3) can be found in the vitellogenic process and precursors for the enzymatic and hormonal processes within the shrimp. Production of ecdysone for molting, growth and egg production require highly mobile and flexible energy sources as found in PUFA/HUFA. Fish phosphoglycerides generally contain 50% of their total fatty acids as n-3 PUFA/HUFA with a ratio of 22:6 (n-3): 20:5 (n-3) of about 2:1. This is seen most clearly in the phosphoglycerides of fish eggs. The lipids of diatoms contain large quantities of 20:5 (n-3) with appreciable quantities of C_{16} (n-3) PUFA/HUFA, but negligible amounts of 22:6 (n-3), whereas the lipids of dinoflagellates contain large amounts of 22:6 (n-3) and also 18:5 (n-3) (Gara et al. 1998). Most hatcheries do not culture high PUFA/HUFA species of microalgae to supply 22:6 n-3 sources to the larvae and thus the origin of substitution PUFA/HUFA to the diet of shrimp (and other marine eukaryotes) with commercial products containing high levels of these lipids.

Shrimp

Shrimp farming production reached 737,200 tonnes in 1998, an increase of 12% from 1997. This increased production mainly takes place in subtropical regions of America (28%, 457 hatcheries) and south-east Asia (72%, 3,718 hatcheries) (Alam et al. 2009). Microalgae are necessary from the second stage of larval development (zoea) and in combination with zooplankton from the third stage (myses). Naturally occurring microalgal blooms are encouraged in large ponds with low water exchange where the larvae are introduced. Sometimes fertilizers and bacteria are added to induce more favorable conditions. This production system with poor control of microalgae provides a better part of shrimp production (Rosenberry 1991; López Elías et al. 2003). On the other hand, large sized hatcheries require highly paid technicians, multimillion dollar investments and highly controlled medium

conditions. The observed trend is toward specialized production, particularly with the supply of post larvae in the hands of big centralized hatcheries. They open a pathway to new techniques especially the genetic selection of strains with stronger immunity.

Bivalve mollusks

Intensive rearing of bivalves has so far relied on the production of live algae which comprises an average of 30% of the operating costs in a bivalve hatchery. The relative algal requirements of the various stages of the bivalve culture process depend on whether the operation aims at the mass production of larvae for remote setting or growing millions of seed till planting size (Lavens and Sorgeloos 1996). In either case, juveniles consume the largest volumes of algal culture to produce a demand of large biomass with high weight species. Bivalve hatcheries rely on a broad range of algal species such as *Chaetoceros* sp, *Chlorella minutissima, Gomphonema* sp, *Isochrysis galbana, Nitzschia* sp, *Pavlova* sp, *Phaeodactylum tricornutum, Skeletonema* sp, *Thalassiosira pseudonana* and *Tetraselmis subcordiformis.* The algal species that were reported in an international survey among hatchery operators in 1995 are *Isochrysis* sp, *C. gracilis*, *C. calcitrans* and *T. suecica*.

Zooplankton

Microalgae have an important role in aquaculture as a means of enriching zooplankton for feeding to fish and larvae (Chakraborty et al. 2007). As such, the algal and zooplankton strains (Fig. 2) of the correct size and nutritional content have been identified for each of the major aquaculture species. The zooplankton most commonly used are rotifers *Brachionus plicatilis* and *Artemia salina* (Chakraborty et al. 2007). To a much lesser extent, cladocerans (*Moina macrocorpa, Daphnia* sp.) and copepods (*Euterpina acutifrons, Tigriopus japonicus*) are used. For zooplankton to grow and reproduce in the hatchery algal food is necessary and it is provided to the newly hatched rotifers or artemia (Nauplii) until these zooplankton reach the desired size. Then just prior to harvesting, zooplankton may be given a boost of algae or a formulated emulsion to pack the gut of the organism. This increases its nutritional value to the target culture species feeding on it. Hatcheries prefer to use *Artemia* whenever possible, because the cysts are purchased as a dry dormant phase. Upon immersion in seawater, the cysts hatch and can be ingested immediately. In addition to providing protein and energy, they provide other key nutrients such as vitamins, essential PUFAs, pigments and sterols which are transferred through a food chain. 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). Finfish hatcheries producing algae to feed rotifers or *Artemia* typically use the following: *Chlorella* sp, *Chlamydomonas* sp, *Nannochloris oculata, Nannochloropsis oculata, Tetraselmis tetrathele* and *T. chuii.*

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 use of small live plankton feeder preys such as the rotifer, *Brachionus plicatilis* is still a prerequisite for success in hatcheries of small larvae finfish like sea breams. These preys can be raised on yeast-based artificial feeds, but this is much less efficient than with phytoplankton. Microalgae present an interest on three levels: (a) quick (7–13 days) recovery of rotifer populations whereas yeast takes 20–35 days; (b) improved nutritional quality of live prey (c) lower bacterial contamination, especially from *Vibrio*. For numerous fresh- and sea-water animals, introduction of phytoplankton in rearing ponds leads to much better results in terms of survival, growth and transformation index than in clear water. In the case of sea bream, this condition has become an economic necessity and the reasons behind the positive role of microalgae in the larvae-rearing ponds of fish as well as shrimp have not been completely elucidated (Richmond 2004). There is no doubt that the water quality is improved and stabilized by oxygen production, pH stabilization etc., but this does not explain everything. The

Fig. 2 Some zooplankton. **a** Foraminifera sp., **b** *Temora longicornis,* **c** Nauplii sp.

action of some excreted biochemical compounds is generally mentioned, as well as the induction of behavioral processes like initial prey catching, other positive functions (such as regulating the bacterial population, probiotic effects and stimulating immunity) have also been suggested but they are not sufficiently understood.

The future

The high production cost of microalgae remains a constraint to many hatcheries. Improvements in alternative diets may continue but production costs of microalgae may also decrease due to the uptake of new technology by hatcheries. Therefore, it is unlikely that microalgae will be totally replaced at least in the medium term. A good selection of microalgal species is also available to support the aquaculture industries (López Elías et al. 2003). However, for some particular applications or industrial sectors new species with improved nutritional quality or growth characteristics could improve hatchery efficiencies. For instance, copepods are recognized as excellent feeds for fish larvae, but they have proven difficult to produce in intensive systems. The utilization of alternative microalgal species could improve their production rates.

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 clubbed with post-harvest processing such as spray drying or algal concentration to develop off-the-shelf algal biomass for distribution to hatcheries (López Elías et al. 2003). Further research is required to enhance the shelf life of concentrates and for the development of concentrates of popular flagellates such as *Isochrysis* sp. and *Pavlova lutheri*. The use of microalgae either as a full or partial enrichment should be considered for improving the nutritional quality of zooplankton. Microalgae contain an array of essential nutrients that may be transferred through food chains especially PUFAs. Microalgae *Isochrysis* sp. and *P. lutheri* can provide a moderate enrichment of DHA though not as effective as commercial oil emulsions like DHA. The new algae like thraustochytrid products are extremely high in DHA and provide an effective means of enriching zooplankton to produce good DHA: EPA ratios. New thraustochytrids are being investigated with other nutritional characteristics, high concentrations of AA. Some work has been documented on the transfer of AA from microalgae through zooplankton and fish larvae, but much lesser is known about other vitamins.

Though microalgae have generally been proposed here as a good source of vitamins, they can vary significantly in their composition. Therefore zooplankton could be deficient in one or more vitamins when enriched with certain dietary regimens. Future research should focus particularly on this issue and the transfer of other essential nutrients (pigments, sterols) to zooplankton fed different diets and grown under different culture conditions. Finally, a better understanding of the mechanism of green water systems both in intensive and extensive culture condition will aid in optimizing the usage of microalgae in larval culture.

Although genetically engineering 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. Li and Tsai (2009) inserted an algae-codon-optimized bovine lactoferin (an antimicrobial peptide) into *Nannochloropsis oculata* to provide an organism against bacterial pathogenic infection. The average survival rate of the medaka fish fed with the transgenic algae was higher that of those fed with wild type algae (85 versus 5%). 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 uplift in the status of aquaculture.

A study by León et al. (2007) aims at metabolically engineering the β-carotene ketolase cDNA from *H. pluvialis,* involved in the synthesis of astaxanthin into *C. reinhardtii*. Various factors such as slow growth, contamination, and relation between the astaxanthin production and development of red inert aplanospores (hemotocysts) limit the *Haematococcus* cultivation. It was shown that *C. reinhardtii* can act as a good candidate to express foreign carotenogenic genes and synthesize new carotenoids, for both carrying out basic metabolic and regulatory studies of the pathway and for the biotechnological production of interesting carotenoids.

Conclusion

Application of non-living microalgae in aquaculture have provided mitigated or unsuccessful results, making live microalgae the first choice in aquaculture feeding. Only partial replacement for live microalgae is successful in studies using preserved non-living algae, microencapsulated diets, or spray-dried algae. However, no complete replacement has been achieved despite intensive research efforts. According to the scientific literature, live microalgae with high nutritive value and appropriate physical properties can provide a healthy rearing environment to the aquaculture system.

Acknowledgments The authors wish to express their sincere gratitude to Dr. Velusubramani, Senior Scientist, Refining Technology, The British Petroleum Company Ltd., Chicago, USA and Prof. R. Manivasakan, Indian Institute of Technology Madras for their critical review of the manuscript.

References

Alam MS, Watanabe WO, Daniels HV (2009) Effect of different dietary protein and lipid levels on growth performance and body composition of juvenile southern flounder (*Paralichthys lethostigma)* reared in recirculating aquaculture system. J World Aquac Soc 40:513–521

Alvarez JS, Llamas AH, Galindo J, Fraga I, Garca T, Villarreal H (2007) Substitution of fishmeal with soybean meal in practical diets for juvenile white shrimp *Litopenaeus schmitti*. Aquacul Res 38:689–695

Apt KE, Behrens PW (1999) Commercial developments in microalgal biotechnology. J Phycol 35:215–226

Atalah E, Hernández Cruz CM, Izquierdo MS, Rosenlund G, Caballero MJ, Valencia A, Robaina L (2007) Two microalgae *Crypthecodinium cohnii* and *Phaeodactylum tricornutum* as alternative source of essential fatty acids in starter feeds for seabream (*Sparus aurata*). Aquacul 270:178–185

Baker RTM (2002) Canthaxanthin in aquafeed applications: is there any risk? Trends Food Sci Tech 12:240–243

Borowitzka MA (1997) Microalgae for aquaculture opportunities and constraints. J Appl Phycol 9:393–401

Brown MR (2002) Nutritional value of microalgae for aquculture. In: Cruz-Suárez LE, Ricque-Marie D, Tapia-Salazar M, Gaxiola-Cortés MG, Simoes N (eds) Avances en Nutrición Acuícola VI. Memorias del VI Simposium Internacional de Nutrición. Acuícola. 3 al 6 de Septiembre del. ancún. Quintana Roo, México

Carvalho AP, Meireles LA, Malcata XF (2006) Microalgal reactors: a review of enclosed system designs and performances. Biotechnol Prog 22:1490–1506

Catarina Guedes A, Meireles LA, Amaro HM, Xavier Malcata F (2010) Changes in lipid class and fatty acid composition of cultures of *Pavlova lutheri*, in response to light intensity. J Am Oil Chem Soc 87:791–801

Chakraborty RD, Chakraborty K, Radhakrishnan EV (2007) Variation in fatty acids composition of *Artemia salina* nauplii enriched with microalgae and baker's yeast for use in larviculture. J Agric Food Chem 55:4043–4051

Dhont J, Van Stappen G (2003) Live feeds in marine aquaculture. Blackwell Science Ltd. pp 65–121

Dunstan GH, Volkman JK, Barret SM, Garland CD (1993) Changes in the lipid composition and maximization of the polyunsaturated fatty acid content of three microalgae grown in mass culture. J Appl Phycol 5:71–83

Durmaz Y (2007) Vitamin E (α-tocopherol) production by the marine microalgae *Nannochloropsis oculata* (Eustigmatophyceae) in nitrogen limitation. Aquacul 272:717–722

FAO (Food and Agriculture Organization) (2002) Fishery Statistics: Commodities, vol 21. FAO, Rome

Fujii K, Nakashima H, Hashidzume Y, Uchiyama T, Mishiro K, Kadota Y (2010) Potential use of the astaxanthin-producing microalga, *Monoraphidium* sp. GK12, as a functional aquafeed for prawns. J Appl Phycol 22:363–369

Gagneux-Moreaux S, Moreau C, Gonzalez JL, Cosson RP (2007) Diatom artificial medium (DAM): a new artificial medium for the diatom *Haslea ostrearia* and other marine microalgae. J Appl Phycol 19:549–556

Gara B, Shields RJ, McEvoy L (1998) Feeding strategies to achieve correct metamorphosis of Atlantic halibut, *Hippoglossus hippoglossus* L., is using enriched *Artemia*. Aquacul Res 29:935–948

Gentsch E, Kreibich T, Hagen W, Barbara N (2009) Dietary shifts in the copepod *Temora longicornis* during spring: evidence from stable isotope signatures, fatty acid biomarkers and feeding experiments. J Plankton Res 31:45–60

Gill I, Valivety R (1997) Polyunsaturated fatty acids: Part 1. Occurrence, biological activities and application. Trends Biotechnol 15:401–409

Guerin M, Huntley ME, Olaizol M (2003) *Haematococcus* astaxanthin: applications for human health and nutrition. Trends Biotechnol 21:210–215

Hong HA, Duc HL, Cutting SM (2005) The use of bacterial spore formers as probiotics. FEMS Microbiol Rev 29:813–835

Kang CD, Sim SJ (2008) Direct extraction of astaxanthin from *Haematococcus* culture using vegetable oils. Biotechnol Lett 30:441–444

Knauer J, Southgate PC (1999) A review of the nutritional requirements of bivalves and the development of alternative and artificial diets for bivalve aquaculture. Rev Fish Sci 7:241–280

Knuckey RM, Brown MR, Barrett SM, Hallegraeff GM (2002) Isolation of new nanoplanktonic diatom strains and their evaluation as diets for the juvenile Pacific oyster. Aquacul 211:253–274

Knuckey RM, Brown MR, René Robert R, Frampton MFD (2006) Production of microalgal concentrates by flocculation and their assessment as aquaculture feeds. Aquacul Eng 35:300–313

Laing I, Millican PF (1992) Indoor nursery cultivation of juvenile bivalve molluscs using diets of dried algae. Aquacul 102:231–243

Lavens P, Sorgeloos P (1996) Manual on the production and use of live food for aquaculture. FAO Fisheries Technical paper. In: Lavens P, Sorgeloos P (eds) Rome. pp 36–19

León R, Inmaculada C, Emilio F (2007) Metabolic engineering of ketocarotenoids biosynthesis in the unicelullar microalga, *Chlamydomonas reinhardtii*. J Biotechnol 130:143–152

Li SS, Tsai HJ (2009) Transgenic microalgae as a non-antibiotic bactericide producer to defend against bacterial pathogen infection in the fish digestive tract. Fish Shellfish Immunol 26:316–325

Liang H, Gong W-J, Chen Z-L, Tian J-Y, Qi L, Li G-B (2009) Effect of chemical preoxidation coupled with in-line coagulation as a pretreatment to ultrafi ltration for algae fouling control. Desalination Water Treat 9:241–245

López Elías JA, Voltolina D, Chavira Ortega CO, Rodríguez Rodríguez BB, Sáenz Gaxiola LM, Esquivel BC, Nieves M (2003) Mass production of microalgae in six commercial shrimp hatcheries of the Mexican northwest. Aquacultural Eng 29:155–164

Lorenz RT, Cysewski GR (2000) Commercial potential for *Haematococcus* microalgae as a natural source of astaxanthin. Trends in Biotechnol 18:160–167

Lubzens E, Gibson O, Zmora O, Sukenik A (1995) Potential advantages of frozen algae (*Nannochloropsis* sp.) for rotifer (*Brachionus plicatilis*) culture. Aquacul 133:295–309

Martínez-Fernández E, Paul C (2007) Southgate Use of tropical microalgae as food for larvae of the black-lip pearl oyster *Pinctada margaritifera*. Aquacul 263:220–226

Masuda R (2003) The critical role of docosahexaenoic acid in marine and terrestrial ecosystems: from bacteria to human behaviour. The big fish bang. In: Browman HI, Skiftesvik AB (eds) Proceedings of the 26th annual larval fish conference. Published by the Institute of Marine Research, Postboks 1870 Nordnes, N-5817, Bergen, Norway. ISBN 82-7461-059-8

Meireles LA, Catarina Guedes A, Xavier Malcata F (2003) Increase of the yields of Eicosapentaenoic and Docosahexaenoic acids by the microalga *Pavlova lutheri* following random mutagenesis. Biotechnol Bioeng 81:5

Meireles LA, Catarina GA, Barbosa CR, Azevedo JL, Cunhab JP, Malcata FX (2008) On-line control of light intensity in a microalgal bioreactor using a novel automatic system. Enzyme Microbial Tech 42:554–559

Muller-Feuga A (2000) The role of microalgae in aquaculture: situation and trends. J Appl Phycol 12:527–534

Muller-Feuga A (2004) Microalgae for aquaculture: the current global situation and future trends. In: Richmond A (ed) Handbook of microalgal culture. Blackwell Science, pp 352–364

Muller-Feuga A, Moal J, Kaas R (2003) The microalgae of aquaculture. In aquaculture. In: Støttrup JG, McEvoy LA (eds) Live feeds in marine aquaculture. Blackwell Science Ltd. pp 253–299

Yúfera M, Navarro N (1995) Population growth dynamic of the rotifer *Brachionus plicatilis* cultured in non-limiting food condition. Hydrobiologia 313/314, 399–405

New MB, Wagner CV (2000) Freshwater prawn culture. Blackwell Science, Oxford, pp 1–11

Patil V, Källqvist T, Olsen E, Vogt G, Gislerød HR (2007) Fatty acid composition of 12 microalgae for possible use in aquaculture feed. Aquacul Int 15:1–9

Pedro C, Fernández-Díaz JC (2001) Pilot evaluation of freeze-dried microalgae in the mass rearing of gilthead seabream (*Sparus aurata*) larvae. Aquacul 193:257–269

Ponis E, Robert R, Parisi G (2003) Nutritional value of fresh and concentrated algal diets for larval and juvenile Pacific oysters (*Crassostrea gigas*). Aquacul 221:491–505

Ponis E, Probert I, Véron B, Mathieu M, Robert R (2006) New microalgae for the Pacific oyster *Crassostrea gigas* larvae. Aquacul 253:618–627

Pulz O (2001) Photobioreactors: production systems for phototropic microorganisms. Appl Microbiol Biotechnol 57:287–293

Pulz O, Scheibenbogen K (1998) Photobioreactors: design and performance with respect to light energy input. Adv Biochem Eng Biotechnol 59:123–151

Raja R (2003) Studies on *Dunaliella salina* (Dunal) Teod. with special reference to its anticancer properties. Ph.D., thesis, University of Madras, Chennai, India

Raja R (2009) Microalgae [Pourriel probable] a column in the IInd Chapter in 'Un monde invisible' edited by Laurence Bordenave, Publisher: Aubanel-La Martinière, La Martinière Group, ISBN: 978-2-7006-0670-6, France, pp 124–126 (French)

Raja R, Hemaiswarya S (2010) Microalgae and immune potential a chapter in dietary components and immune function–prevention and treatment of disease and cancer. In: Watson RR, Zibadi S, Preedy VR (eds) Humana Press/Springer, ISBN: 978-1-60761-060-1, USA, pp 517–529

Raja R, Anbazhagan C, Ganesan V, Rengasamy R (2004a) Efficacy of *Dunaliella salina* (Volvocales, Chlorophyta) in salt refinery effluent treatment. Asi J Chem 16:1081–1088

Raja R, Anbazhagan C, Lakshmi D, Rengasamy R (2004b) Nutritional studies on *Dunaliella salina* (Volvocales, Chlorophyta) under laboratory conditions. Seaweed Res Utili 26: 127–146

Raja R, Hemaiswarya S, Balasubramanyam D, Rengasamy R (2007a) PCR-identification of *Dunaliella salina* (Volvocales, Chlorophyceae) and its growth characteristics. Microbiol Res 162: 168–176

Raja R, Hemaiswarya S, Balasubramanyam D, Rengasamy R (2007b) Protective effect of *Dunaliella salina* (Volvocales, Chlorophyta) on experimentally induced fibrosarcoma on wistar rats. Microbiol Res 162:177–184

Raja R, Hemaiswarya S, Rengasamy R (2007c) Exploitation of *Dunaliella* for β-carotene production. Appl Microbiol Biotechnol 74:517–523

Raja R, Hemaiswarya S, Ashok Kumar N, Sridhar S, Rengasamy R (2008) A perspective on the biotechnological potential of microalgae. Cri Rev Microbiol 34:77–88

Richmond A (2004) Handbook of microalgal culture: biotechnology and applied phycology. Blackwell Science Ltd. pp 1–544

Robert R, Parisi G, Rodolfi L, Poli BM, Tredici MR (2001) Use of fresh and preserved *Tetraselmis suecica* for feeding *Crassostrea gigas* larvae. Aquacul 192:333–346

Rosenberg JN, Oyler GA, Wilkinson L, Betenbaugh MJ (2008) A green light for engineered algae: redirecting metabolism to fuel a biotechnology revolution. Curr Opin Biotechnol 19:430–436

Rosenberry B (1991) World shrimp farming 1991. Aquaculture Digest, San Diego

Sanderson GW, Jolly SO (1994) The value of *Phaffia* yeast as a feed ingredient for Salmonid fish. Aquacul 124:193–200

Sargent JR, McEvoy LA, Bell JG (1997) Requirements, presentation and sources of polyunsaturated fatty acids in marine fish larval feeds. Aquacul 155:117–128

Sayre RT, Wagner RE, Siripornadulsil S, Farias C (2001) Use of *Chalmydomonas reinharditii* and other transgenic algae in food or feed for delivery of antigens

Spolaore P, Joannis-Cassan C, Duran E, Isambert A (2006) Commercial applications of microalgae. J Biosci Bioeng 101:87–96

Tacon AJ (2003) Aquaculture production trends analysis. Review of the state of world aquaculture, Food and Agriculture Organisation of the United Nations, FAO Fisheries Circular No. 886, Rome, pp 5–29

Volkman JK, Jeffrey SW, Nichols PD, Rodgers GI, Garland CD (1989) Fatty acid and lipid composition of 10 species of microalgae used in mariculture. J Exp Mar Biol Ecol 128:219–240

Wen Z-Y, Chen F (2003) Heterotrophic production of eicosapentaenoic acid by microalgae. Biotechnol Adv 21:273–294

Wikfors GH, Ohno M (2001) Impact of algal research in aquaculture. J Phycol 37:968–974

Yamasaki S, Tanabe K, Hirata H (1989) Efficiency of chilled and frozen *Nannochloropsis* sp. (marine *Chlorella*) for culture of rotifer. Mem Fac Fish Kagoshima Univ 38:77–82