Nutritional Value and Use of Microalgae in Aquaculture

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ABSTRACT

This review provides a background on the usage of microalgae in aquaculture, focusing on their nutritional value and transfer of nutrients through food chains. The current status of knowledge is summarized and potential areas of research and industry development are identified. The review is divided into six sections: (1) general attributes of microalgal species used in aquaculture, (2) nutritional properties, (3) production systems, (4) alternatives to fresh algae, (5) use of algae to enrich zooplankton and (6) directions for future research.

GENERAL ATTRIBUTES OF MICROALGAE USED IN AQUACULTURE

Microalgae are utilized in aquaculture as live feeds for all growth stages of bivalve molluscs (eg. oysters, scallops, clams and mussels), for the larval/early juvenile stages of abalone, crustaceans and some fish species, and for zooplankton used in aquaculture food chains. Over the last four decades, several hundred microalgae species have been tested as food, but probably less than twenty have gained widespread use in aquaculture. Microalgae must possess a number of key attributes to be useful aquaculture species. They must be of an appropriate size for ingestion, e.g. from 1 to 15 μm for filter feeders; 10 to 100 μm for grazers (Webb & Chu, 1983; Jeffrey, LeRoi & Brown, 1992; Kawamura, Roberts & Nicholson, 1998) and readily digested. They must have rapid growth rates, be amenable to mass culture, and also be stable in culture to any fluctuations in temperature, light and nutrients as may occur in hatchery systems. Finally, they must have a good nutrient composition, including an absence of toxins that might be transferred up the food chain.

Strains identified by Persoone & Claus (1980) as being successful for bivalve culture included *Isochrysis galbana, Isochrysis* sp. (T.ISO), *Pavlova lutheri, Tetraselmis suecica, Pseudoisochrysis paradoxa, Chaetoceros calcitrans* and *Skeletonema costatu*m. It is noteworthy that now, over 20 years later, hatcheries are still using essentially the same strains for their production (Table 1).

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Table 1. Microalgae commonly used in aquaculture, either as individual diets or components of mixed diets. (++ denotes more popular than +).

	Bivalve molluscs	Crustacean larvae	Juvenile abalone	Zooplankton (used for crustacean, fish larvae)
Isochrysis sp. (T.ISO)	++	+		++
Pavlova lutheri	++	+		++
Chaetoceros calcitrans	++	++		+
C. muelleri or C. gracilis	+	++		+
Thalassiosira pseudonana	+	+		
Skeletonema spp.	+	++		
Tetraselmis suecica	+	+		++
Rhodomonas spp.	+			
Pyramimonas spp.	+			
Navicula spp.	+	+	++	
Nitzschia spp.		+	++	
Cocconeis spp.			+	
Amphora spp.			+	
Nannochloropsis spp.				++

References: Brown *et al*. (1997); Reitan *et al*. (1997); Lee (1997); Kawamura *et al*. (1998); Wikfors & Ohno (2001); Johnston per. comm. (CSIRO Collection of Living Microalgae)

Isochrysis sp. (T.ISO), *Pavlova lutheri* and *Chaetoceros calcitrans* are the most common species used to feed the larval, early juvenile and broodstock (during hatchery conditioning) stages of bivalve molluscs; these are usually fed together as a mixed diet (O'Connor & Heasman, 1997; Richard Pugh, Shellfish Culture Ltd., pers. comm.). Many of the strains successfully used for bivalves are also used as direct feed for crustaceans (especially shrimp) during the early larval stages, especially diatoms such as *Skeletonema* spp. and *Chaetoceros* spp.

Benthic diatoms such as *Navicula* spp. and *Nitzschia* are commonly mass-cultured and then settled onto plates as a diet for grazing juvenile abalone. *Isochrysis* sp. (T.ISO), *Pavlova lutheri*, *T. suecica* or *Nannochloropsis* spp. are commonly fed to *Artemia* or rotifers, which are then fed on to later larval stages of crustacean and fish larvae.

NUTRITIONAL PROPERTIES OF MICROALGAE

Microalgal species can vary significantly in their nutritional value, and this may also change under different culture conditions (Enright *et al*., 1986a; Brown *et al*., 1997). Nevertheless, a carefully selected mixture of microalgae can offer an excellent nutritional package for larval animals, either directly or indirectly (through enrichment of zooplankton). Microalgae that have been found to have good nutritional properties - either as monospecies or within a mixed diet - include *C. calcitrans*, *C. muelleri, P. lutheri, Isochrysis* sp. (T.ISO), *T. suecica, S. costatum* and *Thalassiosira pseudonana* (Enright *et al*., 1986b; Thompson, Guo & Harrison, 1993; Brown *et al*., 1997).

Several factors can contribute to the nutritional value of a microalga, including its size and

shape, digestibility (related to cell wall structure and composition), biochemical composition (eg. nutrients, enzymes, toxins if present) and the requirements of the animal feeding on the alga. Since the early reports that demonstrated biochemical differences in gross composition between microalgae (Parsons, Stephens & Strickland, 1963) and fatty acids (Webb & Chu, 1983), many studies have attempted to correlate the nutritional value of microalgae with their biochemical profile. 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 all the literature data, including experiments where algal diets have been supplemented with compounded diets or emulsions, some general conclusions can be reached (Knauer & Southgate, 1999).

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, Thinh & Parry, 1999). 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, Thompson & Calderwood 1990; Brown *et al*., 1993b). 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*; Enright *et al*., 1986b) and larval scallops (*Patinopecten yessoensis*; Whyte, Bourne & Hodgson, 1989) provided polyunsaturated fatty acids (PUFAs) are also present in adequate proportions. In contrast, high dietary protein provided best growth for juvenile mussels (*Mytilus trossulus*; Kreeger & Langdon, 1993) and Pacific oysters (*Crassostrea gigas*; Knuckey *et al*., 2002).

PUFAs derived from microalgae, i.e. docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) and arachidonic acid (AA) are known to be essential for various larvae (Langdon & Waldock, 1981; Sergeant, McEvoy & Bell, 1997). A summary of the proportion of these important PUFAs in 46 strains of microalgae are shown in Figure 1 (data from Volkman *et al*., 1989; Volkman *et al*., 1991; Volkman *et al*., 1993; Dunstan *et al*., 1994). 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 of EPA (7 to 34%; Fig 1). Prymnesiophytes (eg. *Pavlova* spp. and *Isochrysis* sp. (T.ISO)) and cryptomonads are relatively rich in DHA (0.2 to 11%), whereas eustigmatophytes (*Nannochloropsis* spp.) and diatoms have the highest percentages of AA (0 to 4%). Chlorophytes (*Dunaliella* spp. and *Chlorella* spp.) are deficient in both C20 and C22 PUFAs, although some species have small amounts of EPA (up to 3.2%). Because of this PUFA deficiency, chlorophytes generally have low nutritional value and are not suitable as a single species diet (Brown *et al*., 1997). Prasinophyte species contain significant proportions of C20 (*Tetraselmis* spp.) or C22 (*Micromonas* spp.) - but rarely both.

While the importance of PUFAs is recognized, the quantitative requirements of larval or juvenile animals feeding directly on microalgae is not well established (Knauer & Southgate, 1999). Thompson, Guo & Harrison (1993) found that the growth of Pacific oyster *C. gigas* larvae was not improved by feeding them microalgae containing higher than 2% (total fatty acids) of DHA; moreover the percentage of dietary EPA was negatively correlated to larval growth. However, the authors found a correlation between the percentage composition of the short chain fatty acids 14:0 + 16:0 in microalgae, and larval growth rates. They reasoned that diets with higher percentages of the saturated fats were more beneficial for the rapidly growing larvae, because energy is released more efficiently from saturated fats than unsaturated fats. In late-logarithmic phase, prymnesiophytes, on average, contain the highest percentages of saturated fats (33% of total fatty acids), followed by diatoms and eustigmatophytes (27%), prasinophytes and chlorophytes (23%) and cryptomonads (18%) (Brown *et al*., 1997). The content of saturated fats in microalgae can also be improved by culturing under high light conditions (Thompson *et al*., 1993).

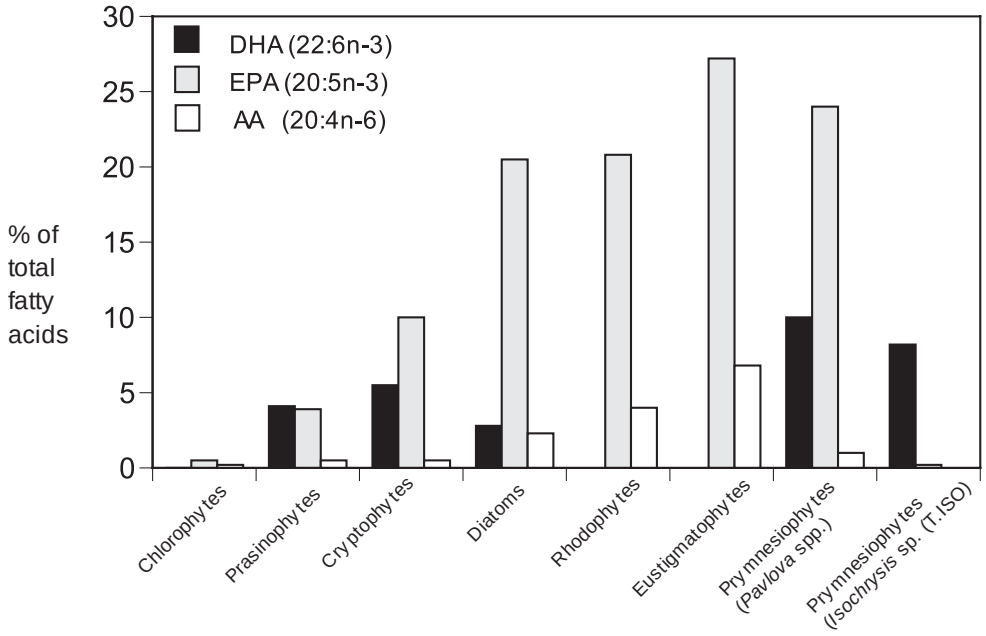

Fig 1. Average percentage compositions of the long-chain PUFAs docosahexaenoic acid (DHA; 22:6n-3), eicosapentaenoic acid (EPA; 20:5n-3) and arachidonic acid (20:4n-6) of microalgae commonly used in aquaculture. Data compiled from over 40 species from the laboratory of CSIRO Marine Research.

The content of vitamins can vary between microalgae. Ascorbic acid shows the greatest variation, i.e. 16-fold (1 to 16 mg g^{-1} dry weight; Brown & Miller, 1992). Concentrations of other vitamins typically show a two- to four-fold difference between species, i.e. β-carotene 0.5 to 1.1 mg g^{-1}, niacin 0.11 to 0.47 mg g^{-1}, α-tocopherol 0.07 to 0.29 mg g^{-1}, thiamin 29 to 109 μg g^{-1}, riboflavin 25 to 50 μg g^{-1}, pantothenic acid 14 to 38 μg g^{-1}, folates 17 to 24 μg g^{-1}, pyridoxine 3.6 to 17 μg g^{-1}, cobalamin 1.8 to 7.4 μg g^{-1}, biotin 1.1 to 1.9 μg g^{-1},

retinol ≤ 2.2 μg g^{-1} and vitamin D < 0.45 μg g^{-1} (Seguineau *et al*., 1996; Brown *et al*., 1999). To put the vitamin content of the microalgae into context, data should be compared with the nutritional requirements of the consuming animal. Unfortunately, nutritional requirements of larval or juvenile animals that feed directly on microalgae are, at best, poorly understood. However, the requirements of the adult are far better known (eg. for marine fish and prawns; Tacon, 1991; Conklin, 1997) and, in the absence of information to the contrary, will have to serve as a guide for the larval animal. These data suggest that a carefully selected, mixed-algal diet should provide adequate concentrations of the vitamins for aquaculture food chains.

The amino acid composition of the protein of microalgae is very similar between species (Brown, 1991) and relatively unaffected by the growth phase and light conditions (Brown *et al.,* 1993a, b). Further, the composition of essential amino acids in microalgae is very similar to that of protein from oyster larvae (*C. gigas*; see Fig.2). This indicates that it is unlikely the protein quality is a factor contributing to the differences in nutritional value of microalgal species.

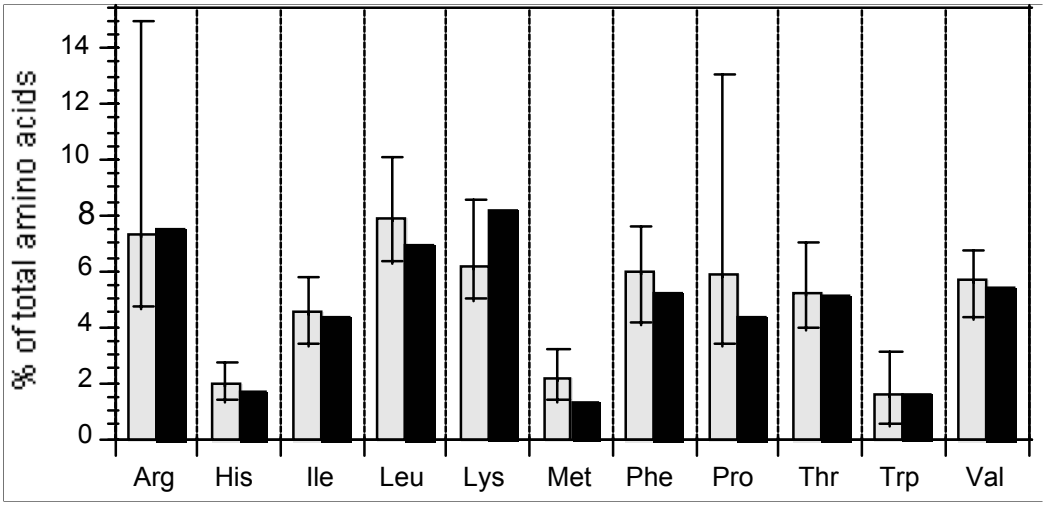

Fig. 2. Comparison of the percentages of essential amino acids in microalgae (grey bars) and Pacific oyster (*C. gigas*) larvae (black bars). Bars for algae represent averages of 47 species; error bars represent the range (Brown 1997).

Sterols (Knauer *et al*., 1999), minerals (Fabregas & Herrero, 1986) and pigments (see discussion in later section of this review) also may contribute to nutritional differences of microalgae.

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, out-door tanks or ponds are used, operated

semi-continuously. Depending on their scale, hatcheries may produce between several hundred to tens of thousands of litres of algae daily. Cells densities range from 10^5 to 10^7 cells mL^{-1} with these standard systems, and production costs can range from US $50 to 200 kg^{-1} dry weight, or 20 to 50% of hatcheries operating costs (Coutteau & Sorgeloos, 1992). There are clear economies of scale with algal production, so that production costs become especially significant for small hatcheries. Consequently, there has been much effort directed at examining alternatives to production of fresh algae, and also more cost-efficient production systems.

Large-scale photobioreactors, either for indoor or outdoor production, have been assessed (Tredici & Materassi, 1992; Chrismadha & Borowitzka, 1994). Essentially these can be considered as variations of the standard culture systems, but with a much higher surface area to volume ratio (SA:VOL). Consequently, light is less likely to become limiting and systems are characterised by high productivity and cell biomass at harvest – potentially eliciting a low production cost. However, these systems do have some disadvantages. Oxygen concentrations (resulting from photosynthesis) can build up because of the high biomass, and therefore give rise to photoinhibition - thus restricting productivity. Because of the high SA:VOL overheating of cultures can be a problem in outdoor systems. Also, because of the high biomass the systems need turbulent flow to ensure nutrient exchange and to avoid light-limitation, thereby making them unsuitable for fragile species. In fact, most aquaculture strains have not been effectively cultured in such systems. Exceptions include *Nannochloropsis* spp. (Tredici & Materassi, 1992) and *Skeletonoma* spp. (Sue Blackburn *et al*., unpub. obs.).

Fermentation technology is well established for low-cost production of bacteria and yeast, and there are some microalgae capable of heterotrophic growth. The advantages include a high-density and biomass production, and elimination of light – a major cost for phototrophic production. Because lower volumes are required for producing the same biomass (compared to conventional algal systems) this provides a greater degree of control and potentially, better economy of production. Production costs of between US $2 to 25 kg^{-1} dry weight microalgae have been projected for this technology (Gladue, 1991). Unfortunately, few aquaculture species have been identified that can grow heterotrophically. *Tetraselmis* spp. are exceptions, though these are generally recognised as having moderate food value, unless forming part of a mixed diet. There is also a high capital cost associated with fermentation; 2 L units can cost US $5 to 10 K, whereas 10,000 L units may exceed US $1M.

ALTERNATIVES TO FRESH ALGAE

Alternatives to microalgae have been sought that may be more cost-effective (Robert & Trintignac, 1997; Knauer & Southgate, 1999). Those tested have included compounded microcapsules (Knauer & Southgate, 1999), lipid emulsions (Coutteau *et al*., 1996), yeasts or yeast-based diets (Nell, Diemar & Heasman, 1996) and bacteria (Douillet, 1993). More recently, several products based on thraustochytrids (microorganisms whose taxonomy may be related to certain algal classes) from the genus *Schizochytrium* have been marketed

through Aquafauna Biomarine Inc. (eg. AlgaMac 2000) and Sanders Brine Shrimp Co. (eg. Docosa Gold). These products have high concentrations of DHA (Barclay & Zeller, 1996), and so are being applied as alternatives to commercial oil enrichments (eg. Selco) for zooplankton fed to larvae (discussed more in the next section). Generally, as direct feeds most of the above products have had lower nutritional value than mixtures of microalgae commonly used in aquaculture, though some performed well as components of a mixed diet with live microalgae (Robert & Trintignac, 1997; Lewis *et al*., 1998; Langdon & Önal, 1999).

A number of processed forms of microalgae have also been assessed as alternatives to live microalgae. One of the first was Algal 161 from CellSys. This was produced from *T. suecica* and cost US $ 180 kg^{-1}. This product had a moderate value as a diet component for molluscs (Laing, Child & Janke, 1990). However, it did not have a high-market penetration and is now unavailable. Algal pastes or concentrates have some potential as alternative diets. The advantage of such products is that they can be used "off-the-shelf", thus providing potential cost-efficiencies to hatcheries. Concentrates are prepared by centrifugation (≈ 1:500 concentration) or flocculation (≈ 1:100 concentration). Concentrates prepared from different microalgae vary in their suitability, with diatoms being the most promising and have a shelf life of between 2 to 8 weeks when stored ≤ 4°C. From a commercial viewpoint, concentrates can be prepared under two different scenarios, i.e. (a) by hatcheries on-site, preparing concentrates as back-up or as a means to store any overproduction of algae, or (b) remote production, centralized at a large facility – with greater economies of scale – and the resultant concentrates dispatched to hatcheries upon request. One company that is currently producing algal concentrates for commercial sale is Reed Mariculture (http://www.seafarm.com). Concentrates fed to the larvae and spat of Sydney rock oyster (Heasman *et al*., 2000) and Pacific oyster (McCausland *et al*., 1999; Brown & Robert, 2002) were effective as partial diets (eg. up to 80%) with growth rates similar to, or marginally inferior to, complete live diets. More R & D on post-harvest preservation methods is required to extend shelf-life beyond 4 to 8 weeks, and also for the preparation of concentrates from flagellate species of microalgae (eg *Isochrysis* sp. (T.ISO) and *P. lutheri*).

USE OF ALGAE TO ENRICH ZOOPLANKTON

Microalgae have an important role in aquaculture as a means of enriching zooplankton for on-feeding to fish and other larvae. In addition to providing protein (essential amino acids) and energy, they provide other key nutrients such as vitamins, essential PUFAs, pigments and sterols, which are transferred through the food chain. For example, rotifers fed microalgae become rapidly enriched with ascorbic acid (AsA). After 24 h, rotifers fed on *Isochrysis* sp. (T.ISO) and *Nannochloropsis oculata* contained 2.5 and 1.7 mg g^{-1} DW, respectively, whereas rotifers fed on baker's yeast (itself deficient in AsA) contained only 0.6 mg g^{-1} DW (Brown, Skabo & Wilkinson, 1998). After an ensuing 16 h of non-feeding, rotifers lost <10% of their AsA, retaining ≈ 50% of total ingested AsA. Similarly, concentration of AsA in *Artemia* may be enriched by feeding with microalgae (Merchie *et*

al., 1995). Little information is available on the transfer of other vitamins from microalgae through the food chain to fish larvae.

PUFA-rich microalgae, such as *Pavlova* spp. and *Isochrysis* sp. (T.ISO) can be fed to zooplankton to enrich them in DHA (Nichols *et al*., 1989). However, often these do not provide the level of enrichment often sought for zooplankton, and commercial oil-emulsions (eg. DHA Selco from INVE) are often used. Recently, "algal-like" products such as AlgaMac 2000 and Docosa Gold (dried preparations of the thraustochytrid *Schizochytrium* sp.) – which contain 5-15% of their DW as DHA – have been utilised. These have produced similar levels of enrichment of DHA within the zooplankton compared to the commercial oils (Gara, Shields & McEvoy, 1998), and also produce DHA to EPA ratios of between 1 and 2, which are considered favourable for fish larval nutrition (Rodríguez *et al*., 1998). Research is also in progress assessing alternate live and dried thraustochytrids as dietary constituents and for enrichment (Tom Lewis, University of Tasmania and Sue Blackburn, CSIRO Marine Research; pers. comm.).

Rønnestad, Helland & Lie (1998) demonstrated that microalgal pigments transferred through to zooplankton may contribute to nutritional value. They found the dominant pigments in the copepod *Temora* sp. were lutein and astaxanthin, whereas in *Artemia* it was canthaxanthin. 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*. The authors ascribed this to the ability of the larvae to convert lutein and/or astaxanthin, but not canthaxanthin, into vitamin A. They recommended that *Artemia* should routinely be enriched with astaxanthin and lutein (the latter pigment is common in "green" microalgae, eg. *Tetraselmis* spp.) to improve their nutritional value.

A common procedure during the culture of both larval fish and prawns is to add microalgae (i.e. "green water") to intensive culture systems together with the zooplankton prey (Tamaru, Murashige & Lee, 1994). Addition of the microalgae to larval tanks can improve the production of larvae, though the exact mechanism of action is unclear. Theories advanced include (a) light attenuation (i.e. shading effects), which have a beneficial effect on larvae, (b) maintenance of the nutritional quality of the zooplankton, (c) an excretion of vitamins or other growth-promoting substances by algae, and (d) a probiotic effect of the algae. Most likely, the mechanism may be a combination of several of these possibilities. A maintenance of NH_3 and O_2 balance has also been proposed, though this has not been supported by experimental evidence (Tamaru *et al*., 1994). The most popular algae species used for green water applications are *N. oculata* and *T. suecica*. More research is needed on the application of other microalgae – especially those species rich in DHA – to green water systems. Green water may also be applied to extensive outdoor production systems by fertilizing ponds to stimulate microalgal growth, and correspondingly, zooplankton production, as food for larvae introduced into the ponds.

AVENUES FOR FUTURE RESEARCH

The high production cost of microalgae remains a constraint to many hatcheries. Despite

efforts over several decades to develop cost-effective artificial diets to replace microalgae as hatchery feeds, on-site microalgal production remains a critical element for most marine hatcheries. Improvements in alternative diets may continue, but production costs of microalgae may also decrease due to the uptake of new technology by hatcheries (eg. continuous bag system, from Seasalter Shellfish). Therefore it is unlikely that microalgae will be totally replaced, at least in the medium term.

A good selection of microalgal species is available to support the aquaculture industry. However for some particular applications or industry sectors, new species with improved nutritional quality or growth characteristics could improve hatchery efficiency. For example, copepods are recognised as excellent feeds for fish larvae, but they have proven difficult to produce in intensive systems. The utilisation of alternate microalgal species could improve their production rates.

Apart from improvements in the cost-efficiencies of on-site algal production, an alternative is the centralization of algal production at specialised mass-culture facilities, using heterotrophic methods or photobioreactors to produce cheaper algal biomass. These technologies could be married with post-harvest processing such as spray-drying, or algal concentration (centrifugation or flocculation) to develop off-the-shelf algal biomass for distribution to hatcheries. More research is required to enhance the shelf life of concentrates and for the development of concentrates of popular flagellates such as *Isochrysis* sp. (T.ISO) and *P. lutheri*.

The use of microalgae either as a full or partial (i.e. in conjunction with products like Selco and AlgaMac 2000) 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 (eg. *Isochrysis* sp. (T.ISO) and *P. lutheri*) can provide a moderate enrichment of DHA, though not as effective as commercial oil emulsions like DHA Selco. The new "algal-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, eg. high concentrations of AA (Tom Lewis, pers. comm.). Some work has been documented on the transfer of AsA from microalgae through to zooplankton and fish larvae, but much less is known about other vitamins. Though microalgae have generally been proposed here as good sources of vitamins, they can vary significantly in their composition. Therefore zooplankton could be deficient in one or more vitamins when enriched using certain dietary regimens. Future research should focus 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 - will aid in optimising the usage of microalgae in larval culture. A broader range of microalgae species, especially mixtures and including species rich in DHA, should be assessed in green water systems.

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