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Review

Microalgae in Eastern Pacific White Shrimp, *Litopenaeus vannamei* (Boone 1931) hatcheries: A Review on Roles and Culture Environments

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Abstract

Demand for shrimp, particularly the eastern Pacific white shrimp *Litopenaeus vannamei* (Boone 1931), will continue to increase in Asian and worldwide seafood markets. Providing shrimp farms with a robust, healthy, and continuous supply of shrimp seed is a challenge that must be addressed to meet the demand. Shrimp feed during hatchery production still relies on live microalgae, despite many years of effort to find suitable full or partial-replacement diet alternatives. Successful mass production of microalgae for hatchery feed to obtain good quality shrimp seedstock depends on a number of environmental factors that determine the growth and nutritional values of various microalgal species. These factors include nutrients in the culture medium, light intensity, temperature, salinity, and pH. An overview of the use and the culture of microalgae in shrimp hatcheries is also presented and outlines the need for research for optimisation of algal diets for the rearing of *L. vannamei* seedstock in Asian hatcheries. Finally, the possibilities of using local isolates for hatchery operation are also highlighted.

Introduction

Despite their critical functions in the aquaculture production chain, microalgae are not documented exhaustively compared to many other cultured organisms. FAO (2012) reported that only small quantities of microalgae are cultured for direct human consumption; this production consists of two species, *Spirulina* sp. and *Haematococcus pluvalis* J. Von Flotow 1844. These two species are cultured mainly for extraction of C-phycocyanin and astaxanthin, respectively.

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Borowitzka (2013) reviewed various high-value products derived from microalgae, from ß-carotene and fatty acids to phycobilins and other unrevealed bioactive compounds, highlighting the potential importance of microalgal research in the future. In aquaculture, microalgal production is mainly conducted to provide live food for mollusc, shrimp, and fish culture. As the production of microalgae still is considered expensive (e.g. Walsh et al. 1987), several approaches have been attempted to replace microalgae in the hatchery with artificial diets. To date, however, microalgae still cannot be replaced fully with other types of feed, such as microbound diets and other enrichment diets such as Nutrokol, S. presso and Algamac (Gallardo et al. 2002; Sanchez et al. 2012; Ma and Qin 2012).

Research on microalgal culture since the early 1940's has led to wide use for feeding early life stages of cultured fish, shrimp, sea urchins (Conceciao et al. 2010; Carboni et al. 2012), and during the entire life cycle of cultured bivalves (Wikfors and Ohno 2001; Gonzalez-Araya et al. 2011). Microalgae are also used for growing and enriching zooplankton live feeds, such as *Artemia*, copepods, and rotifers (Chakraborty et al. 2010) that are fed subsequently to larval fish and shrimp. Microalgal use in the fish hatchery is known as the "green water technique" wherein microalgae are added directly to larval rearing tanks along with the zooplankton that serve as food for the larvae. Green water larviculture has been shown to increase larval quality of cultured fish species (Conceicao et al. 2010; Hemaiswarya 2011; Sanchez et al. 2012). Moreover, research on nutritional requirements of molluscs has led to improved algal culture procedures, particularly with the finding that f/2 culture medium is suitable for various species of microalgae, both in temperate and tropical regions (Wikfors and Ohno 2001). Work showing that cost reductions can be made in mass microalgal cultures by replacing f/2 medium with less expensive agricultural fertilisers (e.g. Valenzuela-Espinoza et. al. 1999; Simental-Trinidad et al. 2001) has expanded nutrient options for microalgal mass culture.

Studies on the biochemical composition of microalgae have enabled aquaculturists to select superior species of microalgae, such as *Isochrysis* spp.,(some now assigned to the new Genus *Tisochrysis* (Bendif et al. 2013), *Pavlova lutheri* (assigned to new name *Diacronema lutheri* (Droop) Bendif and Véron 2011) , *Navicula* spp., and *Chaetoceros calcitrans* (Paulsen) Takano, 1968 , as food for various cultured species, including oysters, scallops, fish, and shrimp larvae (Blackburn et al. 2000; Burke 2000). Research has shown that microalgae with high contents of metabolisable sterols and essential fatty acids are desirable for aquaculture live feeds, particularly for rapid growth of oysters (Wikfors et al. 1984, 1991, 1996). Furthermore, dietary cholesterol is an essential nutrient for crustaceans as they cannot synthesise this sterol *de novo*, thus it or a precursor phytosterol must be supplied in the diet (Teshima 1982). Required sterols may be available naturally in microalgae, as shrimp juveniles cultured with phytoplankton showed better growth and survival compared to shrimp grown in a non-phytoplankton culture method (Sanchez et al. 2012).

Shrimp aquaculture has increased rapidly within the last decades, and cultured shrimp continue to dominate international seafood markets. Aquaculture contributed 52% of the shrimp

supply in the world market in 2009, reaching 3.8 million tonnes by 2010 (FAO 2012). During 2005-2009, world shrimp aquaculture production increased up to 7% annually, but decreased 3% from 2010 to 2011, and then increased again in 2012 (FAO, 2012). It was projected that shrimp aquaculture will have increased up to 10.3% in 2013 (Valderrama and Anderson 2012).

The eastern Pacific white shrimp, *Litopenaeus vannamei* (Boone 1931) is an increasingly popular and important cultured shrimp species after the decline in giant black tiger prawn (*Penaeus monodon* Fabricius 1798) production caused by white spot syndrome disease outbreaks world-wide, most devastatingly in Southeast Asia. As a result, the white shrimp has been introduced to most Southeast Asian countries in the past few years. *Litopenaeus vannamei* has been cultivated widely in Asia, North America, South America and the Pacific Islands because of high survival, rapid growth in intensive culture systems, and disease tolerance (Briggs et al. 2004). The proportional share of *L. vannamei* in global shrimp production continues to increase. *Litopenaeus vannamei* contributed 7% to global production in 2001, with a net volume of 0.2 million tonnes, but in 2009, this contribution had increased to 58%, with a volume of 1.6 million tonnes. The peak production of white shrimp was in 2007 when this species accounted for 68% of global production. It was predicted that in 2013, this species will have contributed up to 69% of global shrimp aquaculture production (Valderrama and Anderson 2012).

As the demand for shrimp products from both local and international markets continues to increase, supply of shrimp larvae and post larvae to farmers is necessary to meet the growing demand. Hatchery seed stock, therefore, is a critical step in the production chain that needs to be addressed, in terms of technology and husbandry protocols. A survey conducted by the Global Aquaculture Alliance in 2011 revealed that seed stock quality and availability was still the main issue limiting production of high-quality shrimp in Asia (Valderrama and Anderson 2012). Accordingly, a research programme to explore the possibility of using local microalgae isolates that meet nutritional demands in shrimp hatcheries can contribute to ensuring that hatchery protocols are conducted appropriately to rear shrimp larvae and post larvae locally as robust, healthy, and cost-effective seed stock for commercial use. Local isolates of microalgae will reduce transportation cost and may help small-scale farmers to increase productivity and thus incomes. Local culture of microalgae will also ensure a continuous supply of live feeds to aquaculture hatcheries, particularly shrimp hatcheries. Finally, a small-scale and local installation of microalgae culture is a promising solution not only to overcome hatchery feed challenges, but will also provide employment that can drive economic growth.

In terms of sustainability, producing microalgae for aquaculture is considered to be more favourable compared to other fish feed production (Taelman et al. 2013). This study found that, based on life-cycle analysis of microalgae production for aquaculture, recycling of nutrients and energy savings were identified as important ways to increase the sustainability of algae production. Up-scaling, reactor design improvements, enhancement of photosynthetic yield, and a good choice of location also contribute to a low resource and carbon footprint. Considering that microalgae still

are an important part of shrimp hatcheries, this present review discusses the use of microalgae to feed early stages of white shrimp, as well as some environmental factors affecting the growth and nutritional content of microalgae necessary for shrimp growth and development. Therefore, the intent of this review is to provide an overview on the use of microalgae in shrimp hatcheries, particularly white shrimp and the possibilities of using local isolates for hatchery operations.

Shrimp nutrition: use of microalgae in early life stages of white shrimp

In natural environments, white shrimp larvae feed initially on phytoplankton and detritus, then on macrophytes, small molluscs, crustaceans, and zooplankton as they grow larger (Senanan et al. 2009). Microalgae are recognised widely to serve as nutritionally important live feeds for rearing shrimp in the hatchery. All nutritional components required for larval shrimp growth may be fulfilled with microalgae in the rearing tanks. Several research projects have shown that, in the culture environment, white shrimp larvae grew faster when co-cultured with phytoplankton (Gallardo et al. 2002; Sanchez et al. 2012; Ju et al. 2009; Nunes et al. 2011), underscoring the importance of microalgae in shrimp nutrition during hatchery rearing.

Approximately 16 genera of microalgae are used in aquaculture; strains used generally have a size range of 2-20 µm with most of the genera being planktonic (Gopakumar and Ignatius 2006). Several genera that are used commonly in shrimp aquaculture are summarised in Table 1. The listed genera of microalgae have been widely used because of good nutritional profiles and ease of culture (Hemaiswarya 2011). Diatoms, particularly *Thalassiosira* spp. and *Amphipora* spp., were found to be ingested and digested readily by white shrimp juveniles (Kent et al. 2011). A cyanobacterial species, *Spirulina platensis* (Gomont) Geitler 1925: 344 (currently accepted name is *Arthrospira platensis* (Nordstedt) Gomont 1892), also was shown to play an important role in reducing nitrogen levels in black tiger shrimp rearing tanks (Chuntapa et al. 2003).

Research on other potential microalgal isolates for use in white shrimp aquaculture hatcheries will continue into the future as we explore local natural resources. For instance, local microalgae isolates from southeast (SE)-Sulawesi, Indonesia are not utilised yet, despite their potential to support aquaculture industries in the region. These local microalgae may have comparable growth rates and nutritional values to the imported counterparts isolated elsewhere in the world. Currently, shrimp aquaculture in SE-Sulawesi relies upon microalgae starters supplied from the south Sulawesi and Java regions (Fig.1) that impose high operational costs to aquaculture farms and hatcheries in the region. The price of microalgae starter culture obtained from Java and south Sulawesi such as *Spirulina* sp., *Chorella,* and *Dunaliella,* is in the range of US$10-30·L^{-1}. That price can be reduced up to 50% if the starter culture were to be provided within SE-Sulawesi region. Importing microalgae from other regions also increases greenhouse gas (GHG) emissions associated with air and ground transportation (Edwards-Jones 2010) and exposes receiving areas to risks from non-native species invasions, including both the algae themselves and any contaminating microorganisms. To reduce both cost and non-native introduction issues, the use of locally-isolated,

cultured microalgae in local hatcheries may be considered. Locally-isolated microalgae with good growth and complete nutritional profiles will help small farmers to increase the productivity of their farms and thus their incomes, which is beneficial to drive economic growth.

Table 1. Genera of microalgae commonly used in shrimp hatchery (modified from Hemaiswarya et al. 2011).

Genus	Class	Utilisation
Isochrysis (Tisochrysis)	Prymnesiophyceae	Feeding of zooplankton such as *Artemia*, and used in some shrimp hatcheries
Tetraselmis	Prasinophyceae	Best for larval shrimp and contains natural amino acids that stimulate feeding in marine animals, complement *Nannochloropsis* for producing rotifers as well as for feeding *Artemia*
Thalassiosira weissflogii	Bacillariophyceae	Applied in the shrimp and shellfish larviculture, considered by several hatcheries to be the single best alga for larval shrimp, also used for feeding copepods and *Artemia*
Dunaliella	Chlorophyceae	Source of vitamin A and B12 in some shrimp hatcheries and also for shrimp coloration
Chaetoceros	Bacillariophyceae	Source of vitamin A and B1 in some shrimp hatcheries
Amphipora spp	Bacillariophyceae	Feeding the white shrimp juveniles
Spirulina platensis	Cyanobacteria	Reducing nitrogen levels in black tiger shrimp rearing tanks

We have isolated four strains of local microalgae from Kendari Bay, SE-Sulawesi. Those strains are noted as Kb1-2, Kb1-3, Kb1-5 and Kb2-6 identified as *Melosira moniliformis* (O.F.Müller) C.Agardh 1824 . Growth experiments on these strains have been conducted in the Coastal Institute, University of Rhode Island. We found that the growth and biomass of these isolates, particularly the Kb1-3 strain, is comparable to *Chaetoceros neogracile* S.L. Vanlandingingham 1968 and *Thalassiosira weissflogii* (Grunow) G. Fryxell& Hasle 1977 at high salinity (35 psu). We are now examining the nutritional content of these strains and identifying the strain using scanning electron microscopy before conducting feeding trials with white shrimp post larvae. These preliminary results suggested that local isolates may be potential for use in shrimp hatcheries, assuming the nutritional compositions of the algae are appropriate. Several studies have been conducted to analyse the nutritional composition of locally isolated microalgae that may be used for tropical aquaculture, e.g., Renaud et al. (1999), Nunez et al. (2002) and Martinez-Fernandez et al. (2006). Renaud et al. (1999) screened 18 strains of local microalgae from tropical waters of Australia and suggested that they may be used for mariculture. Similarly, a study by Nunez et al. (2002) tested the efficacy of employing local isolates of microalgae from north-eastern Venezuela as feed for white shrimp post larvae. They found that local isolates can be utilised by shrimp post larvae. Moreover, shrimp grew better and produced higher biomass under local conditions at large scale production, thereby reducing the production cost.

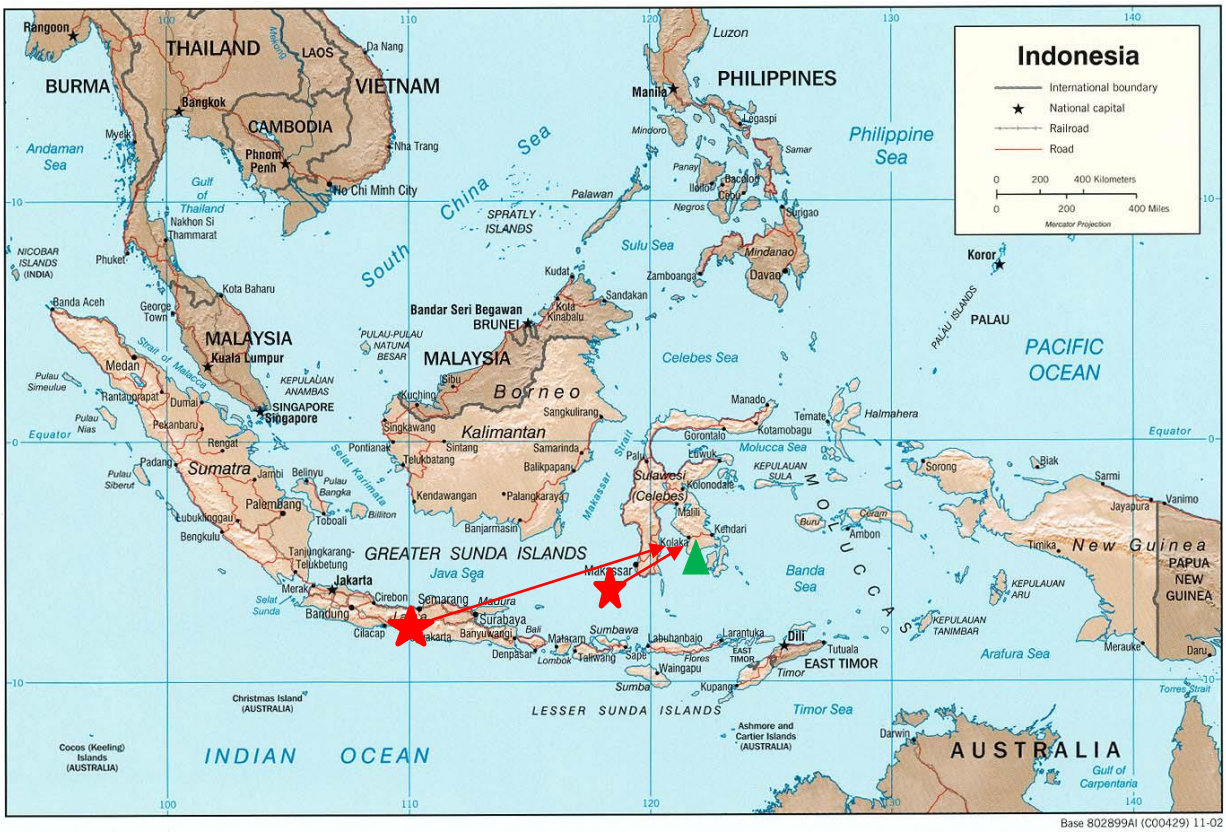

Fig.1. Source of microalgae starter culture (star shapes) for hatchery operation in SE Sulawesi (triangle shape). Image from map collections of the U.S. Central Intelligence Agency (https://www.cia.gov/library/publications/cia-maps-publications/Indonesia.html).

As is true for other cultured animals, shrimp require sufficient levels of proteins, lipids, carbohydrates, minerals, and vitamins for normal growth and development. Nutritional requirements of shrimp larvae and juveniles were reviewed by Kanazawa (1982). Diatoms and brine shrimp have been used commonly for shrimp larval rearing in the hatchery with good results. Shrimp such as *Penaeus japonicus* Spence Bate 1888 need proteins and essential amino acids, with an optimum dietary protein level of 52-57% (Kanazawa 1981). Protein in microalgae is considered to be of good quality, with amino acid profiles comparable to those of other reference food proteins (Gallardo et al. 2002). This may be the reason why shrimp larvae exhibited poor growth when cultured using only a micro-bound diet without microalgae, although Pedroza-Islas et al. (2004) found that a microencapsulated diet with a wall composition of a polysaccharide blend could be used to rear white shrimp mysids. Protein contents of microalgae vary between species and range from 12% to 35%, and this value is influenced by culture medium (Becker 2004). In general, microalgae can be considered to be a good candidate for shrimp aquaculture if they contain a protein higher than 25% of dry weight, 8-30% carbohydrate, and approximately 10% lipid , specially including certain types of lipids such as the fatty acids C20:5ω3 and C22:6 ω3 (Nunez et al. 2002). We summarised the nutritional requirement of shrimp and microalgal strains/species that produce these nutrients in Table 2 and 3.

Table 2. Nutrition requirements of shrimp larvae (modified from Millamena 1996).

Nutrition	Requirement (%)	References
Protein	50-57	Kanazawa 1981
Essential Amino acids		
Arg	4.5%	
Lys	5.3%	
Met + Cys	3.3% (Cys, 0.4)	
Th	3.5%	
Val	3.7%	
Lipid	12-15	
Essential Fatty acids	20:4n-6, 20:5n-3 22:6n-3 2.6% n-3 PUFA < 0.5% 18:2n-6	
Cholesterol	0.05-0.5 (white shrimp) 1 (tiger shrimp)	Castille et al. 2004
Carbohydrate	20	
Carotenoids	Desirable for coloration	
Vitamin C	50 mg as ascorbic acid	

Carbohydrates are utilised by shrimp larvae and juveniles in the form of disaccharides such as sucrose, maltose, and trehalose, and polysaccharides, such as dextrin and starch (Kanazawa 1981). A study with *P. japonicus* juveniles by Kanazawa (1981) showed that maltose was much more readily utilised as an energy source than other sugars. Unlike dietary glucose, that is not converted to trehalose but quickly absorbed from the stomach and released to the blood, maltose is not absorbed from the stomach. Instead, this disaccharide is converted to glucose in the mid gut, then to trehalose in the hepatopancreas, and is finally released gradually into the blood. High variation is found in carbohydrate contents of microalgae, which ranged from 4.6% to 23% (Wikfors and Ohno 2001). This variation is determined by culture procedures employed (Ferreira et al. 2009). Carbohydrate content in *Nannochloropsis gaditana* L.M. Lubián 1982 decreased from 28.47% to 21.01% when this species was grown in semi-continuous culture with daily renewal rates of 10% or 50%, respectively.

Shrimp have been shown to have special needs in lipid metabolism. Fatty acids (FAs) are organic molecules typically found bound to other compounds such as glycerol, sugars, or phosphate head groups to form lipids. Lipids are integral components of cell structures, e.g. membranes, which are made up of phospholipids, and energy stores that often are composed of triglycerides. Through enzyme reactions, FAs can be released from lipids to become free fatty acids (FFAs). The biological activities of FFAs have roles in host defence against potential pathogenic or opportunistic microorganisms, e.g., growth inhibition or the direct killing of bacteria (Desbois and Smith 2010). Shrimp fed on mixtures of formulated and natural feed may have better immunological and nutritional condition when compared to those fed on formulated feed alone because of the greater amount of essential amino acids, fatty acids (Poly Unsaturated Fatty Acids/PUFA and Highly Unsaturated Fatty Acids/HUFA), and other nutritional factors supplied by the live feed component.

Table 3. Known nutrient profiles of some microalgae strain.

Microalgae strains	Nutrition supplied	Level (%)	References
Chaetoceros muelleri	Protein	45.56[a]	Martinez-Fernandez et al. 2006
	Lipid	12.14	
	Carbohydrate	3.74	
	Vitamin C	16	Brown et al. 1997
Chaetoceros sp.	Lipid	17	Renaud et al. 1999
	Protein	36.7	
	Lipid	27.9	
Skeletonema costatum	Protein	13.5	
	Vitamin A	27.07[b]	
Tetraselmis suecica	Vitamin C	17.45	Fabregas and Herrero 1990
	Carbohydrate	5.23[a]	
Pavlova salina	Lipid	30.55	Martinez-Fernandez et al. 2006
	Protein	52.91	
	Vitamin A	5.13[b]	
Chlorella stigmatophora	Vitamin E	69.44	Fabregas and Herrero 1990
	Biotin	0.11	
Thalassiosira pseudonana *T. weissflogii* *Nitzschia breoirostris*	Phytosterol (24-methylenecholesterol and 24-methyl cholesterol)	63-97[c]	Gladu et al. 1991
	Carbohydrate	21.01-28.47[d]	
	Lipid	18.60-35.34	
Nannochloropsis gaditana	Protein	36.19-60.39	Ferreira et al. 2009
	PUFA	12.71-40[e]	
	EPA	6.76-27	
	ARA	3.56-6.90	
Dunaliella salina	β- carotene	65.66-89.77[f]	Abu-Rezq et al. 2010
Isochrysis sp. (T.Iso)	Lipid	29.99a	Martinez-Fernandez et al. 2006
	Carbohydrate	3.33	
	Protein	47.77	
Isochrysis galbana	Vitamin A	19.57b	Fabregas and Herrero 1990
	Vitamin C	30.44	

a= percentage of dry weight, b= percentage of total vitamin, c=percentage of total sterols, d=percentage of total organic content (sum of carbohydrate, lipid and protein), e=percentage of total fatty acids, f=percentage of total carotenoids

Penaeid larvae have an absolute requirement for long-chain, polyunsaturated fatty acids, especially for C20 and C22: ω-3 and ω-6 compounds. In particular, shrimp growth seems to be promoted by docosahexaenoic acid (DHA) 22:6ω-3 and enhanced further by eicosapentaenoic acid (EPA) 20:5ω-3 (Castell 1982). Growth rates of protozoeal stages of *Penaeus semisulcatus* De Haan 1844, *P. monodon*, and *P. japonicus* can be enhanced by dietary supplementation with 20:4ω-6 arachidonic acid (ARA) (D'Souza and Loneragan, 1999). Deficiencies in the ω-3 HUFA contents of microalgae may cause mortalities and/or quality problems in shrimp. Measurable concentrations of EPA generally are present in diatom species (*C. calcitrans*, *C. neogracile*, *Skeletonema costatum* (Greville) Cleve 1873 and *Thalassiosira pseudonana* Hasle & Heimdal 1970), as well as in *Nannochloropsis* sp., *Tetraselmis suecica* (Kylin) Butcher 1959, *Tetraselmis chui* Butcher 1959, and

"*Chlorella minutissima* Fott & Nováková 1969*"* (in reality, a eustigmatophyte) (Brown 1997; Becker 2004).

Cholesterol is an essential nutrient that must be supplied in the diet of shrimp, directly or as a precursor, because crustaceans cannot synthesise this compound *de novo* (Teshima 1982). Cholesterol is the main sterol found in crustaceans and is required for normal development, survival, and growth. Cholesterol also is a precursor for sex hormones, molting hormones, and epidermis constituents in crustaceans (Lim 1998). Sterol content has been investigated by Gladu et al. (1991) for diatom species. These researchers found that five of seven diatom species (*T. pseudonana*, *T. weisflogii*, *Detonula confervacea* (Cleve) Gran 1900, *Cyclotella cryptica* Reimann, Lewin & Guillard and *Nitzschia brevirostris* Hustedt examined in the study contained 24-methylenecholesterol and 24-methyl cholesterol, which together made up 63-97% of the total sterols of these species. Moreover, stigmasterol was the principal sterol in *Amphora coffaeformis* (C. Agardh) Kűtzing 1844 (currently accepted name is *Halamphora coffeaeformis* (Agardh) Levkov 2009), and 24-ethylcholesterol was the major sterol in *Navicula pelliculosa* Hilse 1863: 68 (currently accepted name is *Fistulifera pelliculosa* (Brébisson) Lange-Bertalot 1997). Although cholesterol is not the predominant phytosterol, some phytosterols can partially substitute for cholesterol in the shrimp diet (Teshima 1981; Lim 1998).

Crustaceans, such as shrimp, absorb minerals from water but also need minerals and vitamins included in their diets. Kanazawa (1981) stated that mineral dietary supplement in shrimp is required as these compounds are lost in molted exoskeletons.Vitamin content is a major factor determining the nutritional value of microalgae. The microalgae showing relatively high concentrations of different vitamins were: *T. suecica* for thiamin, pyridoxin, nicotinic acid, pantothenic acid and ascorbic acid and *Chlorella stigmatophora* Butcher for vitamin A, tocopherol (vitamin E) and biotin (Fabregas and Herrero 1990). Some strains of *Dunaliella* such as *Dunaliella tertiolecta* Butcher, 1959 and *Dunaliella salina* (Dunal) Teodoresco, 1905 have been known to be a good source of ß-carotene, riboflavin, cobalamin and folic acid and thus been exploited since 1980s for human consumption and aquaculture (Fabregas and Herrero 1990; Raja et al. 2007; Abu Rezq et al. 2010; Borowitzka 2013). *Tisochrysis iso* (revised to *Tisochrysis lutea* El M.Bendif and I. Probert as in Bendif et al. 2013*)* and *Isochrysis* spp., also is a good source of fat-soluble vitamins (vitamin A and vitamin E, biotin, ascorbic acid, and B-group vitamins (folic acid, nicotinic acid, pantothenic acid, biotin, thiamin, riboflavin, pyridoxine and cobalamin) (Coutinho et al. 2006).

Microalgal isolation and production techniques

Isolation and culture systems

Although several isolated microalgae species are readily available for aquaculture from research centres or large hatcheries, locally-isolated species may be preferred for local use to reduce transportation cost and assure constant availability. Successful isolation of specific microalgae from

the local environment is determined by some important factors, including: environmental conditions (water quality of natural samples are thought to be controlled by habitat conditions and sampling time), taxonomic knowledge (to determine nutrients for culture of isolated species) and elimination of contaminants. Andersen and Kawachi (2011) suggested using caution and multiple methods to isolate unknown species. Microalgal isolation methods commonly used are: enrichment culture, single cell isolation by micropipette, agar isolation, dilution, gravity separation, and phototaxis techniques.

Enrichment culture is the most common culture and isolation method for microalgae; microalgae are induced to "bloom" by fertilisation (addition of nutrients to the water sample). Adding nutrients to the natural water samples is important to overcome possible nutrient deficiencies. Enrichment with nutrients is necessary, as the natural samples often are deficient in one or more nutrients. Microalgae survive in nature because of recycling processes making nutrients continually available in small quantities. Nutrients required for microalgal growth include the macronutrients nitrate or ammonia and phosphate (in an approximate ratio of 16:1), silicate (if growing diatoms), and micronutrients, including various trace metals and the vitamins thiamin (B_1), cyanocobalamin (B_{12}) and sometimes biotin. These nutrients usually are mixed together in specific proportions to create microalgal culture medium (including formulations sold commercially). Walne medium and f/2 medium are the two most extensively used enrichment media and are suitable for the growth of most algae. There are also commercially-available nutrient solutions that are suitable for mass production of microalgae in large-scale, extensive systems. These solutions contain only the most important nutrients and are made of agriculture-grade fertilisers rather than laboratory-grade reagents. These culture media, sometimes together with aqueous soil extract, are the most common enrichment substances for growing microalgae (Andersen and Kawachi, 2011). Generally the amount of nutrients to be added to seawater must be moderate to avoid detrimental effects.

Hemaiswarya (2011) summarised some factors to be considered for production of microalgae, including: the biology of the alga; cost of land, labour, energy, water, and nutrients (climate if the culture is outdoors); and the type of final product needed. Microalgal production remains the main bottleneck to assuring the continuous supply of live feed to shrimp hatcheries. According to Enzing et al. (2014), several factors need to be addressed within 4 to 5 years to achieve the stability of microalgal feed production: 1) lowering the production costs of feed, 2) improvement of production technologies for the safety of food products, 3) stability/reliability of large cultures, 4) suitable strains, and 5) avoiding contamination and subsequent failure of the cultures. Using local isolates for shrimp aquaculture, as suggested earlier in this review, could become a solution to reduce the production cost and prevent contamination of foreign strains, bacteria, and parasites.

Culture of microalgae for aquaculture purposes (rearing of molluscs, shrimp, and fish larvae) takes place mostly on-site, i.e., in the farms where they are utilised, although a new industry is emerging for the production of microalgae and delivery in lyophilised, frozen, or other forms to the

farms (Navarro and Sarasquete 1998; Muller-Fuega 2004). Once in farms, stock cultures are kept under controlled conditions and protected from contamination by other microalgae, ciliates, and potentially harmful bacteria. Bags and cylindrical tanks are quite common approaches for the mass-production of microalgae on farms. In many parts of the world, however, particularly in Southeast Asia, facilities at the farm level are lacking and insufficient. Purchasing healthy seed from a local hatchery that utilises local microalgae would be one solution to reduce the production cost of farmers in developing countries.

Various techniques have been developed to grow microalgae on a large scale, ranging from less-controlled and extensive to mono-specific, intensive cultures. The controlled production of microalgae, however, still is a complex and expensive procedure. Semi-continuous and continuous cultures are commonly used in microalgal production in the hatchery. Semi-continuous culture, in which a percentage of the volume of the culture is harvested periodically and replaced with the same amount of fresh culture medium, is considerably easier and cheaper to establish. Semi-continuous culture management also produces microalgal biomass with high nutritional values (Ferreira et al. 2011).

Based on the intensity of the culture and average cell density from low to high, microalgae culture technology is divided into several types of containers: earth ponds, raceways, plastic polyethylene bags (100-500 L), open cylindrical tanks constructed of polymer fibre glass, and tubular and flat-plate photo-bioreactors. Earth ponds and raceways are more open systems, inexpensive, exposed to weather conditions, and more prone to contamination. In addition, the cell density remains at relatively low levels in comparison with the other culture systems. On the other hand, closed systems are much more expensive to operate, although they can produce high yields of microalgal biomass. Taelman et al. (2013) suggested that the closed photobioreactor for aquaculture purposes will be more profitable and produce higher biomass of microalgae if the design is improved, such as those of Provi APT (Proviron Advanced Photobioreactor Technology) (Fig. 2).

This system basically contains a plastic bag (12 m^2) filled with water and placed on the ground. Each bag contains 35 embedded plastic panels in which the algae grow, yielding a reactive surface of 7 m^2. The Provi APT system is inexpensive to build as it consists entirely of plastic, and automated production can be employed. This design of photobioreactor has been used in trials to culture *Nannochloropsis* sp., which produced approximately 17 tonnes of dried algal biomass per hectare annually (Taelman et al. 2013). This kind of system can be developed on a smaller scale at hatchery level to protect the culture from contamination, to maintain the nutritional value, and also to ensure continuous supply.

Culture conditions and nutritional values

Culturing microalgae under optimal conditions is advantageous to achieve high nutritional values that are beneficial for shrimp larvae. Among other things, light intensity, day length, and

Fig. 2. Typical Provi APT system for efficient microalgae culture (Michiels 2009).

nutrient concentrations are important factors regulating microalgae growth in culture and also affect the nutritional content (Renaud et al. 1991; Lewis et al. 2002; Meseck et al. 2005; Sheng et al. 2011; Huang et al. 2013). Several researchers also have shown that salinity, pH, and temperature are important factors determining the growth of microalgae and controlling the nutritional quality (Goldman et al. 1982; Schmidt and Hansen 2001; Lewis et al. 2002; Renaud et al. 2002; Khatoon et al. 2010; Gu et al. 2012). Table 3 summarises the optimal culture condition for microalgae species commonly used in shrimp aquaculture.

Microalgae most fundamentally need light (intensity and period) for photosynthesis. They need light for a photochemical phase of the photosynthetic process to produce Adenosine triphosphate (ATP), Nicotinamide adenine dinucleotide phosphate-oxidase (NADPH), and use dark periods for biochemical phases synthesising essential molecules for growth (Cheirsilp and Torpee 2012). In the natural environment, microalgae utilise readily available sunlight easily. In controlled, indoor culture conditions, it is difficult to rely upon natural sunlight alone because of seasonal variability. The most common tool to provide illumination in indoor conditions is the fluorescent lamp. Optimal photoperiod to grow microalgae depends upon the place and season. The 12:12 h (light: dark) period may be appropriate to employ in the tropics, but in temperate regions, a 16:8 h photoperiod may result in better microalgal growth. A study by Harrison et al. (1990) found that light intensity at 300 µmol photonnes $m^{-2}s^{-1}$ PAR was optimal for growth of *Isochrysis galbana* Parke 1949 , *T. pseudonana* and *C. calcitrans*. Moreover, the study found that no consistent trend was observed in lipid content of those three microalgae under light limitation (less than 300 µmol photonnes $m^{-2}s^{-1}$ PAR), and protein content was relatively constant for *I. galbana* and *T. pseudonana*, but decreased in *C. calcitrans* with lower illumination. However, a study by Renaud et al. (1991) found high light intensity in culturing *Isochrysis* sp and *Nannochloropsis oculata* (Droop) D.J.Hibberd 1981 decreased the ratio of total unsaturated fatty acids to total saturated fatty acids. A

recent study by Khoeyi et al. (2012) noted that the maximum biomass of *Chlorella vulgaris* Beyerinck (Beijerinck) 1890 was recorded between 0.1 g and 2.05 $g \cdot L^{-1}$ when the culture was exposed to 62.5 µmol photonnes $m^{-2}s^{-1}$ PAR for a 16:8 h light/dark photoperiod; whereas, the maximum percentage of total saturated fatty acids (SFA) was 33.38% at 100 µmol photonnes $m^{-2}s^{-1}$ PAR on a 16:8 h light/dark photoperiod. Other microalgae that are not popular for aquaculture, such as *Lobosphaera incisa* (Reisigl) Karsten, Friedl, Schumannn, Hoyer & Lembcke 2005, showed that at high light intensity (400 µmol photonnes $m^{-2}s^{-1}$ PAR) and complete nutrient culture medium, the cells exhibited higher growth rates and high contents of total fatty acid and arachinoid acid compared to medium (200 µmol photonnes $m^{-2}s^{-1}$ PAR) and low (35 µmol photonnes $m^{-2}s^{-1}$ PAR) light intensity (Solovchenko et al. 2008). For long-term culturing of most microalgal species, light intensity at 10-30 µmol photonnes $m^{-2}s^{-1}$ PAR and low temperature (15°C) have proven to be reliable to keep cultures alive with minimal subculturing (Lorenz et al. 2011).

Table 4. Optimum culture environment of some microalgae species commonly used in shrimp hatchery.

Genus/Species	Optimum Culture Condition						Nutrient Yield (%)	References
	P (Light: Dark)	L(µE photonnes $m^{-2}s^{-1}$)	pH	T (^{0}C)	Salinity (psu)	Medium		
Chlorella vulgaris	16:8	62.5		25-30		f/2	33.38 SFA	Khoeyi et al. 2012
Thalassirosira weissflogii	14:10	~ 100		18	31±1	f/2 f/10	β-carotene	Sheng et al. 2011
Isochrysis galbana		300	8.0-8.3	21±1.5		Enriched seawater	16 DHA 22.62 L	Harrison et al. 1990
Thalassiosira pseudonana		300		18	6	f/2	27.2L	
Isochrysis sp.		300		18	6	f/2	12.07 L	Schwenk et al. 2013
Isochrysis galbana		300		18	35	f/2		
Chaetoceros sp.	12:12	80±2	8.3±0.1	27-30	25±1	f/2	16.8 L	Renaud et al. 2002
Marine diatoms (*Amphora sp.* and *Navicula sp.*)	12:12	31.9	8.5	28	15-25	Conway	40 P 30 L 20 C	Khatoon et al. 2010
Tetraselmis subcordiformis		100		20±1	20	f/2	29.77 L	Huang et al. 2013*
Nannochloropsis oculata		150		25±1	20	f/2	35.85 L	
Pavlova viridis		100		20±1	20	f/2	32.10 L	

SFA = saturated fatty acids, P= Protein, L= lipid and C= Carbohydrate * = nutrient yield is lipid, protein, carbohydrate, DHA, SFA or b-carotene content when nitrogen supplementation at 0.22 mmol $N.L^{-1}$

Nutrient enrichment also controls the growth, protein, carbohydrate, lipid, and fatty acid composition of microalgae. Sheng et al. (2011) showed that, in a P-depleted treatment (f/2-P), the cell size of *T. weissflogii* was 1.48 times larger than that in the full-nutrient-limited (f/100) treatment

and 2.67 times larger than that in P-saturated treatments (f/2 and f/10). Silica is necessary for optimal growth of diatoms and directly affects nutritional contents (Kröger et al. 1999; Ponomorenko et al. 2004; Sumper and Bunner 2006; Schwenk et al. 2013). Although Harrison et al. (1990) found that carbohydrate, lipid, and protein contents of *I. galbana*, *T. pseudonana* and *C. calcitrans* were not changed under 6 h silica (Si) starvation, providing silica in the culture medium is necessary for rapid growth of any diatom (Sumper and Bunner 2006; Schwenk et al. 2013). Moreover, the study by Harrison et al. (1990) also found that, under 2 days of nitrogen (N) starvation, does not change the cellular lipid percentage, but the protein percentage decreased, and the carbohydrate percentage increased. Solovchenko et al. (2008) showed that *L. insica* cultured in nitrogen depleted medium contained increased arachidonic acid as a percentage of total fatty acids. Moreover, Huang et al. (2013) showed that the total lipid content, as well as the proportions of neutral lipid, in *Tetraselmis subcordiformis* (Wille) Butcher 1959 SHOU-S05, *Nannochloropsis oculata* (Droop) D.J.Hibberd 1981 SHOU-S14 and *Pavlova viridis* C.K. Tseng, J. Chen & X. Zhang 1991 (currently accepted name is *Diacronema viridis* (C.K.Tseng, Chen and Zhang) Bendif and Véron 2011) SHOU-S16, decreased significantly with increasing nitrogen supplementation. Eicosapentaenoic acid (EPA (20:5ω3)) and C16 fatty acids were significantly higher in *N. oculata* and *D. viridis*, respectively with increased nitrogen enrichment. This study also showed that *N.oculata* and *D. viridis* accumulated more 16-carbon fatty acids and fewer polyunsaturated fatty acids in nitrogen-limited media. Schwenk et al. (2013) revealed that lipid content of several species of microalgae, including *I. galbana*, *Chaetoceros muelleri* Lemmermann 1898 and *T. pseudonana*, increased during early stationary phase as the nitrogen limited environment shifted the energy supporting cell division to fatty acid biosynthesis and lipid accumulation. These findings imply that knowing growth phase (stationary or log phase) when harvesting microalgae is necessary to select the desired nutritional content of log-phase cells or stationary-phase cells that are either nutrient or light limited.

Salinity is another primary factor influencing the growth and biochemical composition of microalgae. For the purpose of this review, the standard unit for salinity is set to psu (Practical Salinity Unit), although literature has reported salinity in different units such as $g.L^{-1}$ and mM. The gross chemical and fatty acid compositions of *Isochrysis* sp., *N. oculata*, and *Nitzschia* sp. were significantly different at different salinities (Renaud et al. 1992). Khatoon et al. (2010) reported experiments with marine diatoms (*Amphora* sp., *Navicula* sp. and *Cymbella* sp.) and a cyanobacterium (*Oscillatoria* sp.) at different salinities. Diatom growth was significantly higher at 35 psu than at lower salinity, but cyanobacterial growth was better at 25 psu. As for protein and lipid, significantly higher quantities were found in diatoms cultured at low salinities (15-25 psu), and an increase in carbohydrate was seen at high salinities (30-35 psu). Conversely, the cyanobacterium showed a significantly higher lipid content at 30-35 psu compared with other salinity levels, but no significant changes were observed in the protein and carbohydrate contents at different salinity levels. de Castro Araujo and Tavano Garcia (2005) showed that, at high salinity (35 psu), carbohydrates were enhanced, but lipids and protein decreased in *Chaetoceros* cf.

wighamii Brightwell 1856 . Rao et al. (2007) found that growth of *Botryococcus braunii* Kűtzing 1849: 892 (race 'A') and its biochemical composition, i.e. hydrocarbon, carbohydrate, fatty acid, and carotenoids, were influenced by salinity.

Under salinity of 34 mM (2 psu) and 85 mM (5 psu), a 1.7–2.25-fold increase in the relative proportion of palmitic acid, and a two-fold increase in oleic acid, were observed. A two-fold increase in carotenoid content was detected at 85 mM (5 psu) salinity with lutein (75% of total carotenoid) as the major carotenoid followed by ß-carotene.

Another important factor affecting the growth and biochemical composition of microalgae is temperature. Temperature strongly influences cellular chemical composition, the uptake of nutrients, carbon dioxide fixation, and the growth rates for every species of algae. It is known that the growth rate will increase with temperature up to a maximal level. Above this temperature, growth rate decreases drastically (Raven and Geiden 1988). Temperature tolerance in microalgae is species-specific. Renaud et al. (2002) reported that the optimum temperature for growth of *Rhodomonas* sp. was 25–27 °C, but was 27–30 °C for a prymnesiophyte NT19. Furthermore, *Cryptomonas* sp., *Chaetoceros* sp. and *Isochrysis* sp. grew well at 33 and 35 °C. All species had significantly lower percentages of protein when grown at temperatures above 27 °C, but there was no consistent trend in the percentages of carbohydrate. *Chaetoceros* sp. had the highest percentage of lipid when cells were cultured at 25 °C, but *Rhodomonas* sp., *Cryptomonas* sp., NT19, and *Isochrysis s*p., had significantly higher amounts of lipid at temperatures within the range 27-30 °C. The highly unsaturated fatty acid (HUFA), eicosapentaenoic acid, 20:5ω3, was present in all species, with highest amounts in the prymnesiophyte NT19 (19.9% total fatty acids). Percentages of 20:5ω3 were slightly lower at highest growth temperatures for all species. All species had lower percentages of 22:6ω3 at higher growth temperatures. *Chaetoceros* sp. and NT19 had moderate amounts of arachidonic acid, 20:4ω6 in the fatty acid profile (2.7-5.4% of total fatty acids). Highest arachidonic acid percentages were associated with growth temperatures within the range 27-30 °C. Only *Chaetoceros* sp. grew well at 35 °C, maintaining moderate percentages of protein, carbohydrate, lipid, PUFA, and HUFA (9.6% total fatty acids).

The optimum temperature for *C. vulgaris* ranges from 25 to 30 °C. (Sanchez et al. 2008). Several researchers have shown that biomass content, lipid, and fatty acid decreased when the culture conditions were changed from the optimal temperature range. Chinnasamy et al. (2009) reported an increase in biomass content and in chlorophyll content of *Chlorella* sp. at the optimum temperature (30 °C). Converti et al. (2009) reported that lipid content of this species increased from 5.9 to 14.7% when the temperature decreased from 30 °C to 25 °C; at temperatures above 38 °C, oleic acid, a monounsaturated omega-9 fatty acid, production increased.

The optimum temperature to grow *Scenedesmus* sp. is between 20-40 °C (Sanchez et al. 2008). Previously, Christov et al. (2001) studied *Scenedesmus* sp. at temperatures of 15-36 °C and found that, at lower temperatures the chlorophyll and protein levels were reduced, but levels of

carotenoids, saccharides, and lipid were increased. These authors also observed an increase of 30% in sugars and lipids at extremely high temperature (36 °C).

Conclusions

As the demand for white shrimp products in world markets continues to increase, the continuous supply of healthy, inexpensive, and robust shrimp seed stocks to the farmers is important to maintain production of adult shrimp. Microalgae remain an important component of the aquaculture production chain, particularly for hatchery white shrimp feed, despite expensive culture installation and high production cost. Several studies and experiments have been conducted to replace the use of microalgae in white shrimp hatcheries, but to date research has showed that microalgae cannot be replaced completely. Partial substitution with other, low-price enrichment compounds and micro-bound diets is possible, but microalgae must be provided in the shrimp rearing tanks. Semi-continuous culture of microalgae, in which a portion of the biomass is harvested periodically, and nutrient medium is replaced at a constant level, is still the most-efficient procedure to be employed in shrimp hatcheries. Inexpensive outdoor culture installations may also be sufficient to supply microalgae to the hatcheries. In addition, smaller scale, inexpensive design photobioreactors may be installed at the hatchery level to minimize culture contamination, to maintain nutritional profiles, and to ensure continuous supply.

Adequate nutrients, light (intensity and period), salinity, and temperature are important factors in determining maximal growth and high nutritional value of microalgae. Readily available f/2 and Walne medium are the most common culture enrichments with sufficient nutrients to support growth of most algal species. The photoperiod of 12:12 h (light:dark) is suitable for culturing microalgae in the tropics, but in temperate areas a photoperiod of 16:8 generally is more favourable for growth and nutritional content of microalgae. Salinity and temperature optimal levels for culturing microalgae are species specific. Knowledge about log and stationary phase during the culture period is important to ensure that microalgae are harvested when they have the desired nutritional contents.

Although there are approximately 5 genera of microalgae known to serve as good quality feed sources in white shrimp hatcheries, more research on other potential microalgal strains is recommended. This is mainly driven by the concern over sustainability and productivity of local farms. Using local microalgal isolates for white shrimp production will reduce the production cost to farmers, reduce the potential negative impact of shrimp farming on the environment, and increase the dependability and productivity of the farms to the benefit of local economies.

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