Food and Agriculture Organization of the United Nations

COMMISSION ON GENETIC RESOURCES FOR FOOD AND AGRICULTURE

DRAFT

Genetic resources for microorganisms of current and potential use in aquaculture

This document is based on a commissioned study prepared by Russell T. Hill in support of the State of the World on Aquatic Genetic Resources for Food and Agriculture to facilitate the Commission's deliberations when it will review the agenda item on Aquatic Genetic Resources at its Sixteenth Regular Session.

The content of this document is entirely the responsibility of the author, and does not necessarily represent the views of the FAO or its Members.

This draft is being made available as an *Advance Copy* for comments and feedback.

January 2017

Table of Contents

Acronyms and abbreviations

AFLP	Amplified Fragment Length Polymorphism
DHA	docosahexaenoic acid
EPA	Eicosapentaenoic acid
ICN2	Second International Conference on Nutrition
LED	light-emitting diode
RAPD	Random Amplified Polymorphic DNA
RNAi	RNA interference

Abstract

Aquaculture is the farming of aquatic organisms ranging from microbes to shellfish and finfish. World food fish aquaculture production more than doubled from 2000 to 2012 and contributed 42% of total fish production in 2012. Aquatic microorganisms are indispensable resources for growth of shellfish and finfish in natural aquatic ecosystems and in aquaculture. This thematic background study provides information on the genetic resources of key microorganisms on which aquaculture depends. These microorganisms fall into the microbial groups of (1) microalgae and fungal-like organisms, (2) bacteria, including cyanobacteria and (3) zooplankton. Many microalgal species are important in aquaculture, with different species being suitable as feed for shellfish and finfish larviculture, as components of "green water" widely used to enhance survival and growth of larval and adult fish, and as feeds to enhance the nutritional quality of *Artemia* and rotifers. Microalgae are also grown in aquaculture to produce pigments and fatty acids of importance in fish aquaculture and as human nutraceuticals. Bacteria that are used in aquaculture include cyanobacteria such as *Spirulina* used for human diet supplements and a rapidly-growing suite of probiotic bacteria. These probiotic bacteria include species that improve survival and growth of fish and shellfish larval and adult stages. Probiotic bacteria are expected to become increasingly important for disease prevention in aquaculture as antibiotic use is further curtailed and species are grown in more intensive aquaculture systems. Bacteria also play an important role in filtration systems needed in recirculating aquaculture systems. Zooplankton, specifically *Artemia* and rotifers, have a long history and very wide application as feed for the aquaculture industry. Several species of *Artemia* are used, with *Artemia franciscana* being the most important. Of more thanthan 2 000 species of rotifers, *Brachionus plicatilis* and *Brachionus rotundiformis* are most commonly used. Other zooplankton used in aquaculture include copepods that are growing in importance and cladocerans such as *Daphnia* that are widely used in freshwater larviculture.

The future success and growth of aquaculture depends on continued availability and more efficient culture of these important microbes, as well as conservation and expansion of the biological diversity and genetic resources of microbes used in aquaculture. Important issues include the ability to achieve long-term storage of important organisms without them being subject to genetic drift, the role of commercial and public culture collections, and the need for increased use of genomics to characterize all key microbial species used in aquaculture.

1 Introduction

Aquaculture is the farming of aquatic organisms ranging from microbes to shellfish and finfish. Fisheries production from the capture of wild fish has remained fairly constant since the late 1980s and it is the increase in production from aquaculture that has led to substantial growth in fish production for human consumption, with aquaculture contributing more than wild-caught fisheries for the first time in 2014 (FAO, 2016) and this trend is likely to continue. The world aquaculture production of fish was 44.1% of total global fish production, including production for non-food uses, in 2014. The share of fish produced by aquaculture for human consumption increased from 26% in 1994 to about 50% in 2014, with 73.8 million tonnes of fish valued at US$160 billion being harvested from aquaculture in 2014 (FAO, 2016). In facing the challenge of providing food to a growing human population predicted to reach 9.7 billion by 2050, fish consumption, especially fish produced from aquaculture has an important role to play. The Second International Conference on Nutrition (ICN2) held in 2014 adopted the Rome Declaration on Nutrition that highlighted the key role of fish in meeting the nutritional needs of this growing population (FAO, 2016). The world per capita fish consumption has increased from under 10 kg in the 1960s to approach 20 kg in 2014 and 2015 and now provides over 3.1 billion people with approaching 20% of their animal protein intake, enhancing people's diets around the world (FAO, 2016).

Microbes play a critically important role in the cycling of nutrients in terrestrial and aquatic ecosystems globally. Marine microbes are responsible for approximately half of the global primary production and play a huge role in the cycling of carbon, nitrogen, phosphorus and other nutrients (Arrigo, 2005). Microbes have a central role in sustaining life on earth and lie at the center of issues such as sustainability and climate change (Kowalchuk et al., 2008). Microbes also have a direct, central and critically important role in fisheries and aquaculture. Microbes in natural marine and freshwater ecosystems are key components of food webs, primary and secondary production and nutrient cycling. A wide range of microbes are used directly in aquaculture as live feeds, probiotics, and in filtration systems. Aquatic microorganisms are therefore indispensable resources for growth of shellfish and finfish in natural aquatic ecosystems and in aquaculture.

The provision of suitable feeds in aquaculture has been identified as an important constraint in the growth of aquaculture (FAO, 2016). Fishmeal and fish oil are important ingredients in many farmed fish feeds but there has been a reduction in the amount of fishmeal and fish oil included in feeds as the cost of these ingredients has risen. Microbes such as rotifers, *Artemia* and microalgae can be important substituents for fishmeal and fish oil. Suitable aquaculture feeds are particularly important in developing countries where there is a need to ensure that fish farmers have economic and balanced feeds to meet the nutritional needs of various life stages of their production species. Improvements in feed quality and availability could increase production and reduce costs (Hasan and New, 2013). In many cases, in particular for early life stages of fish and shellfish, these feeds include microbes grown specifically to fulfil the nutritional needs of the species being produced in aquaculture. Microbes are also important in "non-fed" aquaculture species that include microalgae being grown as aquaculture crops and also fish species that are filter-feeders consuming microbes present naturally in the production systems. It has been estimated that 30.8% of world fish production by aquaculture comprises non-fed species, including notably carp, and bivalve molluscs such as clams, oysters and mussels (FAO, 2016). This thematic background study on the genetic resources of key microorganisms on which aquaculture depends focuses on the microbes that are grown to be fed to species in aquaculture. However, the microbiology of "non-fed" aquaculture species is also worth considering. The rapid advances in the technologies used to study microbial ecology mean that it is now possible to rapidly determine the community composition of complex natural microbial communities such as those being grazed on by species growing in "non-fed" aquaculture by genomic approaches and to monitor changes in the diversity of those communities.

Application of these genomic monitoring approaches may lead to the ability to alter environmental parameters in growth systems to enhance growth of microbes that are best suited to support the nutritional needs of the production species.

The importance of aquatic microbial genetic resources in current aquaculture production and in the future of aquaculture is often underappreciated. This thematic background study provides information on the genetic resources of key microorganisms on which aquaculture depends.

2 Scope

This thematic background study provides information on the genetic resources of key microorganisms on which aquaculture depends. These microorganisms fall into the microbial groups of (1) microalgae and fungal-like organisms, (2) bacteria, including cyanobacteria and (3) zooplankton. Many microalgal species are important in aquaculture, with different species being suitable as feed for shellfish and finfish larviculture, as components of "green water" widely used to enhance survival and growth of larval and adult fish, and as feeds to enhance the nutritional quality of *Artemia* and rotifers. Microalgae are also grown in aquaculture to produce pigments and fatty acids of importance in fish aquaculture and as human nutraceuticals. Bacteria that are used in aquaculture include cyanobacteria such as *Spirulina* used for human diet supplements and a rapidly-growing suite of probiotic bacteria. These probiotic bacteria include species that improve survival and growth of fish and shellfish larval and adult stages. Probiotic bacteria are expected to become increasingly important for disease prevention in aquaculture as antibiotic use is further curtailed and species are grown in more intensive aquaculture systems. Bacteria also play an important role in filtration systems needed in recirculating aquaculture systems. Zooplankton, specifically *Artemia* and rotifers, have a long history and wide application as feed for the aquaculture industry. Several species of *Artemia* are used, with *Artemia franciscana* being the most important. Of more than 2 000 species of rotifers, *Brachionus plicatilis* and *Brachionus rotundiformis* are most commonly used. Other zooplankton used in aquaculture include copepods that are growing in importance and cladocerans such as *Daphnia* that are widely used in freshwater larviculture.

The scope of this report is generally limited to production and use of specific microorganisms in aquaculture. The scope excludes the role of microbes in diseases in aquaculture as well as the critically important roles of microbes in natural aquatic ecosystems, including as natural food sources for "non-fed" aquaculture species such as fish and shell-fish species that are filter-feeders consuming the microbes that occur naturally in some production systems. However, this latter topic of the microbial communities that sustain "non-fed" aquaculture species is considered in the context of new technologies (Section 7: Genetic and genomic characterization) where it is pointed out that it is now possible to rapidly determine the community composition of complex natural microbial communities by genomic approaches and this may have application in future improvements in production of "non-fed" aquaculture species.

The geographic scope of this report is global and examples of the use and production of microbes in aquaculture are given from many countries. Particular emphasis has been placed on the genetic resources of key microorganisms on which aquaculture depends in the major aquaculture nations. China is by far the world's biggest aquaculture producer. Other major aquaculture producer countries include Indonesia, India, Viet Nam and the Philippines and together with China these countries make up over 80% of world global aquaculture production (FAO, 2016).

3 Microorganisms used in aquaculture

3.1 Microalgae and fungal-like organisms

3.1.1 Uses of microalgae and fungal-like organisms

Uses of microalgae

The uses of microalgae and in aquaculture can be divided into four categories: 1) "green water" approaches, 2) direct feeding, 3) indirect feeding and 4) production of pigments for aquaculture. Each is considered separately below.

"Green water" approaches

"Green water" is water in which microalgae are naturally growing or to which microalgae are added. The latter approach in which cultured microalgae are added to fish larval rearing tanks is sometimes termed "pseudo-green water" (Shields and Lupatsch, 2012). Extensive freshwater fish and shrimp culture is done in man-made enclosures that are fertilized by agricultural or domestic waste or commercial fertilizer to produce blooms of microalgae. The resultant "green water" has been described as an important sector in world aquaculture because of the huge size of freshwater fish aquaculture that it supports (Neori, 2011, Neori, 2013). Several of the most important freshwater fish species consume "green water" plankton which is comprised of mainly microalgae, although zooplankton, protozoa and bacteria are also present in this "green water" (Neori, 2011). These fish and shrimp are raised without additional aquaculture feed. The quantity of "green water" microalgae consumed by fish and shrimp has been conservatively estimated at about 250 million tonnes per annum, based on the tonnage of fish and shrimp produced in "green water" polyculture ponds, the food conversion ratio, and the proportion of microalgae that are first consumed by zooplankton before being eaten by fish or shrimp. The productivity of these complex communities of microalgae in open ponds can be high. There are few reports in which the microalgal species composition of "green water" has been determined. Species composition is likely to be dynamic, changing according to environmental parameters, grazing by protozoa and ingestion by fish and shrimps and other factors. Application of molecular approaches for rapid identification and monitoring of microalgal communities may enable insights into optimal microalgal community composition.

Production of "green water" is also used on a smaller scale by the addition of microalgae to tanks in which larval fish or prawns are being raised. This "green water" technique is used by many fish and shrimp hatcheries. The benefits derived from this approach include improved larval growth and survival although the reasons for these improvements have not always been scientifically studied. Zmora et al. (2013) list documented benefits that include enhancement of the nutritional quality of live prey such as *Artemia* and rotifers, antibacterial activity and improvement of water quality by acting as an *in situ* biological filter. The microalgal species that are widely used for "green water" production are *Nannochloropsis* sp., *Chlorella vulgaris*, *Isochrysis* sp. and *Tetraselmis* sp.

Direct feeding

Microalgae are produced by many aquaculture facilities for direct feeding to finfish, shrimp, crab, bivalve, abalone and sea cucumber larval and juvenile stages. Of the many hundreds of microalgal species, a large number have been tried as aquaculture feeds and around 20-30 strains are now widely used. Hemaiswarya et al. 2011 lists *Nannochloropsis*, *Pavlova*, *Isochrysis*, *Tetraselmis*, *Thalassiosira weisflogii*, *Dunaliella* and *Chaetoceros* as seven microalgal strains that are commercially used. Zmora et al. (2013) lists some additional microalgal strains that are important in feeding crab and shrimp larvae, abalone juveniles and bivalves, including *Thalassiosira*

pseudonana, *Skeletonema*, *Rhodomonas*, *Pyramimonas*, *Navicula*, *Nitzschia*, *Cocconeis* and *Amphora*. See Table 1 for a compiled listing from several recent reviews of microalgal strains used in aquaculture and the applications for which they are used.

Table 1 Microalgal strains used in aquaculture. Compiled from reviews by Zmora et al., 2013[1]; Becker, 2013; Muller-Feuga, 2013; Hemaiswarya et al., 2011; Shields, 2011;

Phylum or Class	Genus and species	Application	Citation
Chlorophyta	*Chlamydomonas khaki*	Bivalve mollusks; rotifer and freshwater zooplankton live prey	Becker, 2013
	Chlorococcum sp.	Bivalve mollusks	
	Chlorella sp.	Bivalve mollusks and crustacean larvae; formulated feed ingredient; rotifer and *Artemia* live prey	Shields, 2011; Becker, 2013
	Chlorella vulgaris	Formulated feed ingredient; rotifer live prey	Shields, 2011
	Chlorella vulgaris B12	Rotifer live prey; "greenwater"	Zmora et al. 2013
	Chlorella (marine)	Crustacean (crab) larvae	Zmora et al. 2013
	Chlorella minutissima	Formulated feed ingredient; rotifer live prey	Shields, 2011
	Chlorella virginica	Formulated feed ingredient; rotifer live prey	Shields, 2011
	Chlorella grossii	Formulated feed ingredient; rotifer live prey	Shields, 2011
	Dunaliella tertiolecta	Bivalve mollusks; formulated feed ingredient; rotifer and *Artemia* live prey	Shields, 2011; Becker, 2013
	Dunaliella salina	Formulated feed ingredient	Shields, 2011
	Dunaliella sp.	Mollusk hatcheries	Hemaiswarya et al., 2011; Muller-Feuga, 2013
	Haematococcus pluvialis	Bivalve mollusks; formulated feed ingredient	Shields, 2011; Becker, 2013
	Micromonas pussila	Bivalve mollusks	Becker, 2013
	Pyramimonas virginica	Bivalve mollusks	Muller-Feuga, 2013; Becker, 2013
	Scenedesmus obliquus	Rotifer and *Artemia* live prey	Becker, 2013
	Scenedesmus quadricauda	Rotifer and *Artemia* live prey	Becker, 2013
	Tetraselmis sp.	Bivalve mollusks and crustacean larvae; rotifer live prey	Hemaiswarya et al., 2011
	Tetraselmis chui	Bivalve mollusks and crustacean larvae	Shields, 2011
	Tetraselmis suecica	Bivalve mollusks and crustacean larvae; Rotifer and *Artemia* live prey	Shields, 2011; Muller-Feuga, 2013; Becker, 2013
Ochrophyta	*Nannochloropsis oculata*	Rotifer live prey; "greenwater"	Shields, 2011
	Nannochloropsis sp.	Crustacean (crab) larvae; zooplankton feed fin fish hatcheries; shrimp hatcheries	Zmora et al. 2013 Hemaiswarya et al., 2011; Muller-Feuga, 2013
	Olisthodiscus luteus	Bivalve mollusks	
Labyrinthulomycetes	*Schizochytrium* sp.	Rotifer and *Artemia* live prey	Shields, 2011
	Ulkenia sp.	Rotifer and *Artemia* live prey	Shields, 2011
Bacillariophyta (diatoms)	*Actinocyclus normanii*	Bivalve mollusks	Becker, 2013
	Amphora sp.	Gastropod mollusks and sea urchins	Shields, 2011
	Amphora ovalis	Crustacean larvae	Becker, 2013.
	Bellerochea polymorpha	Bivalve mollusks	Becker, 2013.
	Chaetoceros affinis	Bivalve mollusks and crustacean larvae; *Artemia* live prey	Becker, 2013.
	Chaetoceros calcitrans	Bivalve mollusks and crustacean larvae	Shields, 2011, Zmora et al. 2013; Muller-Feuga, 2013; Becker, 2013.
	Chaetoceros gracilis	Bivalve mollusks and crustacean larvae	Shields, 2011, Zmora et al. 2013; Muller-Feuga, 2013
	Chaetoceros muelleri	Bivalve mollusks and crustacean larvae; *Artemia* live prey	Becker, 2013.
	Chaetoceros neogracile	Bivalve mollusks	Zmora et al. 2013

	Chaetoceros sp.	Shrimp hatcheries	Hemaiswarya et al., 2011
	Cocconeis duplex	Abalone larvae	Becker, 2013.
	Cyclotella cryptica	Aquaculture, unspecified	Muller-Feuga, 2013
	Cyclotella nana	*Artemia* live prey	Becker, 2013.
	Cylindrotheca closterium	Crustacean larvae	Becker, 2013.
	Navicula sp.	Bivalve mollusk larvae, astropod mollusks and sea urchins	Shields, 2011, Zmora et al. 2013; Becker, 2013.
	Nitzschia sp.	Gastropod mollusks and sea urchins	Shields, 2011, Zmora et al. 2013
	Nitzschia closterium	*Artemia* live prey	Becker, 2013.
	Nitzschiapaleacea	*Artemia* live prey	Becker, 2013.
	Phaeodactylum sp.	Crustacean (crab) larvae	Zmora et al. 2013
	Phaeodactylum tricornutum	Mollusk hatcheries; Bivalve mollusks and crustacean larvae	Muller-Feuga, 2013; Becker, 2013.
	Skeletonema costatum	Bivalve mollusks and crustacean larvae	Shields, 2011; Muller-Feuga, 2013; Becker, 2013.
	Skeletonema sp.	Crustacean larvae	Zmora et al. 2013
	Thalassiosira pseudonana	Bivalve mollusks and crustacean larvae	Shields, 2011; Becker, 2013.
	Thalassiosira pseudonana, clone 3H	Mollusk hatcheries	Muller-Feuga, 2013
	Thalassiosira weissflogii	Bivalve mollusks and crustacean larvae; copepod and *Artemia* live prey	Hemaiswarya et al., 2011
Haptophyta	*Coccolithus huxleyi*	Bivalve mollusks	Becker, 2013
	Cricosphaera elongata	Bivalve mollusks	Becker, 2013
	Dicrateria sp.	Bivalve mollusks	Becker, 2013
	Isochrysis sp.	Copepod and *Artemia* live prey; bivalve mollusks	Hemaiswarya et al., 2011
	Isochrysis galbana	Mollusk hatcheries	Muller-Feuga, 2013
	Isochrysis galbana affinis "Tahiti" *(T.* iso*)*	Bivalve mollusks and crustacean larvae Bivalves; rotifer and *Artemia* live prey	Shields, 2011; Muller-Feuga, 2013 Zmora et al. 2013; Becker, 2013
	Olisthodiscus luteus	Freshwater zooplankton live feed	Becker, 2013
	Pavlova lutheri	Bivalve mollusks Rotifer and *Artemia* live prey Mollusk hatcheries	Shields, 2011; Zmora et al. 2013; Muller-Feuga, 2013; Becker, 2013
	Pavlova pinguis	Bivalve mollusks Rotifer and *Artemia* live prey Mollusk hatcheries	Becker, 2013
	Pavlova sp.	Bivalve mollusks Rotifer live prey	Hemaiswarya et al., 2011
	Pseudoisochrysis paradoxa	Bivalve mollusks and crustacean larvae	Becker, 2013
Cryptophyta	*Cryptomonas* sp.	Bivalve mollusks	Becker, 2013
	Rhodomonas salina	Bivalve mollusks	Becker, 2013
	Chroomonas salina	Bivalve mollusks	Becker, 2013
Dinophyta	*Crypthecodinium cohnii*	Rotifer and *Artemia* live prey	Shields, 2011

[1]For Zmora et al. 2013, microalgae with applications designated as "most popular" are included.

A group that warrants separate mention is the Thraustochytrids. Thraustochytrids are marine microorganisms that were previously classified as fungi but are here considered as microalgae, although there is on-going debate about the classification of thraustochytrids. Many

thraustochytrids are rich sources of long chain polyunsaturated fatty acids and therefore have potential as feed additives in aquaculture. Unlike other microalgae used in aquaculture that are photosynthetic, thraustochytrids are heterotrophs that can grow on rich media in the absence of light and can therefore be readily cultured using industrial fermenters. The genus *Schizochytrium* within the thraustochytrids is the genus most commonly used in aquaculture. Several products based on thraustochytrids from the genus *Schizochytrium*, have been marketed through Aquafauna Biomarine and Sanders Brine Shrimp. These products have high concentrations of docosahexaenoic acid (DHA), which is important for human health (Guedes and Malcata, 2012). Dried algae *Schizochytrium* from Advanced BioNutrition Corp. was shown to be effective in enhancing weight gain, feed efficiency ratio and long chain polyunsaturated fatty acid content when included in the diets of channel catfish *Ictalurus punctatus* (Li et al., 2009). *Schizochytrium* has been used as a replacement for fish oil in the diets of jade perch (*Scortum barcoo*) juveniles which stored more n-3 long chain polyunsaturated fatty acids, in particular DHA (Van Hoestenberghe et al. 2014). In a recent study, fish oil was completely replaced with dried whole cells of *Schizochytrium* in the diet of Nile tilapia, resulting in significantly higher weight gain and an improved feed conversion ratio, as well as improved deposition of long chain polyunsaturated fatty acids in the tilapia filets (Sarker et al. 2016). Although the genus *Schizochytrium* is now most commonly used, new isolates from the diverse thraustochytrid group have high future potential. For example, the newly isolated microalga *Aurantiochytrium* sp. KRS101 was found to have a high DHA content and ability to grow on cheap substrates such as molasses (Hong et al., 2011). An interesting approach to reduce the cost of thraustochytrid biomass is to grow these organisms on wastewater from marine aquaculture, which has the added advantage of removing nutrients from the wastewater prior to discharge (Jung and Lovitt, 2010). The dinoflagellate *Crypthecodinium cohnii* can also be grown autotrophically and has the advantage of producing DHA in high concentrations, and without a mixture of other polyunsaturated fatty acids (Mendes et al. 2009).

Indirect feeding

Indirect feeding includes feeding microalgae to zooplankton to improve nutritional value. Microalgae are used as a food source for zooplankton such as rotifers and *Artemia*, both to grow the zooplankton and to enrich their nutritional value once they have reached the appropriate size to be fed to the species being grown in aquaculture.

Rotifers are grown in batch, semi-continuous or continuous culture, and commonly used microalgal diets for these rotifers include *Chlorella vulgaris*, *Isochrysis*, *Pavlova*, and *Nannochloropsis* sp. (Hemaiswarya et al. 2011; Guedes and Malcata, 2012; Zmora et al., 2013).

Thraustrochytrids have also been used to enrich *Artemia* and rotifers with long chain polyunsaturated fatty acids (Yamasaki et al., 2007). *Schizochytrium limacinum*, a thraustochytrid with high DHA content, was found to be effective in increasing the DHA content of rotifer *Brachionus plicatilis* and *Artemia franciscana*. Turbot (*Scophthalmus maximus*) juveniles fed with these enriched rotifers and *Artemia nauplii* were found to have a reduced rate of pseudoalbinism compared with a control group that received yeast-fed rotifers and *Artemia* (Song et al., 2007).

Crypthecodinium cohnii phospholipid extract and meal has also been used to enrich rotifers and *Artemia*, resulting in high levels of DHA. A 60% replacement of menhaden oil with *Crypthecodinium cohnii*-derived algal oil resulted in no change in growth rates in striped bass (Harel et al., 2002).

Production of pigments of importance to aquaculture.
The microalga *Haematococcus pluvialis* is increasingly used as a natural source of the carotenoid pigment astaxanthin that has application as a nutraceutical for human health and as a pigment to provide a desirable pink or red colour in species grown in aquaculture. The biology and commercial aspects of astaxanthin pigment production by *H. pluvialis* has been well reviewed by Han et al. (2013). Natural astaxanthin produced by *H. pluvialis* competes in the marketplace with synthetic astaxanthin. Synthetic astaxanthin costs about US$2 000/kg (Li et al., 2011) and dominates the market that was estimated to total US$447 million in 2014 (Wade et al., 2015). Natural astaxanthin fetches a much higher price and has advantages as a feed additive in aquaculture because it provides better pigmentation in some fish species and is preferred by consumers because of perceived safety benefits (Han et al., 2013). Natural and synthetic astaxanthins are often used in diet formulations for crustaceans such as shrimp that are grown in intensive aquaculture systems and are also widely used for farmed salmon and trout (Wade et al. 2015).

Uses of fungal-like organisms
Some species of yeast have been used as probiotics, dietary supplements and sources of pigments in aquaculture. Many species of yeast have been found as part of the normal microbiota of fish. Most of the reports of probiotic effects of yeast are focused primarily on two species, *Saccharomyces cerevisiae* and *Debaryomyces hansenii* (Navarrete and D. Tovar-Ramirez, 2014). A number of commercial products comprising yeast and yeast components are available commercially, including the preparations MacroGard® Betagard A®, EcoActiva®, NuPro® (a yeast-derived protein source), Nutriferm®, Fibosel®, Levucell® (derived from *S. boulardii*), and Bactocell® (derived from *Pediococcus acidilactici*). These products are sold as additives for agriculture, including in pig and poultry diets, as well as human nutraceuticals. The products MacroGard® Betagard A® and Levucell® SB20 all provided some increased resistance to challenges with the bacterial pathogen *Edwardsiella ictaluri* in juvenile channel catfish (*Ictalurus punctatus*) (Welker et al. 2012). The effects of β-glucans derived from yeast on fish immunity was recently reviewed by Vetvika et al. (2013) and found to be satisfactory in eliciting immunity and already to be widely used in commercial aquaculture, although there is a need for more efficient administration methods before glucans are prophylactically used routinely in aquaculture. Yeast extracts added to diets have been shown to have beneficial effects on health and growth rate in both marine fish (e.g. cobia; Lunger et al. 2006) and freshwater fish (e.g. Nile tilapia; Berto et al, 2015).

The yeast *S. cerevisiae* has also been tested as a dietary component. Addition at 15% dry weight substitution of fish meal was palatable to tilapia juveniles without affecting body composition (Ozório et al. 2012). Yeast can be an economic substitution because it is available cheaply as brewers yeast and as a by-product from ethanol fermentations, although dried yeast from these sources may vary in quality from batch to batch.

The red yeast *Rhodosporidium paludigenum* was found to enhance the growth performance and anti-oxidant performance of the widely cultured tropical shrimp *Litopenaeus vannamei* and has the potential to be a promising probiotic (Yang et al. 2010).

The pink yeast *Phaffia rhodozyma*, contains astaxanthin at about 0.4% and was used in the past as a source of this pigment for colouring of salmonids (Choubert et al. 1995). More recently the microalgae *Haematococcus* that contains 1.5–4.0% has been used instead (see above). In a study comparing commercially synthesized astaxanthin with that derived from *P. rhodozyma* marketed as Ecotone™, Ecotone™ was found to be a more effective astaxanthin source for pigmentation of Atlantic salmon muscle than the synthetic astaxanthin (Bjerkeng et al., 2007). The major market

for this pigment is aquaculture, more than 95% of the market share belonging to synthetic astaxanthin but with interest in natural rather than synthesized astaxanthin increasing because of consumer preference for natural products.

3.1.2 Production strategies and systems

Microalgae

Microalgae are divided into photoautotrophic microalgae that require light for growth and heterotrophic microalgae that grow on organic nutrients and are not light-requiring. Many different systems are used for in-house production of microalgae by aquaculture hatcheries, including carboys, hanging polyethylene bags, and bubble or airlift columns. Generally, production at a small scale is by batch culture, although continuous culture has been proposed as an attractive alternative because it can achieve reduced costs through automation and provide better environmental controls leading to more consistent quality of microalgae (Marchetti et al., 2012). For larger scale production, the two major cultivation systems are open ponds, tanks or raceways and closed photobioreactors. Open outdoor systems are susceptible to contamination and are therefore generally used for microalgae that can be grown under selective conditions, such as *Chlorella* that can tolerate high concentrations of nutrients or *Dunaliella* that thrives under high-salt conditions. For closed systems, many configurations of photobioreactors have been used, generally comprising flat panel photobioreactors or tubular photobioreactors. Important variables in all these systems are the type of aeration that is provided and the light source, ranging from natural light in open outdoor systems to incandescent, fluorescent and light-emitting diode (LED) lighting of varied intensity and wavelengths in closed systems. Microalgal culture facilities for aquaculture are reviewed in more detail in Zmora et al. 2013 and Guedes and Malcata, 2012.

Neori (2011; 2013) has described "green water" production as the most important sector within aquaculture because of the vast freshwater fish aquaculture that this approach supports. "Green water" production strategies include reliance on natural algal blooms and addition of fertilizer and domestic waste to ponds to stimulate natural bloom production. Production of "pseudo-green water" by addition of microalgae to fish larval rearing tanks relies on microalgal production by one of the approaches described above.

Heterotrophic microalgae including thraustrochytrids such as *Schizochytrium* sp. and the dinoflagellate *Crypthecodinium cohnii* can be grown in fermenters. Growth in fermenters can achieve very high cell densities and have economic advantages. *Crypthecodinium cohnii* is grown on an industrial scale to produce DHA by Royal DSM (previously Martek Corp.) in Maryland, USA. The freshwater photoautotrophic microalga *Chlorella* sp. can also be grown heterotrophically. *Chlorella* sp. is grown commercially in heterotrophic systems in Taiwan Province of China and Japan with an annual production of about 1 100 tonnes (Liu and Hu, 2013) and the total annual production of *Chlorella* biomass in both phototrophic and autotrophic conditions, exceeds 2 000 tonnes pa. (Spolaore et al. 2006).

Fungal-like organisms

Because of the importance of fungi in a wide range of commercial processes, there is an extensive base of knowledge for the intensive cultivation of fungi. Most of the species of fungal-like organisms of potential application in aquaculture can readily be cultivated economically on a large scale by tapping into the existing technologies for fungal cultivation.

3.1.3 Genetic technologies

Microalgae

Genetic technologies can be applied to microalgae of importance in aquaculture in several different ways. Genome sequencing and transcriptomics can be used to understand the systems biology of important strains, including patterns of gene expression under different conditions. Sequencing of genes that serve as phylogenetic markers, such as the cytochrome *c* oxidase subunit 1 (CO1),18S and 16S rRNA genes can be used to rapidly and unequivocally identify individual strains. A study of 16S and 18S rRNA gene molecular markers showed that 18 strains of microalgae could be well differentiated at the genus level but that better databases of reference sequences may be needed before species-level identification can be reliably achieved using these marker genes (Alonso et al., 2012). It is important to correctly identify microalgae in order to obtain reproducibility and reliability in their application in aquaculture and gene-based identifications are accurate and can be particularly useful to differentiate strains that are morphologically similar. Metagenomic approaches based on sequencing of large numbers of genes that provide phylogenetic information can be used to fully characterize the species composition of complex microalgal communities (Uyaguari et al., 2016), such as in "green water". Even more extensive sequencing of total DNA and RNA extracted from complex communities such as those found in "green water" could be used to reveal phylogeny and patterns of gene expression in all of the microalgae and bacteria making up these complex communities.

Rapidly advancing genetic and genomic technologies offer the potential to genetically modify microalgal strains to enhance desired characteristics, including optimizing microalgal quality for aquaculture feeds and other applications. The greatly reduced cost of DNA sequencing has facilitated the sequencing of the genomes of many microalgae. Recently, for example, the genomes of five species of *Nannochloropsis* were sequenced, giving new information on the diversity of lipid synthesis genes in this microalgal genus that is widely used in aquaculture (Wang et al., 2014). Bioinformatic analysis of genome sequences can give new insights into the pathways encoding compounds of interest and facilitate genetic manipulation.

Much of the impetus for research on genetic manipulation of microalgae over the past decade has come from the interest in exploiting microalgae for biofuel production. The ability of microalgae to synthesize many useful products and for genetic manipulation to establish microalgae as a widely used platform for the production of many high-value products was reviewed by Rosenberg et al. (2008). The best studied microalga in terms of genetic manipulation is *Chlamydomonas reinhardtii*, in which many tools have been developed but unfortunately these tools are often not applicable in other species of microalgae.

A critical step in genetic manipulation of microalgae is genetic transformation to get DNA into the cells. Widely used methods for introduction of DNA into microalgal cells include "biolistics" in which cells are bombarded with DNA-coated particles and electroporation in which electric current is used to permeabilize the cell membrane. Other methods that have been used include agitation of microalgae lacking cell walls with glass beads coated with DNA and agitation with silicon carbide "whiskers" that can pierce through cell walls and inject DNA into algal cells. Many species of microalgae have now been successfully transformed with DNA introduced into either the nucleus, chloroplast or mitochondrion of the microalga. Successful genetic transformation has now been demonstrated in more than 30 species including most of the microalgae that are important in aquaculture (see Enzing et al. [2012] for a list).

Once transformation is achieved, selection methods are needed to select the cells that have been successfully transformed. Several selection systems are available for *C. reinhardtii* and *Volvox carteri* but few systems exist for other microalgae. A widely used selection system is *ble*, a protein conferring resistance to bleomycin, phleomycin and zeomycin (Stevens et al. 1996). Marker and reporter genes for use in microalgae are listed by Gangl et al. (2015).

Several promoters are available to drive the expression of genes that are inserted into microalgae. Promoters from highly expressed endogenous microalgal genes such as ribulose bisphosphate carboxylase are used for nuclear expression (Walker et al., 2005) Genes encoding core photosynthetic subunits are used for chloroplast expression (Purton, 2007).

Even once genes are successfully inserted into green algae, stable long-term expression of transgenic proteins has seldom been obtained except in *C. reinhardtii* and *Volvox carteri*, possibly due to microRNA gene regulatory systems causing transgene silencing (Rosenberg et al., 2008). One of the first examples of successful genetic manipulation was the transformation of *V. carteri* with a hexose transporter gene that resulted in the microalga being able to grow heterotrophically on hexose rather than by photosynthesis (Hallmann et al. 1996). Trophic conversion was also achieved in the diatom *Phaeodactylum tricornutum* by introducing a gene encoding a glucose transporter (Zaslavskaia et al. 2001). These cases of trophic conversion demonstrate the potential of genetic manipulation of microalgae to change production from light-dependent photosynthesis to heterotrophic fermentation for large-scale commercial growth, which may have economic benefits for some production systems.

The microalgal genetic manipulation workhorse *C. reinhardtii* has provided many new products, including erythropoietin, interferon, proinsulin and human fibronectin (Rasala et al. 2010). There are few examples of development of transgenic algae for food and feed production because of challenges including public acceptance and regulatory issues (Enzing et al, 2014).

Gressel (2013) comprehensively reviews the characteristics that are beneficial to genetically engineer into algal "platform" strains for wide applicability in aquaculture (and for other applications, including biofuels). If strains are to be grown on a large scale in open ponds, resistance to contamination is important; the approach that Gressel (2013) discusses to prevent contamination by competing algae is to engineer the desired strain with genes encoding herbicide resistance and then apply herbicide treatment to maintain the desired strain. Engineering of microalgal strains to produce short-chain antimicrobial peptides could be useful in reducing contamination by bacteria and fungi (Gressel, 2013). The antimicrobial peptide lactoferrin was successfully produced in a transgenic strain of *Nannochloropsis oculata* and shown to be effective when fed to medaka fish in reducing infection of those fish by the bacterial pathogen *Vibrio parahaemolyticus* (Li and Tsai, 2008). Gressel (2013) proposes that the insecticide avermectin may be effective against many species of zooplankton and algae engineered to produce avermectin may be resistant to zooplankton contamination that can very rapidly decimate dense cultures of microalgae. He and others previously patented a method to attain resistance to viral infections, by isolating and amplifying the viral nucleic acid, splicing it into an expression cassette and transforming into the microalgae. Survivors with the right orientation of the cassette would become resistant to the viral infection (Gressel et al. 2010).

Other desirable traits listed by Gressel (2013) that could be engineered into microalgae are the ability to overcome the quorum sensing processes that may cause algal crashes once high densities are reached, heat tolerance to resist the high temperatures in closed bioreactors or ponds in sunny

climates and changes to photosynthetic efficiency that overcome photoinhibition at high light intensities. Truncated light-harvesting chlorophyll antenna size *(tla)* strains in *Chlamydomonas reinhardtii* operated with improved solar energy conversion efficiency (Melis, 2009). Reducing or truncating the size of the chlorophyll antennae has been shown to increase the light intensity at which photosynthesis saturates in two strains of the diatom *Cytothella* sp. (Huesemann et al., 2009). A strain of *Chlorella vulgaris* in which reduced chlorophyll antennae size was obtained by chemical mutagenesis achieved 44.5% improvement in biomass productivity under high light conditions (Shin et al., 2016). Random mutagenesis was also effective in producing a strain of *Chlorella sorokiniana* with reduced chlorophyll content and truncated antennae that showed higher productivity that the wildtype and yielded 30% higher biomass in photobioreactors (Cazzaniga et al. 2014). RNA interference (RNAi) technology was used to silence all 20 light harvesting complex genes in *C. reinhardtii* resulting in a chlorophyll/cell reduction of 68% and cells that were less susceptible to light inhibition and grew at a faster rate (Mussgnug et al., 2007). Gene silencing technology such as RNAi is emerging as a useful tool in genetic engineering and functional analysis of microalgae but progress is limited by incomplete understanding of the highly diverse silencing systems present in most microalgal species (Kim et al. 2015).

Fungal-like organisms

The genome of the yeast *S. cerevisiae* that has probiotic applications in aquaculture was the first eukaryote to have its genome sequenced, in the early 1990s (Goffeau et al. 1996) and there are extensive genetic and genomic resources available for this yeast. The yeast *Debaryomyces hansenii* that also has probiotic effects has a draft genome sequence for two strains that will provide insights into the halotolerance of this yeast (Kumar et al., 2012). Although no genome sequence is available for the *Phaffia rhodozyma* that has potential as a source of the important pigment astaxanthin, genetic manipulation of this yeast has been achieved, including methods for isolation of mutants that are affected in carotenoid biosynthesis as well as techniques for isolation and analysis of carotenoids (Lin et al., 2012). These approaches could be applied to enhance pigment production.

3.1.4 Producer countries and production trends

Microalgae

China is the major global producer of microalgal biomass and production trends in China are covered in detail in the recent extensive review by Chen et al. (2016). The four major microalgae produced in order of tonnage are *Arthrospira* (see section 3.2.4), *Chlorella*, *Dunaliella*, and *Haematococcus*. Commercial production of all of the species of microalgae of significance to aquaculture has recently been reviewed in individual chapters in Richmond and Hu (2013).

3.2 Bacteria

3.2.1 Uses of bacteria

Arthrospira (*Spirulina*)

Arthrospira (*Spirulina*) is a genus of photosynthetic cyanobacteria and is therefore correctly discussed under Bacteria rather than Microalgae, although in many texts, *Arthrospira* (*Spirulina*) is categorized under Microalgae. Members if the genus *Arthrospira* are filamentous, multicellular cyanobacteria with a characteristic helical filament shape. The two most important species in terms of production for food and feed are *Arthrospira platensis* and *Arthrospira maxima*, often incorrectly categorized as *Spirulina platensis* and *Spirulina maxima*. In an analysis based on 16S rRNA gene sequence analysis of five commercial strains of "Spirulina", four of the strains were found to be closely related to the genus *Arthrospira* and the fifth strain was affiliated with the genus *Halospirulina* (Kwei et al. 2011). This and other recent phylogenetic studies based on molecular,

morphological and biochemical characterization are all consistent with the most important producer organisms being two closely related species in the genus *Arthrospira, A. platensis* and *A. maxima*.

A. platensis and *A. maxima* are found naturally occurring in alkaline waters with high carbonate and bicarbonate concentrations, in tropical and sub-tropical waters. Under the extreme conditions in alkaline and saline lakes, *Arthrospira* can grow to high concentrations and be the dominant microbe present. The growth characteristics of *Arthrospira* and useful in maintaining almost pure cultures of this genus in production facilities (see 3.2.2. below).

Arthrospira has been used as a vitamin and protein supplement in aquaculture (Habib et al., 2008). The widespread use and potential of *Arthrospira* in aquaculture is reviewed by Becker (2013). Raw *A. platensis* was effective as a feed for larval tilapia (Lu et al. 2002). *A. platensis* was used as a probiotic for effective growth and immunity promotion in Nile tilapia (*Oreochromis niloticus*) that were challenged with the bacterial pathogen *Aeromonas hydrophila* (Abdel-Tawwab & Ahmad, 2009). In tests of *Arthrospira* as feed for several species of carp fry, *Cyprinus carpio* (common carp), *Hypophthalmichthys molitrix* (silver carp), and *Ctenopharyngodon idella*, addition of 10% *Arthrospira* to other diet ingredients generally resulted in better performance of the fry (Ayyappan, 1992). Additional examples are given in Becker (2013) who points out that *Arthrospira* probably has the broadest range of applications of all algae employed in commercial aquaculture.

Probiotic bacteria

Bacteria are increasingly used in aquaculture as probiotics to reduce the effects of the many pathogens that can infect aquacultured species. Probiotic bacteria have been most extensively used in China and in South America. There are now many hundreds of examples in the literature of the use of bacteria to improve disease resistance or growth of aquacultured species. The use of probiotics for disease control in aquaculture has recently been comprehensively reviewed by Newaj-Fyzul et al. (2014) who list 18 species of Gram-negative bacteria and 19 species of Gram-positive bacteria that have been considered for use in aquaculture. Modes of action may include competitive exclusion or immuno-stimulation as well as improvement in appetite or feed conversion that leads to better growth (Newaj-Fyzul et al. 2014).

Here, some very recent examples are provided as well as a detailed description of some use of probiotic bacteria in China because of the importance of the aquaculture industry in that country. In recent work, supplementation of the diets of the important tropical freshwater fish Indian carp (*Labeo rohita*) with the Gram-positive bacteria *Bacillus subtilis* and *Terribacillus saccharophillus* was found to significantly increase the immune and humoral response, suggesting an improvement in fish innate immunity (Sumathi et al. 2016).

With the demand of environmental friendly aquaculture practices, the use of probiotic products is an increasingly common practice in many fish or shellfish hatcheries and farms in China (Zhang et al., 2014; Han & Sun, 2016). The probiotics used in Chinese aquaculture are mainly photosynthetic bacteria (PSB), antagonistic bacteria, microorganisms for nutritional and enzymatic contribution to the digestion (lactic acid bacteria, yeast, etc.), bacteria for improving water quality (nitrifying bacteria, denitrifiers, etc.), *Bdellovibrio*, and other probiotics. The species of photosynthetic bacteria currently used in Chinese aquaculture include *Rhodopseudomonas palustris*, *Rubrivivax gelatinosa*, *Rhodobacter capsulata*, *R. spaheroides*, and *Phaeospirillum fulvum* (Qi et al., 2009). Purple non-sulfur bacteria were traditionally used in aquaculture in China since 1980s (Zhang et al., 1988). These bacteria were reported to be able to stimulate shrimp and fish growth, increase the survival rate of fish larvae and elevate the production of scallop seeds. Instead of using homemade

photosynthetic bacterial products, many farmers today are using concentrated and encapsulated commercial photosynthetic bacterial products (Qi et al., 2009).

In addition to the above applications, photosynthetic bacteria are being applied to improve the water quality of aquaculture ponds (Li et al. 2011). Bacterial antagonism plays an increasing major role in the equilibrium between competing beneficial and potentially pathogenic microorganisms (Qi et al., 2009). *Flavobacterium odoratum* (Mo et al. 2007), *Alteromonas sp.* (Li et al., 2001), *Phaeobacter inhibens* (Dong et al., 2007), *Vibrio natriegens, V. alginolyticus* (Li, 2008) were isolated and identified as effective aquaculture antagonistic bacteria that are capable of inhibiting pathogens in the culture ponds for aquaculture animals (Mo et al. 2007; Dong et al., 2007; Li et al. 2001). Several strains of the genera *Bacillus* and *Rhodobacter* have recently been identified from healthy *Litopenaeus vannamei* and shown to have safe digestive enzyme ability in juvenile shrimp (Dou et al. 2016). At present, probiotics research and development is focusing more onto (1) the added amount, additive type, safety and drug compatibility issues of discovered probiotic bacteria; (2) the exploration and development of new probiotic resources to protect aquatic animals from specific pathogens; (3) the *in vivo* proliferation characteristics of probiotics in aquatic animals and the function and interaction of these probiotics with aquatic animals' immune system (Han & Sun, 2016).

Bacteria used in filtration systems

Bacteria play an important role in filtration in aquaculture systems. Recirculating aquaculture systems have great promise as a sustainable way for farming marine fish. A fully contained recirculating aquaculture system achieved efficient biological waste treatment and water recycling by combining aerobic nitrification with simultaneous anaerobic denitrification and anaerobic ammonium oxidation mediated by efficient microbial filters. In addition, excess organic carbon remaining after denitrification was converted to methane gas by methanogenic microbes (Tal et al., 2009). The microbial diversity of biological filters in recirculating aquaculture systems is extensive and includes the genera *Nitrosomonas* (ammonium oxidation), *Nitrospira* (nitrite oxidation), *Thiomicrosporia*, *Thiothrix, Rhodobacter*, and *Hydrogenophaga* (autotrophic sulfide-dependent denitrification), *Pseudomonas* and *Paracoccus* (heterotrophic denitrification), various Proteobacteria and Firmicutes (dissimilatory nitrate reduction to ammonia), *Planctomycetes* and *Brocadia* (anaerobic ammonium oxidation), *Desulfovibrio*, *Dethiosulfovibrio*, *Fusibacter* and *Bacteroides* (sulfate reduction), *Thiomicrospira* (sulfide oxidation) and methanogenic archaea (methanogenesis) (Schreier et al. 2010). Metagenomic approaches that provide insights into metabolic functions and studies to quantify expression of individual genes for the entire community will assist in design optimization and guide bioaugmentation strategies (Schreier et al. 2010).

3.2.2 Production strategies and systems

Arthrospira (Spirulina)

There is a history of *Arthrospira* harvesting from blooms in natural ecosystems such as the soda lakes in Kenya and other parts of East Africa and in Lake Texcoco, near Mexico City, Mexico. Industrial production strategies have been reviewed in an FAO report (Habib et al., 2008) and by Belay (2013). Small-scale production has advantages over traditional agriculture in yielding a protein rich product, requiring no arable land and little water and being efficient in terms of energy use (Habib et al., 2008). Commercial and mass cultivation has four key steps 1. Growing the algae 2. Harvesting 3. Drying and 4, packaging of the biomass (Belay, 2013). Production is generally carried out in shallow race-way ponds with mixing achieved by paddlewheels. Critical parameters include use of an appropriate strain and control of pH, nutrient concentration, the light environment, and contamination (Belay, 2013). Harvesting is generally by filtration and this step is important in

the overall economics of the process. Careful and quick drying is essential to maintain a high quality product. Some of the technology being developed in attempts to grow microalgae very economically and on large scales may have application in improving *Arthrospira* production systems (Belay, 2013).

Arthrospira sp. are phototrophic organisms and have traditionally been considered to be obligate autotrophs that are dependent on light for growth and cannot grow in the dark (Habib et al., 2008). However, 34 of 35 axenic *Arthrospira* strains were found to be capable of heterotrophic growth on glucose as a carbon source and ten strains tested for photoheterotrophy grew with glucose and maltose but not with fructose or sucrose (Muhling et al., 2005). This raises the interesting possibility that *Arthrospira* could be commercially grown to high biomass concentrations under mixotrophic conditions (Belay, 2013).

Probiotic bacteria

The very wide range of bacteria that have been proposed as probiotics (Newaj-Fyzul et al. 2014) mean that many different media and growth conditions are used to grow these bacteria. One example of bacteria that are used on a commercial scale is the growth of the photobacteria *Chromatium perty* and *Chromatium okenii* in flat panel photobioreactors by the Chinese company Yantai Rich-Bio Science and Technology Ltd. These *Chromatium* spp. bacteria are used to reduce mortality in juvenile sea cucumbers (Zmora et al. 2013).

3.2.3 Genetic technologies

Arthrospira (Spirulina)

Many cyanobacteria, in particular members of the genus *Arthrospira*, contain large numbers of repeat sequences dispersed throughout their genomes and this characteristic makes sequencing of *Arthrospira* genomes more challenging. Draft genome sequences are now available for several strains of *Arthrospira*, including *Arthrospira* sp. PCC 8005 (Janssen et al. 2010), *A. platensis* NIES-39 (Fujisawa et al. 2010), and *A. platensis* C1 (PCC9438) (Cheevadhanarak et al., 2012). *A. platensis* C1 is a widely used laboratory strain that has the advantage of forming single colonies on agar plates because it is non-motile, without the ability to glide on surfaces (Cheevadhanarak et al., 2012). Whole genome sequencing was recently done on *A. platensis* YZ, followed by detailed comparative genomic analysis with the other available draft sequences of *Arthrospira* sp. (Xu et al., 2016). This study revealed extensive lateral transfer between different species of *Arthrospira* sp., as well as abundant restriction modification systems.

Genetic manipulation of *Arthrospira* has been challenging and little progress has been made because of difficulties in transformation of cyanobacteria in this genus. The extensive restriction-modification systems found in this genus are likely one of the factors that have resulted in limited success in introducing DNA into *Arthrospira* sp. There are a few reports of transformation of *A. platensis* strains (Kawata et al. 2004; Gaoge et al., 2004).

3.2.4 Producer countries and production trends

Arthrospira (Spirulina)

Since large-scale production of Arthrospira started in Japan in the 1960s, Arthrospira production has expanded to at least 22 countries according to the FAO; production figures vary widely and better monitoring of global Arthrospira production is needed (Habib et al., 2008). It is generally

accepted that the largest Arthrospira production nation is China, with estimates of annual production ranging from 1 000 tonnes to 3 500 tonnes (Lu et al., 2011) to the most optimistic figure for Chinese production, based on the websites of a number of companies of about 10 000 tonnes (Belay, 2013). A large producer is Hainan Simai Enterprising Ltd. in Hainan Province with an annual production of 200 tonnes (Mostafa, 2012). It was estimated in 2012 that there were more than 60 Arthrospira production facilities in China producing around 10 000 tonnes per year (Zhang & Xue, 2012) with an annual growth rate of about 10% (Chen et al., 2016).

The two largest producers in the USA are Earthrise Nutritionals in California and Cyanotech Corp. in Hawaii. Other significant producers are Taiwan Province of China, India, and Thailand.

3.3 Zooplankton

3.3.1 Uses of zooplankton

Artemia

Artemia is a genus of planktonic crustaceans in the class Branchiopoda found around the world occurring naturally in hypersaline environments such as salt lakes. *Artemia*, also known as brine shrimp, produce cysts that are highly resistant to desiccation, thermal fluctuations and UV radiation and retain viability for many years. On rehydration, *Artemia* cysts hatch to produce larval nauplii of about 0.4 mm in size that are very widely used as live feed in aquaculture. The use of *Artemia* as feed for fish larvae in place of their natural diets began in the 1930s and was an important step in the establishment of commercially important aquaculture (Sorgeloos, 1980).

Artemia comprises both zygogenetic and parthenogenetic groups. Seven zygogenetic species are generally recognized (Dhont & Van Steppen, 2003). Several species of *Artemia* are used in aquaculture, with *Artemia franciscana* found in the Americas being the most important. There are also many geographic strains with their own strain-specific characteristics that have developed in the many hundreds of salt lakes and artificial salterns around the world and these strains provide a resource for selection of characteristics desirable in aquaculture, in particular nutritional value (Dhont & Van Steppen, 2003). Artemia can be grown on many different food sources, including microalgae, dried algae, bacteria and yeasts and particulate products from food processing (Dhont & Van Steppen, 2003).

Several different life stages of *Artemia*, including decapsulated cysts, non-feeding nauplii (instar I), enriched nauplii (instar II and subsequent stages) and the adult stages, can be used as feed for fish larvae (Dhont & Van Steppen, 2003). *Artemia* is used as live feed for many species in fish larviculture, including seabream and seabass, halibut, flounder and commercially important crustaceans including shrimp, crabs and lobsters (Dhont & Van Steppen, 2003). In 1997, 80-85% of *Artemia* went to shrimp hatcheries with the rest going to marine fish larviculture (FAO, 2011). Because of the cost and as a result of periods of limited *Artemia* supply, there has been some reduction in use. Consumption of cysts in shrimp hatcheries fell from about 10 kg per million postlarvae to less than 5 kg by 2011. In seabream and seabass hatcheries, the reduction has been even more dramatic, from 600–700 kg of cysts per million larvae in 1990 to less than 100 kg in 2011 (FAO, 2011).

Rotifers

The phylum Rotifera comprises three classes, Monogonta, Bdelloidea and Seisonidea, with the Monogonta being the largest class with about 1 500 genera, including the genus *Brachionus* which is the most important genus used in aquaculture although other genera are being used for example by farmers in Asia (X. Zhou, pers. comm.). Two members of this genus, *Brachionus plicatilis* and *Brachionus rotundiformis* are most commonly used as feed for larval stages of marine fish and are euryhaline, able to grow in a wide range of salinities including seawater. *B. plicatilis* ranges in size from 200-360 µm and is known as the L-strain (large) and *B. rotundiformis* is 150–220 µm in size and known as the S-strain (small). *Brachionus calyciflorus* and *Brachionus rubens* are two freshwater rotifers that have been produced in freshwater mass cultures (FAO, 1996). Rotifers have the advantages of small size, the characteristic of growing to very high densities in mass culture systems and can serve vessels for desired nutrients because their nutrient composition can be improved by feeding them with specialized enrichment diets (Delbos & Schwarz. 2009).

B. plicatilis was first identified by Japanese researchers as a pest in eel aquaculture in the 1950s. Soon after it was used as a live food organism for the larval stages of fish species and is suitable for this application because of its small size and slow swimming velocity that make them suitable prey for fish larvae that have just resorbed their yolk sacs but are not yet large enough to be able to feed on *Artemia* (FAO, 1996). Rotifers have been important as feed for the larval stages of many major fish species in aquaculture, including yellowtail (*Seriola quinqueradiata*), red sea bream (*Pagrus major*), Asian sea bass (*Lates calcarifer*), gilthead sea bream (*Sparus aurata*) and the European sea bass (*Dicentrarchus labrax*) as well as penaeid shrimp and crab (Lubzens and Zmora, 2003). Challenges in the use of rotifers include the fact that very large numbers, up to several billions per day, can be required for raising marine fish larvae in commercial aquaculture and the nutritional quality of the rotifers must be carefully controlled by appropriate enrichment methods (Lubzens and Zmora, 2003). One of the most important parameters in the nutritional quality of rotifers is their lipid composition. The lipid content of rotifers typically varies between 9 and 28% of their dry weight and phospholipids and triacylglycerols are affected by the lipids provided in their diet. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are well known as essential fatty acids for the survival of marine fish larvae. The ratio of these and other fatty acids can be optimized in rotifers depending on the fish larval requirements. Rotifers cultured on yeast can be nutritionally inadequate because they lack sufficient essential fatty acids; the appropriate lipid content can be achieved by feeding the rotifers on lipid emulsions or on specific algae that provide the desired lipids, such as EPA-rich *Nannochloropsis* or DHA-rich *Isochrysis* (Lubzens and Zmora, 2003).

Copepods and other zooplankton

Copepods are the dominant zooplankton in marine waters, are highly diverse comprising about 2 400 genera and are the natural food source for most marine fish larvae. Free-living copepods most commonly used in aquaculture belong to three of the ten copepod orders: Calanoida, Harpacticoida and Cyclopoida (Støttrup, 2003). The interest in using copepods in aquaculture has been stimulated by efforts to expand the diversity of fish species grown in aquaculture, including ornamental species, some of which have larval stages that accept only copepod-sized prey. The calanoid copepod *Acartia tonsa* is widely cultured for research and has been used as feed for fish larvae in mariculture (SInterest in harpacticoid copepods has been stimulated because some of these copepods, including *Tisbe biminiensis*, have fast population growth and high concentrations of highly unsaturated fatty acids such as EPA and DHA, compared to rotifers and *Artemia* (de Lima et al. 2013). In general, mass production of copepods as live feed is still at the experimental stage and success has been achieved with a limited number of species. The tropical harpacticoid *Pararobertsonia* in terms of its rapid reproduction (Zaleha & Busra, 2013). Other zooplankton used in aquaculture include cladocerans such as *Daphnia* that are widely used in freshwater larviculture.

3.3.2 Production strategies and systems

Artemia

The two primary methods of *Artemia* production are the harvesting of naturally occurring *Artemia* blooms in salt lakes and the intentional production of *Artemia* cysts in man-made solar saltworks or salterns. Initially, there were only two commercial sources of *Artemia*, the Great Salt Lake, Utah USA and the San Francisco Bay coastal saltworks, California USA. Demand for *Artemia* had increased by the 1970s and at the same time harvests from the Great Salt Lake had decreased, causing a shortage and concomitant price increase that stimulated harvesting of natural *Artemia* resources from other sources, including Argentina, Australia, Canada, Columbia, and France (Dhont & Van Steppen, 2003) as well as southern Siberia, Kazakhstan and China (FAO, 2011).

Intensive *Artemia* cyst production in salterns was started in the 1970s in Brazil, followed by the Philippines, China and Thailand and is now widespread in East Asia and Latin America with particular success in Viet Nam. The process involved deliberate transplantation of *Artemia* cysts and is beneficial for salt production because the *Artemia* control populations of hypersaline microalgae that can interfere with the salt production process (FAO, 2011).

Rotifers

Production strategies for rotifers are largely determined by the need to a continuous supply of rotifers from live cultures. Rotifers can deteriorate rapidly in their nutritional quality as cultures age. Generally, small stock cultures are maintained separately from the main growth facility to serve as a reserve if the main cultures "crash" or fail as a result of technical errors of infection with pathogens. The systems for mass culture of rotifers have been described in detail by Lubzens and Zmora (2003). The three methods commonly used are batch culture, semi-continuous culture and continuous culture. Some batch culture is done at very high density by feeding with condensed *Chlorella* enriched with vitamin B_{12}. These high density compact systems have the advantage of enabling maintenance of different rotifer strains or growth to different sizes, for feeding to different fish species. Semi-continuous systems are generally in tank volumes ranging from 3 000–300 000 L with low rotifer density of about 100–300 rotifers ml^{-1}, with harvesting at the rate of about 6–7% of rotifer biomass per day. Continuous cultures are highly controlled and based on the chemostats used in microbial fermentations. Compact continuous culture systems of 1 000 litres can provide, for example, 1.7–3.5 billion *B. rotundiformis* per day but these systems have a high initial capital cost (Lubzens and Zmora, 2003). The commercial diets for rotifers have been shown to vary considerably in quality and this variation can result in rotifers that are below the minimum requirements for feeding fish larvae (Hamre, 2016). Recently, high density stable cultures of about 20 000–30 000 rotifers ml^{-1} has been achieved on a commercial scale in Japan, with ultra-high density culture of 160 000 rotifers ml^{-1} also being successfully attained (Yoshimatsu & Hossain, 2014). Important growth parameters, depending on the species and strain of rotifer being grown and its intended use, typically include salinity, temperature, dissolved oxygen, pH, and ammonia concentration (FAO, 1996). One challenge in all rotifer production systems is to maintain the health of the rotifer cultures. Early warning on deterioration of rotifer culture health can be obtained by careful monitoring of parameters such as egg ratio, swimming velocity, ingestion rate, viscosity of the culture medium which increases with the age of the culture, enzyme activity, and direct detection of diseases that could lead to the eventual collapse of the culture (Lubzens and Zmora, 2003).

Copepods and other zooplankton

Production methods for copepods are reviewed in detail by Støttrup (2003). In many cases, the copepods that have been used to culture marine fish species have been collected from the wild, in fjords or confined water bodies where the copepods occur naturally at high densities. Filtration devices with appropriate mesh sizes have been developed to facilitate collection. Some copepod production has occurred in enclosed areas in Norway where cod larvae have been successfully raised. Production in outdoor ponds or large tanks has been carried out in Europe and Asia for the culture of cod, grouper and turbot, using filtered seawater that contains phytoplankton but from which the zooplankton grazers have been excluded. There have also been some attempts at the intensive culture of copepods. Small calanoid copepods with fast generation times in the genera *Acartia*, *Centropages*, *Eurytemora*, and *Temora* have been successfully cultured. These copepods originate in coastal waters and are tolerant of variations in salinity and temperature (Støttrup, 2003). Some harpacticoid copepods, in particular *Tigriopus japonicas*, have been successfully cultured and Støttrup, 2003) lists several advantages of using harpacticoids, including their high tolerance to a wide range of environmental conditions, ability to live on a range of inert or live diets, high reproductive capacity and short life cycles, and ability to be cultured at high densities.

3.3.3 Genetic technologies

Artemia

Genetic technologies are not yet well developed for *Artemia*. Most of the genetic studies of *Artemia* have focused on the phylogeny of this genus. The mitochondrial DNA sequence is available for *A. franciscana* (Valverde et al. 1994). A study using Random Amplified Polymorphic DNA (RAPD) analysis confirmed the separation and homologous clustering within four known bisexual species, the American *A. franciscana* and *A. persimilis*, the Mediterranean *A. salina*, and the *Artemia* species from China (Badaracco et al., 1995). RAPD analysis was also used to show that 14 *Artemia* strains from the Caribbean belonged to *A. franciscana* and were divergent from *A. persimilis* (Argentina) (Camargo et al. 2002). Phylogenetic analysis has also been done by Amplified Fragment Length Polymorphism (AFLP) marker analysis of 15 strains of *Artemia* demonstrating that *Artemia tibetiana* could be differentiated from *Artemia sinica* (Sun et al., 1999). Use of AFLP analysis also showed that all bisexual European and North African *Artemia* populations are conspecific (Triantaphyllidis et al., 1997). Microsatellite markers were developed for characterization of two populations of *A. franciscana* and two populations of diploid parthenogenetic *Artemia*, demonstrating the utility of these microsatellites in studies on population genetics and tracking invasive processes in *Artemia* (Muñoz et al., 2008). Phylogenetic analysis using DNA barcoding based on the cytochrome c oxidase subunit 1 (COI) gene revealed clear differences between *Artemia* from five salt lakes on the Tibetan Plateau and other *Artemia* populations in China (Wang et al. 2008). One recent study moved beyond phylogenetic analysis to establish an AFLP-based genetic linkage map for *A. franciscana* and use this map to explore the sex-determining region as well as provide a genome size estimation for *A. franciscana* of ca. 0.93 Gb (De Vos et al., 2013). This comparatively small genome size makes the genome sequencing of *A. franciscana* an obvious next step.

Rotifers

In a major study, the phylogeny of the *B. plicatilis* species complex was investigated by analysis of CO1 and internal transcribed spacer 1 (ITS1) gene sequences from 1 273 isolates of the *B. plicatilis* complex, revealing the existence of 15 species within the complex. This study showed that some traits such as body length were related to phylogeny whereas other such as genome size were not (Mills et al. 2016). The genome sizes within the *B. plicatilis* species complex were found to be highly variable with a seven-fold range from 55 to 407 megabases. This range of variation was unexpected and is even higher than that among distantly related rotifer species belonging to

different genera. There were indications that whole genome duplications have played a role in the evolution of the *Brachionus* 'Austria' lineage (Stelzer et al. 2011). The heterogeneity in genome size would make it difficult to select a "typical" *B. plicatilis* as the best candidate for full genome sequencing.

Copepods and other zooplankton

Genomics research on copepods has been reviewed by Bron et al. (2011). In 2011, there were eight mitochondrial genome sequences but no assembled genomes for copepods. Genomic resources comprised mainly expressed sequence tags (ESTs) for the parasitic species *Lepeophtheirus salmonis* and *Caligus rogercresseyi* (Bron et al. 2011).

3.3.4 Producer countries and production trends

Artemia

The total global demand for *Artemia* cysts is currently 2 500- 3 000 tonnes per annum and is likely to increase. Reliable production statistics are available for the Great Salt Lake, Utah USA and the Mekong Delta, Viet Nam. For the Great Salt Lake ecosystem, total weight in tonnes of raw biomass harvested varied from less than 1 000 tonnes to almost 12 000 tonnes in annual production figures from 1985 to 2009 (FAO, 2011). The unpredictable and widely varying harvest from the Great Salt Lake is largely a result of changing natural hydrological and climactic conditions. This large variation causes major fluctuations in price and availability. Production in tonnes wet weight from the Vinh Chau and Bac Lieu districts of the Mekong Delta, Viet Nam ranged from less than 1 tonne to more than 50 tonnes and stabilized around 15 tonnes from 2004–2009 (FAO, 2011).

The largest demand is from China with an annual consumption of 1 500 tonnes of which approximately half is domestically produced and the other half imported from Russia and Kazakhstan (Dhont et al., 2013). In China, the *Artemia* can be divided into two broad groups, one in the coastal salt pans to the north of the Yangtzi River, including the coastal areas at Liaodong Bay, Bohai Bay, and Laizhou Bay; the other in inland salt lakes, such as Ebi Lake in Xinjiang Uyghur Autonomous Region, Yuncheng Salt Lake in Shanxi Province, as well as the salt pans at west coast of Hainan Island (Yan, 2008). China possesses extensive *Artemia* sources as it has coastal salt pans with an entire area of over 7 billion square meters and over 500 inland salt lakes whose area is over 1 000 square meters individually. These unique geographic characteristics have supported the discovery and establishment of over 70 *Artemia* strains and an annual productivity of 800–1 200 tonnes (dry weight) that consist one third of the annual global productivity. Some provinces in China are constituting various regional laws or regulations to protect their local *Artemia* bioresources. Qinghai Province, which has the most abundant salt lakes in China, constituted the Interim Measure for *Artemia* Source Protection in 2003 and later on amended this to become an official measure in 2009.

A study on the diversity of *Artemia* strains in salt pans along the coast of Hebei Province Coast revealed *A. franciscana*, and *A sinica* both of which reproduce sexually as well as local strains which have parthenogenetic reproduction. (Kexin 2006) Another study focusing on the *Artemia* bioresources in inland salt lakes of *Alxa* League in Inner Mongolia discovered the local *Artemia* strains possess oocytes of medium size diameter, appear red, and resist high temperature. Since they live in salt lakes in the natural massive deserts, their productivity and quality are heavily impacted by local severe weather and climate conditions (Fuyi, 2005).

Artemia production in Russia is focused on about 100 *Artemia* lakes in western Siberia with harvesting of cysts typically taking place in 20–40 of these lakes each year. The total annual harvest is 550 tonnrs, with 350 tonnes in the Altai region and 200 tonnes from other Russian regions (Litvinenko et al., 2015).

Farmed production of *Artemia* in salterns has been particularly successful in Viet Nam, starting in the Mekong Delta in the 1980s and expanding to more than 1 000 hectares of salterns in the Vinh Chau and Bac Lieu areas and resulting in around 50 tonnes per annum of high quality *Artemia* cysts for domestic use and export (FAO, 2011).

There is potential for growth of *Artemia* production in sub-Saharan Africa. *Artemia* populations are present along the coast of Kenya and *A. franciscana* occurs in eight salt works in Kenya. Artemia production in Kenya has been proposed as an important asset to the local aquaculture industry that could create thousands of employment opportunities (Ogello et al., 2014).

Rotifers

Because of the need for a continuous supply of rotifers from live cultures, rotifer production is a highly distributed process with large hatcheries typically producing their own rotifers. The distributed nature of rotifer production makes it difficult to obtain good figures for production levels in various countries. Rotifer mass production in three locations in Israel was reported at ca. $1.2–3.0 \times 10^{10}$ rotifers per day (Lubzens et al., 1997).

Copepods and other zooplankton

Information on production of copepods is limited. A few culture methods have been applied to mass culture in commercial hatcheries Støttrup (2003) gives the example of the Danish hatchery Maximus A/S that was producing half a million turbot juveniles per year based on copepods supplemented with *Artemia* for later larval stages.

4 Drivers affecting production of microorganisms

The role of microorganisms in aquaculture is likely to expand, with key drivers being the growth of the aquaculture industry, economic factors in cases where use of microbes can reduce costs and the need to reduce the environmental impact of aquaculture. The environmental impact and scarcity of fish meal and fish oil as feed for aquaculture is a major driver in development of alternative, microbe-based feeds. There is great potential in exploring the about 2 400 genera of copepods to identify additional copepod species that can be readily grown in mass production systems to economically produce copepods for live feed.

Increased use of recirculating aquaculture systems for fresh-water and marine species will benefit from rigorous studies on the diversity of bacteria used in their filtration systems, including establishment of defined inocula to be able to rapidly commission new recirculating systems and recommission systems after maintenance and rapidly establish obtain optimal filtration efficiencies.

The potential of microalgae to be grown on large scales to produce biofuel has received intensive renewed interest over the past decade. Microalgae can be grown on non-arable land using brackish water and have the potential to produce high concentrations of lipids that can be converted to biodiesel or the microalgal biomass can be converted to biocrude by hydrothermal liquefaction. There are still major challenges to produce algal biofuels economically at a scale that would have a meaningful impact on the supply of liquid fuels for transportation (Hannon et al., 2010).

Nevertheless, the investment in this field is providing new technologies for economically growing microalgae at large scales that may benefit aquaculture by reducing costs of microalgal production. It is also possible that if microalgal biofuels are produced at scale. There may be synergies between algal biofuels and availability of microalgae for nutrition in aquaculture.

5 Culture collections and conservation strategies

There is a great need for maintenance of microbes that have critically important roles in the aquaculture industry to be maintained and generally available in culture collections. Because of the diversity of microbes that are used in aquaculture and the fact that some of them are difficult to preserve, this is not a straightforward issue. Good conservation strategies makes it less labour-intensive and more economical to preserve strains. For each class of microbes, preservation strategies are discussed and examples of culture collections and other sources for these microbes are provided.

5.1 Microalgae and fungal-like organisms

Gressel (2013) points out that "domesticated" microalgal strains have a tendency to revert back to their "wild-type" characteristics, making it important to maintain stock cultures and to check cultures before using them as starters for new cultures. It is not always straight-forward to determine appropriate conditions for long-term storage of microalgae. Cryopreservation is by far the preferable method for long-term storage because it preserves the genetic integrity of strains and is more economic. However, conditions for cryopreservation have to be established for each algal species and strain.

The Bigelow National Center for Marine Algae and Microbiota (https://ncma.bigelow.org/) maintains the largest collection of publically available marine algal strains in the world, with very good taxonomic and geographic representation. The collection has more than 1 700 cryopreserved algal strains maintained in liquid nitrogen. Another major algal strain collection in the USA is UTEX, the Culture Collection of Algae at the University of Texas at Austin (https://utex.org).

Microalgal culture collections in China include the Freshwater Algae Culture Collection at the Institute of Hydrobiology (http://algae.ihb.ac.cn/english/). In Taiwan Province of China, 31 species (49 strains) of microalgae are maintained at the Tungkang Marine Laboratory (Su et al. 1997).

Microalgal collections in Japan include the Culture Collection of the National Institute for Environmental Studies https://www.nies.go.jp/kenkyu/yusyo/index.html (Japanese website) and Chlorella Industry Co. Ltd. That has a large culture collection of *Chlorella* species and provide condensed *Chlorella vulgaris* product for culturing rotifers.

The Australian National Algae Culture Collection, CSIRO (http://www.csiro.au/en/Research/Collections/ANACC) holds living cultures of more than 1 000 strains of more than 300 microalgal species.

A useful list of microalgal culture collections in several countries is at http://mcc.nies.go.jp/AOACC/Facilities.html

5.2 Bacteria

A very large number of bacterial species have been proposed as probiotic agents. Also, many different microbial species including bacteria and archaea, the majority of which are still poorly characterized, are important in biological filters in recirculating aquaculture systems. Fortunately,

bacteria and archaea are generally readily preserved for long periods of time using cryopreservation or lyophilization. Cryopreservation is often accomplished by addition of glycerol at 15–30% in the growth medium that is suitable for the bacterium, followed by freezing at -80˚C in an ultra-low temperature freezer or in liquid nitrogen. Freeze-drying or lyophilization effectively preserves many species of bacteria although not all strains can be recovered after lyophilization so individual testing is required. A major challenge here is that many different research groups have isolates that have been described as having probiotic properties and in many cases these strains are maintained by the individual research group, which does not provide for long-term security of the strains. Most countries have national culture collections and it is very important that significant strains are deposited in the culture collections to ensure their long-term availability to the research community and the aquaculture industry. Once the necessary intellectual property protection has been obtained and strains are described in the literature, they should be deposited in culture collections. This is a requirement for patent filing. For those strains on which patents are not obtained and are described in the scientific literature, it is a requirement of some journals that strains be deposited in culture collections. For example the instructions to authors for the American society for Microbiology journal "Applied and Environmental Microbiology" (AEM) state that "AEM expects authors to deposit important strains in publicly accessible culture collections and to refer to the collections and strain numbers in the text." If this requirement was also put in place by all journals publishing work on bacterial strains of significance in aquaculture, key bacterial strains would be better secured for future research and industrial application.

The World Federation for Culture Collections (http://www.wfcc.info/index.php/collections/display/) lists 589 culture collections in 68 countries.

5.3 Zooplankton

Artemia

The highly resistant cysts of *Artemia* sp. make this organism eminently suited to easy long-term storage. INVE Aquaculture (http://www.inveaquaculture.com/) offers a wide range of *Artemia* cysts.

Rotifers

Rotifer eggs can be cryopreserved after treatment with suitable cryoprotectant agents such as dimethylsulfoxide or propane-diol (Lubzens and Zmora, 2003). Short-term storage can be achieved at 4˚C but this is for periods of weeks to months rather than long-term storage.

In Japan, Chlorella Industry Co. Ltd. sell rotifers as culture starters http://www.chlorella.co.jp/ (only Japanese website). The laboratory of Dr. Atsuchi Hagiwara at the Graduate School of Fisheries and Environmental Sciences, Nagasaki University, maintains rotifer cultures of 110 strains together with some copepod and crustacean species, which are used mainly for academic research and provided only for academic purposes. http://www2.fish.nagasaki-u.ac.jp/FISH/KYOUKAN/hagiwara/custom1e.html

Many of the microbes that are important in aquaculture reside in companies that sell products to the aquaculture industry. These companies obviously have an important interest in maintaining the stocks of these microbes. This system also has the advantage of maintaining dispersed stocks on microbes in various locations.

6. Research, education and training

There is clearly a need for increased research in the area of genetic resources for microorganisms of current and potential use in aquaculture. Advances in molecular technologies in microbiology, including genomics and metagenomics, are not yet being extensively exploited for the benefit of the aquaculture industry. Aquaculture companies could find it to be beneficial in the mid to long-term to invest more heavily in research in microbiology to broaden their product base and maintain competitive advantages. Countries that want to be leaders in aquaculture into the future should provide competitive funding opportunities for academic researchers working in the area of genetic resources for microorganisms of use in aquaculture.

A challenge in education and training in genetic resources for microorganisms of current and potential use in aquaculture is that this area is highly interdisciplinary, requiring skills in microbiology of a wide range of microbial species as well as in molecular approaches and knowledge of aquaculture. Aquaculture microbiology is emerging as a new area of specialization and yet there are few training programs dedicated to this specialty. This may be an area that warrants establishment of new graduate programs for advanced training.

The FAO has an important role to play in education of professionals involved in the aquaculture industry in the importance of microbial systems. One useful step here would be to include more microbial resources in the Cultured Aquatic Species Information Program.

7. Stakeholders and resources

Academic and private contacts for microorganisms of current and potential use in aquaculture is provided in Table 2 [in preparation].

8. Future Prospects

For aquaculture to continue to diversify and expand to meet the challenge of providing high-quality seafood to a growing human population predicted to reach 9.7 billion by 2050, the contributions of microbiology are essential. Fortunately, this is an exciting time for the fields of microbial ecology and microbial genomics and metagenomics. By using molecular approaches to study the diversity and phylogeny of microbes, the range of microbes that can be of use in aquaculture can be rapidly extended. Existing and newly discovered microbes of importance in aquaculture can be characterized increasingly rapidly at a genomic level.

The use of metagenomic approaches makes it feasible to characterize complex microbial communities, including those found in natural “green water’ systems and in biological filters in recirculating aquaculture systems. By monitoring the diversity of these microbial communities and maintaining desired microbial community structures, it may be possible to increase the productivity of aquaculture systems in the future.

The significant insights that have been made into the links between human microbiomes and health may have analogous benefits in species that are important in aquaculture. Perhaps by analysing the gut microbiomes of fish species grown in aquaculture, it will soon be possible to manipulate those microbiomes to optimize feed conversion ratios or to maintain better health. The technology is certainly in place to be able to rapidly and economically determine the diversity of fish gut microbiomes.

However, it is important to also keep pace with traditional techniques in microbiology. The fundamental importance of maintaining diverse culture collections of all microbes of importance to aquaculture cannot be overemphasized. It is quite possible that changing environmental conditions, including those brought about by climate change, may mean that some key microbes can no longer be isolated from the environments in which they were once found and can only be recovered from culture collections.

Close integration of microbiology and aquaculture and the curation of key microbial species that are used in the aquaculture industry as well as expansion of the diversity of microbes used in aquaculture are critically important for the aquaculture industry to continue to flourish and prosper and to play its part in providing healthy food for the expanding world population.

9 References

Abdel-Tawwab, M. & M. H. Ahmad. 2009. Live *Spirulina* (*Arthrospira platensis*) as a growth and immunity promoter for Nile tilapia, *Oreochromis niloticus* (L.) challenged with pathogenic *Aeromonas hydrophila*. *Aquatic Research*, 40:1037-1046.

Alonso, M., F. C. Lago, J. M. Vieites, & M. Espiñeira. 2012. Molecular characterization of microalgae used in aquaculture with biotechnology potential. *Aquaculture International*, 20:847-857.

Arrigo, K. R. 2005. Marine microorganisms and global nutrient cycles. *Nature*, 437:349-355.

Ayyappan, S. 1992. Potential of *Spirulina* as a feed supplement for carp fry. In: *Spirulina*, Ecology, Taxonomy, Technology, and Applications. C. V. Seshadri & N. Jeeji Bai (Eds.) National Symposium, MCRC, Madras, India. pp. 171-172.

Badaracco, G., M. Bellorini and N. Landsberger. 1995. Phylogenetic study of bisexual *Artemia* using random amplified polymorphic DNA. *Journal of Molecular Evolution*, 41:150-154.

Becker, E. W. 2013. Microalga for aquaculture: Nutritional aspects. In Handbook of Microalgal Culture: Applied Phycology and Biotechnology. A. Richmond & Q. Hu (Eds.) 2nd Edition. Wiley Blackwell. Oxford, UK. pp. 671-691.

Belay, A. 2013. Biology and industrial production of *Arthrospira* (*Spirulina*). In Handbook of Microalgal Culture: Applied Phycology and Biotechnology. A. Richmond & Q. Hu (Eds.) 2nd Edition. Wiley Blackwell. Oxford, UK.

Berto, R. G. Pereira, J. L. P. Mouriño, M. L. Martins & D. M. Fracalossi. 2015. Yeast extract on growth utilization and haemato-immunological responses on Nile tilapia. *Aquaculture Research*, 47:2650-2660.

Bjerkeng, B., M. Peisker, K. von Schwartzenberg, T. Ytestøyl & T. Asgard. 2007. Digestibility and muscle retention of astaxanthin in Atlantic salmon, *Salmo salar*, fed diets with the red yeast *Phaffia rhodozyma* in comparison with synthetic formulated astaxanthin. *Aquaculture*, 269:476-489.

Bron, J. E., D. Frisch, E. Goetze, S. C. Johnson, C. E. Lee & G. A. Wyngaard. 2011. Observing copepods through a genomic lens. *Frontiers in Zoology*, 8:22.

Camargo, W. N., P. Bossier, P. Sorgeloos and Y. Sun. 2002. Preliminary genetic data on some Caribbean *Artemia franciscana* strains based on RAPD's. *Hydrobiologia*, 468:245-249.

Cazzaniga S., L. Dall'Osto, J. Szaub, L. Scibilia, M. Ballottari, S. Purton, & R. Bassi. 2014. Domestication of the green alga *Chlorella sorokiniana*: reduction of antenna size improves light-use efficiency in a photobioreactor. , 7:157.

Cheevadhanarak, S., K. Paithoonrangsarid, P. Prommeenate, et al. 2012. Draft genome sequence of *Arthrospira platensis* C1 (PCC9438). *Standards in Genomic Sciences*, 6:43-53.

Chen, F. 1996. High cell density culture of microalgae in heterotrophic growth. *Trends in Biotechnology*, 14:421-426.

Chen, J., Y. Wang, J. R. Benemann, X. Zhang, H. Hu & S. Qin. 2016. Microalgal industry in China: challenges and prospects. *Journal of Applied Phycology*, 28:715-725.

Choubert, G., Milicua, J.C., Gomez, R., Sancé, S., Petit, H., Genevieve, N.S., Castillo, & R., Trilles, J.P. 1995. Utilization of carotenoids from various sources by rainbow trout:

muscle colour, carotenoid digestibility and retention. *Aquaculture International*, 3:205–216.

De Lima, L. C. M., D. M. A. F. Navarro & L. P. Souza-Santos. 2013. Effect of diet on the fatty acid composition of the copepod *Tisbe biminiensis*. *Journal of Crustacean Biology*, 33:372-381.

Delbos B. & M. H. Schwarz. 2009. *Artemia* culture as a live feed item for intensive finfish and crustacean larviculture. *Virginia Sea Grant*: VCE publication # 600-105.

De Vos, S., P. Bossier, G. Van Stappen, I. Vercauteren, P. Sorgeloos & M. Vuylsteke. 2013. A first AFLP-based genetic linkage map for brine shrimp *Artemia franciscana* and its application in mapping the sex locus. *PLoS ONE*, 8(3):e57585.

Dhont, J., K. Dierckens, J. Støttrup, G. Van Stappens, M. Wille & P. Sorgeloos. 2013. Rotifers, *Artemia* and copepods as live feeds for fish larvae in aquaculture. *In* Advances in Aquaculture Hatchery Technology. G. Allan and G. Burnell (Eds). Woodhead Publishing, Cambridge.

Dhont, J. & G. Van Steppen. 2003. Biology, tank production and nutritional value of *Artemia*. *In* Live Feeds in Marine Aquaculture. J. G. Støttrup and L. A. McEvoy (Eds.). Blackwell Publishing, Oxford.

Dong, Y., X. Zhang, J. Liu, Z. Hu, C.J. 2007. Identification and inhibitory activity to pathogenic Vibrio species of a marine bacterium Phaeobacter DL2. *Journal of Fishery Science of China*, 14, 996.

Dou, C. et al. 2016. 2016. Isolation and screening of digestive enzyme producing probiotics from intestine of *Litopenaeus vannamei*. *Journal of Fisheries of China*, 40, 537.

Enzing, C., A. Nooijen, G. Eggink, J. Springer & R. Wijffels. 2012. Algae and genetic modification; research, production and risks. Netherlands Commission on Genetic Modification. Accessed on 1 December 2016 at http://www.cogem.net/index.cfm/en/publications/publication/research-report-algae-and-genetic-modification-research-production-and-risks?action=search&&count=9&containerid=542A633C-BBE0-1D96-94BAB7DADAD79A1F&lng=en_US&offset=82&q=&category=&from=30-09-1998&to=02-12-2016&order=date_desc

FAO. 1996. P. Dhert. Rotifers. Pp.49-78. *In* Manual on the production and use of live food for aquaculture. P. Lavens and P. Sorgeloos (Eds). FAO Fisheries Technical Paper 361.

FAO. 2011. Cultured aquatic species information program. *Artemia* spp. (Leach, 1819)

FAO. 2016. The state of world fisheries and aquaculture 2016.
Contributing to food security and nutrition for all. Rome. 200 pp.

Fujisawa, T., R. Narikawa, S. Okamoto et al. 2010. Genetic structure of an economically important cyanobacterium *Arthrospira* (*Spirulina*) *platensis* NIES-39. *DNA Research*, 17:85-103.

Fuyi B. The source and strain characters of Artemia in inland salt lakes of Alxa League in Inner Mongolia In: Zeng H, Chen H, Wu Y, Pan H, editors. Proceeding of the National Marine High-Tech Industry Forum 2005; Haikou, China: China High-tech Industrilization Association; 2005. p. 206-14.

Gamboa-Delgado, J. & J. M. Márquez-Reyes. 2016. Potential of microbial-derived nutrients for aquaculture development. Reviews in Aquaculture doi:10.1111/raq.12157

Gangl, D., J. A. Z. Zedler, P. D. Rajakumar, E. M. Ramos Martinez, A. Riseley, A. Włodarczyk, S. Purton, Y. Sakuragi, C. J. Howe, P. E. Jensen & C. Robinson. 2015. Biotechnological exploitation of microalgae. *Journal of Experimental Botany*, 66:6975-6990.

Gaoge, W., Z. Xuecheng, D. Delin & T. Chengkui. 2004 . Study on recipient system for transgenic manipulation in *Spirulina platensis* (*Arthrospira*). *Japanese Journal of Phycology*, 52: 243–245.

Goffeau A., B. G. Barrell, H. Bussey, R. W. Davis, B. Dujon, et al. 1996. Life with 6000 genes. *Science*, 274: 546–567.

Gressel, J. 2013. Transgenic marine microalgae: A value-enhanced fishmeal and fish oil replacement. In Handbook of Microalgal Culture: Applied Phycology and Biotechnology. A. Richmond & Q. Hu (Eds.) 2nd Edition. Wiley Blackwell. Oxford, UK.

Gressel, J., O. Chen & M. Danon. 2010. Method and system for protection and cross protection of algae and cyanobacteria from virus and bacteriophage infections. U. S. Patent Application 2010/0068721.

Guedes A. C. & F. X. Malcata. 2012. Nutritional value and uses of microalgae in aquaculture. In: Muchlisin, Z., Ed., Aquaculture, InTech.
http://www.intechopen.com/books/aquaculture/nutritional-value-and-uses-of-microalgae-in-aquaculture.

Habib, M. A. B., M. Parvin, T. C. Huntington & M. R. Hasin. 2008. A review on culture, production and use of Spirulina as food for humans and feeds for domestic animals and fish. FAO Fisheries and Aquaculture Circular C1034. FAO, Rome. 33 pp.

Hallmann, A. & M. Sumper. 1996. The *Chlorella* hexose/H+ symporter is a useful selectable and biochemical reagent when expressed in *Volvox*. *Proc Natl Acad Sci U S A*, 93:669-673.

Hamre, K. 2016. Nutrient profiles of rotifers (*Brachionus* sp.) and rotifer diets from four different marine fish hatcheries. *Aquaculture*, 450:136-142.

Han, D., Y. Li & Q. Hu. 2013.Biology and commercial aspects of *Haematococcus pluvialis*. In Handbook of Microalgal Culture: Applied Phycology and Biotechnology. A. Richmond & Q. Hu (Eds.) 2nd Edition. Wiley Blackwell. Oxford, UK.

Han, Z. & J. Sun. 2016. Screening and application of probiotics in aquaculture. *Fisheries Science*, 35, 93.

Hannon, M., J. Gimpel, M. Tran, B. Rasala & S. Mayfield. 2010. Biofuels from microalgae: challenges and potential. *Biofuels*, 1:763-784.

Harel, M., W. Koven, I. Lein, Y. Bar, P. Behrens, J. Stubblefield, Y. Zohar & A. R. Place. 2002. Advanced DHA, EPA and ArA enrichment materials for marine aquaculture using single cell heterotrophs. *Aquaculture*, 213:347-362.

Hasan, M. R. & M. B. New, M.B., eds. 2013. On-farm feeding and feed management in aquaculture. FAO Fisheries and Aquaculture Technical Paper No. 583. Rome, FAO. 67 pp.

Hemaiswarya, S., R. Raja, R. Ravi Kumar, V. Ganesan & C. Anbazhagan. 2011. Microalgae: A sustainable feed source for aquaculture. 27:1737-1746.

Hong, W-K, D. Rairakhwada, P-S Seo, S-Y Park, B-K hur, C. H. Kim & J-W Seo. 2011. Production of lipids containing high levels of docosahexaenoic acid by a newly isolated microalga, *Aurantiochytrium* sp. KRS101. *Applied Biochemistry and Biotechnology*, 164:1468-1480.

Huesemann, M. H., T. S. Hausmann, R. Bartha, M. Aksoy, J. C. Weissman, & J. R. Benemann. 2009. Biomass productivities in wild type and pigment mutant of *Cyclotella* sp. (Diatom) Applied Biochemistry and Biotechnology, 157:507-526.

Janssen, P. J., N. Morin, M. Mergeay, et al. 2010. Genome sequence of the edible cyanobacterium *Arthrospira* sp. PCC 8005. *Journal of Bacteriology*, 192: 2465-2466.

Jung I. S & R. W. Lovitt. 2010. Integrated production of long chain polyunsaturated fatty acids (PUFA)-rich *Schizochytrium* biomass using a nutrient supplemented marine aquaculture wastewater. *Aquacultural Engineering*, 43:51-61.

Kalarani V., V. Sumathi, J. K. Roshan, D. Sowjanya & D. C. Reddy. 2016. Effect of dietary supplementation of *Bacillus subtilis* and *Terribacillus saccharophillus* on innate immune responses of a tropical freshwater fish, *Labeo rohita*. *Journal of Clinical Cellular Immunology*, 7: 395.

Kawata, Y., S. Yano, H. Kojima & M. Toyomizu. 2004. Transformation of *Spirulina platensis* strain C1 (*Arthrospira* sp. PCC 9438) with Tn5 transposase–
transposon DNA-cation liposome complex. *Marine Biotechnology*, 6:355-363.

Kexin Z, Muqi X, Xiangchu Y. 2006. Constitution of Artemia strains in coastal salterns of Hebei Province. *Chinese Journal of Zoology*, 41(4):1-5. In Chinese.

Kim, E-J., X. Ma & H. Cerutti. 2015. Gene silencing in microalgae: Mechanisms and biological roles. *Bioresource Technology*, 184:23-33.

Kowalchuk, G. A., S. E. Jones and L. L. Blackall. 2008. Microbes orchestrate life on Earth. *ISME Journal*, 2:795-796.

Kumar, S., A. Randhawa, K. Ganesan, G. P. Raghava & A. K. Mondal. 2012. Draft genome sequence of the salt-tolerant yeast *Debaryomyces hansenii* var. *hansenii* MTCC 234. *Eukaryotic Cel*, 11:961-962.

Kwei, C. K., D. Lewis, K. King, W. Donohue & B. A. Neilan. 2011. Molecular classification of commercial *Spirulina* strains and identification of their sulfolipid biosynthesis genes. *Journal of Microbial Biotechnology*, 21:359-365.

Li, M. H., E. H. Robinson, C. S. Tucker, B. B. Manning & L. Khoo. 2009. Effects of dried algae *Schizochytrium* sp., a rich source of docosahexaenoic acid, on
growth, fatty acid composition, and sensory quality of channel catfish
Ictalurus punctatus. *Aquaculture*, 292:232-236.

Li, S. S. & H. J. Tsai. 2009. Transgenic microalgae as a non-antibiotic bacteriocide producer to defend against bacterial pathogen infection in the fish digestive tract. *Fish and Shellfish Immunology*, 26:316-325.

Li, J., D. L. Zhu, J. F. Niu, S. D. Shen & G. C. Wang. 2011. An economic assessment of astaxanthin production by large scale cultivation of *Haematococcus pluvialis*. *Biotechnology Advances*, 29:568-574.

Li, X., et al. Aquaculture Industry in China: Current State, Challenges, and Outlook. *Reviews in Fisheries Science*, 19: 187.

Li, H. Ocean University of China (2008).

Lin, G., J. Bultman, E. A. Johnson & J. W. Fell. 2012. Genetic manipulation of *Xanthophyllomyces dendrorhous* and *Phaffia rhodozyma*. *Methods in Molecular Biology*, 898:235-249.

Litvinenko, L. I., A. I. Litvinenko, E. G. Boiko & K. Kutsanov. 2015. *Artemia* cyst production in Russia. *Chinese Journal of Oceanology and Limnology*, 33:1436-1450.

Liu, J & Q. Hu. 2013. *Chlorella*: Industrial production of cell mass and chemicals. In Handbook of Microalgal Culture: Applied Phycology and Biotechnology. A. Richmond & Q. Hu (Eds.) 2nd Edition. Wiley Blackwell. Oxford, UK.

Lu, Y. M., Xiang &Y. H. Wen. 2011. *Spirulina* (*Arthrospira)* industry in Inner Mongolia of China: current status and prospects. *Journal of Applied Phycology*, 23:265-269.

Lu, J., G. Yoshizaki, K. Sakai & T. Takeuchi. 2002. Acceptability of raw *Spirulina platensis* by larval tilapia, *Oreochromis niloticus*. *Fishery Science*, 68:51-58.

Lubzens, E. & O. Zmora. 2003. Production and nutritional value of rotifers. *In* Live Feeds in Marine Aquaculture. J. G. Støttrup and L. A. McEvoy (Eds.). Blackwell Publishing, Oxford.

Lubzens, E., G. Minkoff, Y. Barr & O. Zmora. 1997. Mariculture in Israel – past achievements and future directions in raising rotifers as food for marine fish larvae. *Hydrobiologia*, 358:13-20.

Lunger, A. N., S. Craig & E. McLean. 2006. Replacement of fish meal in cobia (*Rachycentron canadum*) diets using an organically certified protein. *Aquaculture*, 257:393-399.

Marchetti, J., G. Bougaran, L. Le Dean, C. Mégrier, E. Lukomska, R. Kaas, E. Olivo, R. Baron, R. Robert & J. P. Cadoret. 2012. Optimizing conditions for the continuous culture of *Isochrysis* affinis *galbana* relevant to commercial hatcheries. *Aquaculture*, 326-329:106-115.

Melis, A. 2009. Solar energy conversion efficiencies in photosynthesis: Minimizing the chlorophyll antennae to maximize efficiency. *Plant Science*, 177:272-280.

Mendes, A., A. Reis, R. Vasconcelos, P. Guerra, & T. Lopes da Silva. 2009. *Crypthecodinium cohnii* with emphasis on DHA production: A review. *Journal of Applied Phycology*, 21:199-214.

Mills, S., J. A. Alcántara-Rodríguez, J. Ciros-Pérez, A. Gómez, A. Hagiwara, K. H. Galindo, C. D. Jersabek, R. Malekzadeh-Viayeh, F. Leasi, J-S. Lee, D. B. M. Welch, S. Papakostos, S. Riß, H. Segers, M. Serra, R. J. Shiel, R. Smolak, T. W. Snell, C-P. Stelzer, C. Q. Tang, R. L. Wallace, D. Fontaneto, & E. J. Walsh. 2016. Fifteen species in one: deciphering the *Brachionus plicatilis* species complex (Rotifera, Monongononta) through DNA taxonomy. Hydrobiologia (in press) DOI:10.1007/s10750-016-2725-7

Mo, Z., et al. 2001. Selection of Vibios-Antagonism Bacteria. *Journal of Ocean University of Qingdao*, 31, 225.

Mostafa, S. S. M. 2012. Microalgal biotechnology: Prospects and applications. Chapter 12. InTech. http://dx.doi.org/10.5772/53649

Muhling, M., N. Belay & B. A. Whitton. 2003. Screening *Arthrospira* (*Spirulina*) for heterotrophy. *Journal of Applied Phycology*, 17:129-135.

Muller-Feuga, A. 2013. Microalgae for aquaculture: The current global situation and future trends. In Handbook of Microalgal Culture: Applied Phycology and Biotechnology. A. Richmond & Q. Hu (Eds.) 2nd Edition. Wiley Blackwell. Oxford, UK.

Munoz J., A. J. Green, J. Figuerola , F. Amat & C. Rico. 2009. Characterization of polymorphic microsatellite markers in the brine shrimp *Artemia* (Branchiopoda, Anostraca). *Molecular Ecology Resources*, 9: 547–550.

Mussgnug, J. H., S. Thomas-Hall, J. Rupprecht, A. Foo, V. Klassen, A. McDowall, P.M. Schenk, O. Kruse & B. Hankamer. 2007. Engineering photosynthetic light capture: impacts on improved solar energy to biomass conversion. *Plant Biotechnology Journal*, 5:802-814.

Navarrete P. & D. Tovar-Ramirez. 2014. Use of yeasts as probiotics in fish aquaculture. In: M. P. Hernandez- Vergara and C. I. Perez-Rostro (Eds.) Sustainable Aquaculture Techniques, Publisher: INTECH.

Neori, A. "Green water" microalgae: the leading sector in world aquaculture. J. Appl. Phycol. 23:143-149.

Neori, 2013. Greenwater aquaculture: The largest aquaculture sector in the world. *World Aquaculture*, 44:26-30.

Newaj-Fyzul, A., A. H. Al-Harbi & B. Austin. 2014. Review: Developments in the use of probiotics for disease control in aquaculture. *Aquaculture*, 431:1-11.

Ogello, E. O., E. Kembenya, C. M. Githukia, B. M Nyonje & J. M. Munguti. 2014. The occurrence of the brine shrimp, Artemia franciscana (Kellog 1906) in Kenya and the potential economic impacts among Kenyan coastal communities. *International Journal of Fisheries and Aquatic Studies*, 1:151-156.

Ozório, R. O. A., L. Portz, R. Borghesi & J. E. P. Cyrino. 2012. Effects of dietary yeast (*Saccharomyces cerevisiae*) supplementation in practical diets of tilapia (*Oreochromis niloticus*). *Animals*, 2:16-24.

Palanichamy, S. & V. Rani. 2004. Observation on the long-term preservation and culture of the marine microalgae *Nannochloropsis oculata*. *Journal of the Marine Biological Association India*, 46:98-103.

Purton, S. 2007. Tools and techniques for chloroplast transformation of *Chlamydomonas*. *Advances in Experimental Medicine and Biology*, 616:34-45.

Qi, Z., X.-H. Zhang, N. Boon, P. Bossier. 2009. Probiotics in aquaculture of China -Current state, problems and prospect. *Aquaculture*, 290, 15.

Rasala, B. A., M. Muto, P. A. Lee, M. Jager, R. M. Cardoso, C. A. Behnke, P. Kirk, C. A. Hokanson, R. Crea, M. Mendez & S. P. Mayfield. 2010. Production of therapeutic proteins in algae, analysis of expression of seven human proteins in the chloroplast of *Chlamydomonas reinhardtii. Plant Biotechnology Journal*, 8:719-733.

Richmond, A. & Q. Hu (Eds.). 2013. Handbook of Microalgal Culture: Applied Phycology and Biotechnology. 2nd Edition. Wiley Blackwell. Oxford, UK.

Rosenberg, J. N., G. A. Oyler, L. Wilkinson & M. J. Betenbaugh. 2008. A green light for engineered algae: redirecting metabolism to fuel a biotechnology revolution. *Current Opinion in Biotechnology*, 19:430-436.

Sarker, P. K., A. R. Kapuscinski, A. J. Lanois, E. D. Livesey, K. P. Bernhard & M. L. Coley. 2016. Substitution of fish oil with marine microalga *Schizochytrium* sp. improves growth and fatty acid deposition in juvenile Nile tilapia (*Oreochromis niloticus*). *PLoS ONE*, 11: e0156684.

Schreier, H. J., N. Mirzoyan 7 K. Saito. 2010. Microbial diversity of biological filters in recirculating aquaculture systems. *Current Opinion in Biotechnology*, 21:318-325.

Shields R. J. & I. Lupatsch. 2012. Algae for aquaculture and animal feeds. Technikfolgenabschätzung – Theorie und Praxis 21. Jg., Heft 1, Juli 2012.

Shin, W-S., B. Lee, B-r. Jeong, Y. K. Chang & J-H. Kwon. 2016. Truncated light-harvesting chlorophyll antennae size in *Chlorella vulgaris* improves biomass efficiency. *Journal of Applied Phycology*, DOI 10.1007/s10811-016-0874-8

Song, X., X. Zhang, N. Guo, L. Zhu & C. Kuang. 2007. Assessment of marine thraustochytrid *Schizochytrium limacinum* OUC88 for mariculture by enriched feeds. *Fisheries Science*, 73:565-573.

Sorgeloos, P. 1980. Life history of the brine shrimp *Artemia*. *In* The Brine Shrimp *Artemia*. G. Persoone, P. Sorgeloos, O. Roels & E. Jaspers (Eds.). Universa Press, Wetteren.

Spolaore P., C. Joannis-Cassan, E. Duran & A. Isambert. 2006. Commercial applications of microalgae. *Journal of Bioscience and Bioengineering*, 101:87–96.

Stelzer, C-P., S. Riss & P. Stadler. 2011. Genome size evolution at the speciation level: The cryptic species complex *Brachionus plicatilis* (Rotifera). *BMC Evolutionary Biology*, 11:90.

Stevens D. R. , J. D. Rochaix & S. Purton. 1996. The bacterial phleomycin resistance gene *ble* as a dominant selectable marker in *Chlamydomonas*. *Molecular and General Genetics*, 251:23-30.

Støttrup, J. G. 2003. Production and nutritional value of copepods. *In* Live Feeds in Marine Aquaculture. J. G. Støttrup and L. A. McEvoy (Eds.). Blackwell Publishing, Oxford.

Støttrup, J. G. 2006. A review on the status and progress in rearing copepods for marine laviculture: advantages and disadvantages among calanoid, harpacticoid and cyclopoids copepods, Avances en nutrición acuicola. In: Memorías del Octavo Simposium Internacional de Nutrición Acuicola, Mazatlán, Sinaloa, México, pp 62-83.

Su, H. M., M. S. Su & i. C. Liao. 1997. Collection and culture of live foods for aquaculture in Taiwan. *Hydrobiologia*, 358:37-40.

Sun Y., W-Q Song, Y-C. Zhong, R-S. Zhang, T. J. Abatzopoulos & R. Y. Chen. 1999. Diversity and genetic differentiation in *Artemia* species and populations detected by AFLP markers. *International Journal of Salt Lake Research*, 8: 10.

Tal, Y., H. J. Schreier, K. R. Sowers, J. D. Stubblefield, A. R. Place & Y. Zohar. 2009. Environmentally sustainable land-based marine aquaculture. *Aquaculture*, 286:28-35.

Triantaphyllidis G. V., G. R. J. Criel, T. J. Abatzopoulos, K. M. Thomas, J. Peleman, J. A. Beardmore and P. Sorgeloos. 1997. International Study on *Artemia*. LVII. Morphological and molecular characters suggest conspecificity of all bisexual European and North African *Artemia* populations. *Marine Biology*, 129: 477–487.

Uyaguari, M. I., M. Chan, B. L. Chaban, M. A. Croxen, J. F. Finke, J. E. Hill, M. A. Peabody, T. Van Rossum, C. A. Suttle, F. S. L. Brinkman, J. Isaac-Renton, N. A. Prystajecky & P. Tang. 2016. A comprehensive method for amplicon-based and metagenomic characterization of viruses, bacteria, and eukaryotes in freshwater samples. *Microbiome*, 4:20.

Valverde J. R., B. Batuecas, C. Moratilla, R. Marco & R. Garesse. 1994. The complete mitochondrial DNA sequence of the crustacean *Artemia franciscana*. *Journal of Molecular Evolution*, 39: 400–408.

Van Hoestenberghe, S., C-A. Fransman, T. Luyten, D. Vermeulen, I. Roelants, S. Buysens & B. M. Goddeeris. *Schizochytrium* as a replacement for fish oil in a fishmeal free diet for jade perch, *Scortum barcoo* (McCulloch & Waite).
Aquaculture Research, 47:1747-1760.

Vetvicka, V., L. Vannucci & P. Sima. 2013. The effects of β-glucan on fish immunity. *North American Journal of Medical Science*, 5:580-588.

Wade, N. M., J. Gabaudan & B. D. Glencross. 2015. A review of carotenoid utilization and function in crustacean aquaculture. *Reviews in Aquaculture*, DOI: 10.1111/raq.12109.

Walker T. L., C. Collet, & S. Purton. 2005. Algal transgenics in the genomic era. *Journal of Phycology*, 41:1077-1093.

Wang, W., Q. Luo, H. Guo, P. Bossier, G. Van Stappen, P. Sorgeloos, N. Xin, Q. Hu & J. Y. 2008. Phylogenetic analysis of brine shrimp (*Artemia*) in China using DNA barcoding. *Genomics, Proteomics Bioinformatics*, 6:155-162.

Wang, D., K. Ning, J. Li, J. Hu, D. Han, H. Wang, X. Zeng, X. Jing, Q. Zhou, X. Su, X. Chang, A. Wang, W. Wang, J. Jia, L. Wei, Y. Xin, Y. Qiao, R. Huang, J. Chen, B. Han, K. Yoon, R. T. Hill, Y. Zohar, F. Chen, Q. Hu, & J. Xu. 2014. *Nannochloropsis* genomes reveal evolution of microalgal oleaginous traits. PLOS Genetics.10(1):e1004094. doi:10.1371/journal.pgen.1004094

Welker, T. L., C. Lim, M. Yildirim-Aksoy, & P. H. Klesius. Effect of short-term feeding duration of diets containing commercial whole-cell yeast or yeast subcomponents on immune function and disease resistance in channel catfish, *Ictalurus punctatus. Journal of Animal Physiology and Animal Nutrition*, 96:159-171.

X. Zhang *et al.* 2014. Effect of photosynthetic bacteria on water quality and microbiota in grass carp culture. *World Journal of Microbiology and Biotechnology*, 30, 2523.

Xu, T., S. Qin, Y. Hu, Z. Song, J. Ying, P. Li, W. Dong, F. Zhao, H. Yang & Q. Bao. 2016. Whole genomic DNA sequencing and comparative genomic analysis of Arthrospira platensis: high genome plasticity and genetic diversity. *DNA Research*, DOI: 10.1093/dnares/dsw023

Yamasaki, T., T. Aki, Y. Mori, T. Yamamoto, M. Shinozaki, S. Kawamoto & K. Ono. 2007. Nutritional enrichment of larval fish feed with thraustochytrid producing polyunsaturated fatty acids and xanthophylls. *Journal of Bioscience and Bioengineering*, 104: 200–206.

Yan L. 2008. Differential proteomic analysis in different development stages of Artemia sinica: Liaoning Normal University.

Yang, S-P, Z-H Wu, J-C Jian & X-Z Zhang. 2010. Effect of marine red yeast *Rhodosporidium paludigenum* on the growth and antioxidant competence of *Litopenaeus vannamei*. *Aquaculture*, 309:62-65.

Yoshimatsu, T. & M. A. Hossain. 2014. Recent advances in the high-density rotifer culture in Japan. *Aquaculture International*, 22:1587.

Zaleha K. & I. Busra. 2012. Culture of harpacticoid copepods: Understanding the reproduction and effect of environmental factors. *In* Aquaculture, Z. Muchlisin (Ed.), InTech, DOI: 10.5772/28373. Available from: http://www.intechopen.com/books/aquaculture/copepods-in-aquaculture

Zaslavskaia, L.A., J. C. Lippmeier, C. Shih, D. Ehehardt, A. R. Grossman & K. E. Apt (2001) Trophic conversion of an obligate photoautotrophic organism through metabolic engineering. *Science*, 292:2073-2075.

Zhang, D., Q. Shan, N. Chen, Z. Qiao. 1988. Isolation and cultivation of photosynthetic bacteria of Rhodospirillaceae and its application as additive in feeds of fish and prawn. *Journal of Fisheris of China*, 12, 367.

Zmora, O., D. J. Grosse, N. Zou, & T. M. Samocha. 2013. Microalga for aquaculture: Practical implications. *In* Handbook of Microalgal Culture: Applied Phycology and Biotechnology. A. Richmond & Q. Hu (Eds.) 2nd Edition. Wiley Blackwell. Oxford, UK.