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Nutritional Value and Uses of Microalgae in Aquaculture

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1. Introduction

Microalgae (i.e. single-celled algae or phytoplankton) represent the largest, yet one of the most poorly understood groups of microorganisms on Earth. As happens with plants relative to terrestrial animals, microalgae represent the natural nutritional base and primary source of bulk nutrients in the aquatic food chain.

Microalgae play indeed a crucial nutritional role with regard to marine animals in the open sea, and consequently in aquaculture. Most marine invertebrates depend on microalgae for their whole life cycle, so commercial and experimental mollusc or fish hatcheries have included a microalga production system in parallel to their animal production itself. Microalgae are utilized as live feed for all growth stages of bivalve molluscs (e.g. oysters, scallops, clams and mussels), for larval/early juvenile stages of abalone, crustaceans and some fish species, and for zooplankton used in aquaculture food webs at large. It should be emphasized that the productivity of any hatchery is directly related to the quantity and quality of the food source used therein.

On the other hand, the concept of aquaculture as a set of engineered systems in terms of wastewater treatment and recycling has received an impetus over the past few years. They are designed to meet specific treatment and wastewater specifications, and may simultaneously solve environmental and sanitary problems along with economic feasibility [1,2]. A renewed interest has also been experienced by high rate microalgal ponds for treatment of wastewater – where photosynthetic microalgae supply oxygen to heterotrophic bacteria, and where wastewater-borne nutrients are converted into biomass protein [2,3]. Therefore, microalga culturing is likely to play an increasingly important role in aquatic food production modules, specifically to produce (or be used as) feed for fish, convert CO_2 to O_2 and remediate water quality.

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2. General attributes of microalgal species in aquaculture

Unlike air-breathing animals, those living in aquatic media and used for large scale human consumption as food are seldom herbivorous at the adult stage; most farmed animals are indeed carnivorous from their post-larval stage on, or omnivorous at best. The associated food web is accordingly longer, so only filtering molluscs and a few other animals truly depend on plankton throughout their lifetime. However, microalgae are required for larva nutrition during a brief period – either for direct consumption in the case of molluscs and penaeid shrimps, or indirectly for the live prey fed to small-larval fish. In these cases, the post-larvae specimens are hatched, bred and raised by specialized establishments (hatcheries) – which are particularly complex to operate because they involve intensive production of microalgae and, in the case of small-larval fish, production of such small live prey as rotifers. Aquacultured animals for which rearing does not exhibit these constraints are seldom found; this is the case of salmonids, whose eggs have sufficient reserves to hatch big larvae capable of feeding directly on dry particles [4].

Over the last decades, several hundred microalga species have been tested as feed, but probably less than twenty have experienced a widespread application in aquaculture. In fact, microalgal species vary significantly in their nutritional value – which is also dependent on culturing conditions [5,6]. To provide a better balanced nutrition package and more effectively improve animal growth, a carefully selected mixture of microalgae should be fed to fish, directly or indirectly (through enrichment of zooplankton) – as this leads to better results than a diet composed of a single microalga [7,8].

Microalga production for use as feed is divided into intensive monoculture – for larval stages of bivalves, shrimp and certain fish species, and extensive culture – for growth of bivalves, carp and shrimp. Favored genera for the former include *Chaetoceros, Thalassiosira, Tetraselmis, Isochrysis*, *Nannochloropsis*, *Pavlova and Skeletonema* [6,9,10]. These organisms are fed directly or indirectly to the cultured larval organism; indirect means of providing them are usually through artemia, rotifers and *Daphnia* – which are, in turn, fed to the target larval organisms. It is widely accepted that microalgae are actively taken up by shrimp larvae, and play an important role in nutrition at that life stage; however, it is uncertain whether juveniles and adults do actively feed on microalgae as well. Some reports suggest that microalgae are found in their gut because shrimp accidentally ingest them together with debris [11,12].

The nutritional value of a microalgal diet is critically related to its ability to supply essential macro- and micronutrients to the target animal consumer. As emphasized above, a mixed microalgal diet – as routinely used in the hatchery and nursery phases of oyster cultivation [13], is likely to outperform monoalgal diets [14]. However, the nutritional requirements of bivalves are poorly defined; feeding experiments with microalgae of partially defined compositions have shown that carbohydrate and polyunsaturated fatty acid (PUFA) levels are major factors for growth of oysters [5]. Supply of additional dietary carbohydrate was found to increase oyster growth rate, provided that adequate protein and essential fatty acids were concomitantly supplied [9]. Supplementation of juvenile mussel diets with protein microcapsules led to a positive growth response, and indicated that a protein content below 40 %(w/w) significantly contrains mussel growth rates [15].

Diatoms and haptophytes (prymnesiophytes) are nutritious microalgae that are frequently used as feed for oysters [13]. The prymnesiophytes *Isochrysis* sp. and *P. lutheri* are rich sources of docosahexaenoic acid (DHA, 22:6n-3) – comprising 8-10% total fatty acids [16], while diatoms are a rich source of eicosapentaenoic acid (EPA, 20:5n-3) [17]. Mixed microalgal diets of prymnesiophytes and diatoms are common in bivalve hatcheries, and considered as highly nutritious in terms of requirements for essential PUFAs [18].

Microalgae should, in general, possess a number of key attributes to be useful for aquacultured species: they should be of an appropriate size and shape for ingestion and ready digestion (i.e. they should have a digestible cell wall to make nutrients easily available); they should undergo fast growth rates, and be amenable to mass culture; they should be stable to fluctuations in temperature, light and nutrient profile, as often occur in hatchery systems; and they should exhibit appropriate nutritional qualities, including absence of toxins (that might otherwise accumulate through the food chain). A major challenge faced by algologists is thus to reduce production costs, while maintaining reliability of microalgal feed.

Microalgae provide food for zooplankton, but they can also help stabilize (and even improve) the quality of the culture medium. For numerous freshwater and seawater animal species, introduction of phytoplankton to rearing ponds (the so-called green-water technique) produces much better results in terms of survival, growth and transformation index than the classical clear-water technique [19-21]. The rationale behind this observation is not entirely known, yet it may include water quality improvement by oxygen production and pH stabilization, and action of some excreted biochemical compounds, along with induction of behavioral processes such as initial prey catching and regulation of bacterial population [4,22], probiotic effects [23], and stimulation of immunity [24].

3. Nutritional features of microalgae

Microalgal species can vary significantly in nutritional value, as a function of the prevailing culture conditions. Only a reduced number of species have been used, primarily for historical reasons and ease of cultivation – rather than supported by scientific evidence of any superior performance as nutritional or therapeutical supplements. Hence, formulations more carefully selected of microalgal origin may offer the opportunity for development of improved nutritional packages aimed at larval animals.

Several factors contribute to the nutritional value of a microalga – including its size and shape, and digestibility as related to cell wall structure and composition (as mentioned above), as well as biochemical composition (e.g. accumulation compounds, enzymes and toxins) and specific requirements of the target animal. For this reason, several studies have attempted to correlate the nutritional value of microalgae to their chemical profile. However, results from feeding experiments are often difficult to interpret because of the confounding effects of other formulation additives. An examination of literature data – including those pertaining to microalga-based, compounded diet emulsions, have meanwhile allowed a few general conclusions to be reached [25].

As primary producers in the aquatic food chain, microalgae provide many phytonutrients, including in particular PUFAs – e.g. EPA, arachidonic acid (AA) and DHA, which are known to be essential for various marine animals [25], as well as for growth and

metamorphosis of many larvae [8,26]. However, the ratios of DHA, EPA and AA may actually be more important than their absolute levels [24,27]. Most microalgal species exhibit moderate to high percents of EPA (7 to 34%); and prymnesiophytes (e.g. *Pavlova* spp. and *Isochrysis* sp.) and cryptomonads are relatively rich in DHA (0.2 to 11%), whereas eustigmatophytes (e.g. *Nannochloropsis* spp.) and diatoms have the highest percentages of AA (up to 4%). Chlorophytes (*Dunaliella* spp. and *Chlorella* spp.) are deficient in both C20 and C22 PUFAs, although some species have small amounts of EPA (up to 3.2%); because of such a PUFA deficiency, chlorophytes are in general ascribed a poor nutritional value, so they are not suitable for use as single species-diet [6]. Prasinophyte species contain significant proportions of C20 (*Tetraselmis* spp.) or C22 (*Micromonas* spp.), but rarely of both. Therefore, the fatty acid contents of microalgae exhibit systematic differences according to taxonomic group – although there are examples of significant differences between microalgae, even within the same class.

The contents of antioxidants are also not uniform among microalgae; e.g. the concentrations of vitamins and carotenoids convey significant variations among species. Note that any mixed-algal diet should provide adequate concentrations of vitamins and carotenoids to be effective in aquaculture; unfortunately, the nutritional requirements of larval or juvenile animals that feed directly on microalgae are still poorly understood at present. In fact, artificial diets often lack natural pigments that allow such organisms as salmon or trout acquire their characteristic red color (muscle), which, in nature, is a result of eating microalgae containing red pigments; without such a color, a lower market value results. One way to alleviate this shortcoming is by adding astaxanthin to fish feed, with a consequently growing market for microalga-based sources, e.g. *Haematococcus pluvialis* [24,28].

On the other hand, the amino acid composition of microalgal proteins is rather similar between species [29], and relatively unaffected by their intrinsic growth phase and extrinsic light conditions [30,31]. Furthermore, the content in essential amino acids of microalgae is similar to that of oyster larvae. Overall, this indicates that protein quality is unlikely a factor that contributes to differences in nutritional value among microalgae. Finally, sterols [32], minerals [33] and pigments of microalgae also contribute to their nutritional performance in aquaculture.

Several studies have indicated that, in the late-logarithmic growth phase, microalgae contain typically 30-40 %(w/w) protein, 10-20 %(w/w) lipids and 5-15 %(w/w) carbohydrates [6,34]. When cultured through the stationary phase, the proximate composition of microalgae may significantly change; e.g. nitrate limitation leads carbohydrate levels to double at the expense of protein [31,35]. Hence, a strong correlation exists between composition of microalgae and their measurable nutritional value – even though diets containing high levels of carbohydrates have been reported to produce the best growth of juvenile oysters [9] and larval scallops [36], as long as PUFAs are also present to adequate proportions. Conversely, high dietary protein provides maximum growth for juvenile mussels [15] and oysters [18].

Another relevant issue is that marine environments are typically filled with bacteria and viruses that can attack fish and shellfish, and thus potentially devastate aquaculture farms. Bacteria and viruses can also attack single-celled microalgae, so these microorganisms have developed biochemical mechanisms for self-defense; such mechanisms involve secretion of compounds that inhibit bacterial growth or viral

attachment. For instance, compounds synthesized by *Scenedesmus costatum*, and partially purified from its organic extract exhibited activity against aquacultured bacteria because of their fatty acids longer than 10 carbon atoms in chain length – which apparently induce lysis of bacterial protoplasts.

The ability of fatty acids at large to interfere with bacterial growth and survival has been known for some time, and recent structure-function relationship studies have proven that said ability depends on both their chain length and degree of unsaturation. Cholesterol and other compounds can antagonize antimicrobial features [37], so both composition and concentration of free lipids should be taken into account [38]. The activity of extracts of *Phaeodactylum tricornutum* against *Vibrio* spp. was attributed to EPA – a compound synthesized *de novo* by diatoms [39]; this PUFA is found chiefly as a polar lipid species in structural cell components (e.g. membranes) and is toxic to grazers [40], as well as a precursor of aldehydes with deleterious effects upon such consumers as copepods [41]. Similarly, unsaturated and saturated long chain fatty acids isolated from *S. costatum* [42] and organic extracts from *Euglena viridis* [43] display activity against that bacterial genus.

4. Microalgal biomass production systems

Commercial culture of microalgae targeted at their metabolites has been taking place for over 40 years, and the main microalgal species grown are *Chlorella* and *Spirulina* for healthy foods, *Dunaliella salina* for β-carotene, *H. pluvialis* for astaxanthin and several species for aquaculture [44].

There are several reactor configurations that met with success in mass cultivation of microalgae – chosen according to such factors as physiology of the microalga, cost of layout land, intensity of labour, cost of energy, availability of water, cost of nutrients, suitability of climate (if the culture is implemented outdoors) and specification of final product(s). Large-scale culture systems should be compared on the basis of such indicators as efficiency of light utilization, controllability of temperature, hydrodynamic stress allowable, ability to maintain unimicroalgal and/or axenic cultures and feasibility of scale-up.

A major decision to be made is whether to use closed photobioreactors (PBRs) or open ponds to cultivate a given microalga. The latter may entertain a large area, and are relatively cheap to build and easy to operate – but contamination is hard to control, stable environmental conditions (particularly temperature) are difficult to maintain, and the attainable cell density is relatively low because of mutual shading effects. On the other hand, extensive areas of land will be needed for commercial exploitation, besides substantial costs of harvesting afterwards [45,46]. The final choice of system is always a compromise between these parameters, aimed at achieving an economically acceptable outcome [44].

A common feature of most microalgal species produced commercially (i.e. *Chlorella*, *Spirulina* and *Dunaliella*) is that they grow in highly selective environments – which means that they can be grown in open air cultures and still remain relatively free of contamination by other microalgae and protozoa [47-49]. Species of microalgae that do not possess this selective advantage must be grown in closed systems; this includes most marine algae grown as aquaculture feeds (e.g. *Skeletonema*, *Chaetoceros*, *Thalassiosira*, *Tetraselmis* and *Isochrysis*), as well as the dinoflagellate *Crypthecodinium cohnii* [44].

Typical systems used indoors for microalgal mass culture include carboys (10 to 20 L), polythene bags (100 to 500 L) and tubs (1000 to 5000 L); these are usually operated batch- or continuouswise [44]. For larger volumes, outdoor tanks or ponds are preferred, which are operated semicontinuously; depending on their scale, hatcheries may produce between several hundreds to tens of thousands of liters of microalgal biomass per day. However, the culture systems employed at present are still fairly unsophisticated: e.g. *D. salina* is cultured in large (up to ca. 250 ha) shallow open-air ponds with no artificial mixing; and *Chlorella* and *Spirulina* are also grown outdoors, in either paddle-wheel mixed ponds or circular ponds (up to 1 ha each) with a rotating mixing arm. The production of microalgae for aquaculture occurs generally on a much lower scale. Other commercial large-scale systems include tanks used in aquaculture, the cascade system developed in the Czech Republic [50] and heterotrophic fermenter devices used for culture of *Chlorella* in Japan and Taiwan [47,51], and for culture of *C. cohnii* in USA [52,53].

The choice of which configuration is preferable depends obviously on the objective function; e.g. wastewater treatment might preclude open systems, owing to the unacceptably high costs arising from the large volumes to be processed and the low added value of the resulting products [54]. There has been a major effort directed at examining alternatives for the production of fresh microalgae, and also at more cost-efficient production systems.

4.1 Open cultivation systems

Microalgae cultivation in open ponds has been in current used since the 1950s [44]; these systems have been categorized as natural waters (lakes, lagoons and ponds), and artificial ponds or containers – with raceway ponds being the most frequently used artificial system [55]. The four major types of open-air systems currently in use (i.e. shallow big ponds, tanks, circular ponds and raceway ponds) have all advantages and disadvantages. This type of system usually consists of either circular ponds with a rotating arm to mix the culture, or long channels in a single or multiple loop configuration stirred by paddle wheels [56] – although simpler configurations have also met with sucess [54]. Raceway ponds are usually built in concrete, but compacted earth-lined ponds with (white) plastic have also been proposed. In a continuous production cycle, broth and nutrients required by microalgal growth are introduced in front of the paddlewheel, and circulated through the loop to the harvest extraction point; said paddlewheel undergoes a continuous motion to prevent sedimentation. The CO_2 requirement is usually satisfied using the open atmosphere as source – yet submerged aerators may be installed to enhance CO_2 supply, and thus absorption yield [57].

Compared to closed photobioreactors, open ponds represent a less expensive investment for large-scale production of microalgal biomass. On the other hand, open pond production does not necessarily compete with agricultural crops for land, since it can be implemented in areas with marginal crop production potential [58]. Open ponds also have low energy input requirements [59], and regular maintenance and cleaning are easier [60].

Open ponds and raceways were the first large-scale designs implemented, and are still the most widely applied in industrial processing. The main constraints related to their operation are the difficulty to control contamination and to keep the culture environment

steady, and the cost associated with harvesting. Furthermore, the open character of the system makes it possible for naturally occurring microalgae or their predators to infiltrate, and thus compete with microalgae intended for cultivation. Therefore, a monoculture can only be maintained under extreme conditions of pH, salinity or temperature that guarantee dominance by the desired strain (e.g. *D. salina* dominance requires highly salted media, whereas *Spirulina platensis* demands high pH values). Unfortunately, high pH, temperature and salt concentration are not compatible with most microalgal species of interest.

Regarding biomass productivity, however, open pond systems are less efficient than closed photobioreactors [61]. This can be attributed to such parameters as evaporation losses, temperature variation, CO_2 deficiency, inefficient mixing and light limitation. Although evaporation losses make a net contribution to cooling, they may also cause significant changes in the ionic composition of the medium – with detrimental effects upon microalga growth [62]. Although this type of reactor is extensively used in industrial microalgal production – e.g. to produce *Spirulina* and *Dunaliella* spp. up to worldwide totals of 5000 and 1200 ton/yr, respectively [24], open systems have apparently reached their upper limit – with little room for further technological improvement.

4.2 Closed cultivation systems

Despite the success of open systems, future advances in microalgal mass culture will require improved closed systems, as the most interesting microalgal species cannot grow in highly selective environments [44]. Hence, photobioreactor technology is on the rise, which is designed to overcome the major constraints associated with open pond production systems [63]; recall that both pollution and contamination risks preclude use of open ponds to prepare high-value products for eventual use as active ingredients in aquaculture feed formulation [60].

Closed systems include tubular, flat plate and fermenter types, among other possibilities. The former two are specifically designed for efficient recovery of sunlight, whereas the latter may require artificial illumination. Owing to the higher cell mass productivities attained, harvesting costs can be significantly reduced. Closed photobioreactors also provide reproducible cultivation conditions, good heat transfer, better biomass yield, higher product quality and opportunity for flexible technical design [44,60]. Note, however, that the costs of closed systems are substantially higher than their open pond counterparts [54]. A variety of closed photobioreactors have been tested (or at least proposed) for industrial microalgal biomass production [64,65], but engineering and economic analyses of such reactors still lag behind the open ponds [66-70].

A typical photobioreactor is essentially a four-phase system, consisting of solid microalgal cells, a liquid growth medium, a gaseous phase and a light radiation field [71]. Its productivity is limited by various design features – but, most importantly, the reactor is to be operated under favorable illumination conditions, with optimized surface-to-volume ratio and light/dark cycle, coupled with adequate mass transfer features [72].

The current consensus is that commercial (photoautotrophic) production of metabolites with interest for aquaculture by microalgae should resort to outdoor enclosed photobioreactors [56,62,65,73,74]. Tredici [65] reviewed the development of those type of reactors over the last

decade; while many types of experimental PBRs have been considered, built and tested, very few have actually succeeded on a commercial level. Commercial application of photobioreactor technology remains indeed restricted to the production of two Chlorophyte microalgae: *Chlorella* and *Haematococcus* [62,75].

Scale up of photobioreactors from bench to commercial scale is not trivial – since it needs changes in illumination, gas transfer and temperature to be taken into account, all of which are severely affected by turbulence in the reactor, and consequently require a tight control. Therefore, scale up appears to be much more of an engineering problem than a biological one; and general recommendations as to possible maximum scales have accordingly been produced [75,76].

5. Alternatives to fresh microalgae

Marine microalgae have been the traditional food component in finfish and shellfish aquaculture, e.g. for larval and juvenile animals [77]; they are indeed essential in hatchery and nursery of bivalves, shrimp and some finfish cultures. Microalgae are also used to produce zooplankton – typically rotifers, which are in turn fed to freshly hatched carnivorous fish [78]. As aquaculture industry expands [79] – and since microalgal biomass cultivation on-site may represent up to 30% of the operating costs [13], there is a demand for marine microalgae that cannot be met by the conventional methods used in hatcheries – thus forcing one to resort to substitutes with mediocre results that bring about several problems [44,80].

Despite the obvious advantages of alive microalgae in aquaculture, the current trend is to avoid using them because of their high cost and difficulty in producing, concentrating and storing them [8,81]. Alternatives that are potentially more cost-effective have been investigated – including nonliving food, viz. microalga pastes, dried microalgae, microencapsulates, cryopreservation, flocculation, bacteria or yeasts; they have been tested *in vitro* and in actual hatcheries, but met with variable degrees of success [82,83]. For instance, in Japan, where *Nannochloropsis oculata* is the most important cultured feed for the rotifer *Brachionus plicatilis*, concentrated suspensions and frozen biomass of this microalga are commercially available [84]; and partial replacement of alive microalgae by microencapsulated and yeast-based diets is indeed a routine practice in hatcheries for penaeid shrimp [24,85]. However, most these approaches have proven unsuitable as major dietary components, because of their lower nutritional value than mixtures of alive microalgae.

Several criteria should be addressed in attempts to find substitutes for alive microalgae as diet in aquaculture. From a nutrition standpoint, alive microalgae possess higher nutritional value and better digestibility than most substitutes; note that the nutritional quality depends critically on such biochemical constituents as PUFAs, vitamins, sterols and carbohydrates [86].

Useful bacteria can provide only a part of the metabolic requirements in aquaculture – by supplying a few organic molecules and vitamins. Under conditions close to those found in rearing facilities, the bacterial input should not represent more than 15% of the microalgal contribution for mollusk larvae and juveniles of many species [87,88]. Yeasts were as well investigated as an alternative food source – but poor results were observed [83,89]. Therefore, these two alternatives are not suitable to fully replace alive microalgae.

An alternative diet with an apparently better potential is microalgal pastes or concentrates [90-92]; these are prepared by centrifugation (up to 1:500 concentration) or flocculation (up to 1:100 concentration). Concentrates prepared from distinct microalgae vary in their suitability – with diatoms being the most promising; and they have a shelf life of between 2 and 8 weeks, when stored below 4°C. Commercially, microalgal concentrates can be prepared under two different scenarios: (a) by hatcheries on-site, which prepare concentrates as back-up or as a means to store overproduction; or (b) by remote production, centralized at a large facility – with a greater economy of scale, with the resulting concentrates dispatched to hatcheries upon request.

The advantage of such concentrates is that they can be used "off-the-shelf", thus contributing favorably to the cost-efficiency in hatcheries. On the other hand, the lower nutritional value of most dried microalgae compared to alive feed, and the limited availability of commercial dried products appear as main shortcomings. Globally speaking, concentrates have low levels (or even absence) of ω3-PUFA, and lead to a difficult digestion by bivalve larvae [93]. The genus *Tetraselmis* seems to be a good candidate for microalgal paste, but its nutritional quality deteriorates quite rapidly [94]; experiments have indicated that such substitutes should be used as supplement only when rations of live microalgae are insufficient. Furthermore, spray-dried microalgae and microalga paste may be useful to replace up to 50% of alive microalgae. Coutteau and Sorgeloos [13] reported that artificial or non-living diets are rarely applied in routine processing of bivalves, and are mostly considered as a backup food source only. Centrifuged concentrates of *P. lutheri*, in combination with *Chaetoceros calcitrans* or *S. costatum*, lead to 85-90% of the growth when a mixed diet of alive microalgae for oyster *Saccostrea glomerata* larvae is used [92].

Centrifugation has been successfully applied to prepare concentrates, but it has some limitations – i.e. the process involves exposing cells to high gravitational and shear forces that damage the cell structure. On the other hand, processing of large culture volumes is time-consuming and requires costly equipment, i.e. a specialized continuous centrifuge. Research on post-harvest preservation is required to extend shelf-life beyond 4 to 8 weeks, and also to prepare concentrates from flagellate species (e.g. *Isochrysis* sp. and *P. lutheri*).

Alternative processes have meanwhile been developed that are potentially less damaging to cells – including foam fractionation [95], flocculation [96,97] and filtration [98]. Sandbank [99] fed microalgae, grown in waste-water and flocculated with aluminium sulfate, to common carp (*Cyprinus carpia*); a diet containing 25% of microalgal meal led to a growth comparable to that by the control diet, with no harmful effects detected upon long term health of the fish. Millamena et al. [96] successfully fed *Penaeus monodon* larvae with dried, flocculated *C. calcitrans* and *Tetraselmis chuii* cells. However, a common disadvantage encountered was that the harvested cells are difficult to disaggregate back to single cells, which is a requirement to feed them to filter-feeding species such as bivalves [100].

A novel technique was developed for flocculation of marine microalgae that appears useful in aquaculture: it entails adjustment of pH of the culture using NaOH, followed by addition of a non-ionic polymer, Magnafloc LT-25; the ensuing flocculate is then harvested and neutralized, thus leading to a final concentration of between 200- and 800-fold. This process was successfully applied to harvest cells of *C. calcitrans* and *C. muelleri, Thalassiosira pseudonana, Attheya septentrionalis, Nitzschia closterium, Skeletonema* sp., *Tetraselmis suecica* and

Rhodomonas salina, with efficiencies above 80%; it proved rapid, simple and inexpensive, and relatively independent of processed volume (unlike concentration by centrifugation). The harvested material was readily disaggregated to single cell suspensions by dilution in seawater, coupled with mild agitation. Microscopic examination proved that the final cells are indistinguishable from the nonflocculated ones; and assay for chlorophyll of the concentrates prepared from cultures of up to 130 L showed marginal degradation by 2 weeks of storage [100].

Cryopreservation has been thoroughly adopted by culture collections to preserve strains, but may also find an application in aquaculture [80]. Viable cryostorage of biological specimens has followed various protocols of cooling/thawing rates and cryoprotectant addition, which have been developed and tuned more or less empirically [101]. Recall that temperatures used for cryostorage are well below freezing – down to even -196 ^{o}C in liquid helium, when biological specimens are to be stored without limit [102]. While cryostorage is generally thought to be innocuous to the cell, the events occurring upon freezing or thawing can lead to severe damage, or even cell death. Moreover, cryoprotectants that enhance the cell viability at cryogenic temperatures are usually toxic at physiological temperatures [103] – an obstacle that is overcome by reducing the exposure time or the temperature of incubation prior to cryopreservation [104]. Knowledge of cryoprotectant tolerance levels for microalgae is still limited [105], as well as for early larval stages and for zooplankton that are cultivated and rely on the availability of microalgae for growth. In general, cryopreservation possesses a high potential for culture collections, and may also offer a solution for reliable supply of microalgae in aquaculture. For instance, marine microalgae used in aquaculture were successfully cryopreserved under 4, -20 and -80 ^{o}C using common cryoprotectants (i.e. methanol, dimethylsulfoxide, propylene glycol and polyvinylpyrrolidone), with promising results at least for *Chlorella minutissima, Chlorella stigmatophora, Isochrysis galbana* and *Dunaliella tertiolecta* [80].

Several products based on thraustochytrids (i.e. microorganisms with a taxonomy related to certain microalgal classes), from the genus *Schizochytrium*, have been marketed through Aquafauna Biomarine and Sanders Brine Shrimp. These products have high concentrations of DHA [106], and have accordingly been applied as alternatives to commercial oil enrichment of zooplankton fed to larvae. As direct feeds, most such products have a lower nutritional value than mixtures of microalgae, yet some performed well as components of a mixed diet with alive microalgae [83,107].

In general, substitutes of alive microalgae should present an appropriate physical behavior – and this constitutes a significant challenge; in particular, they should not aggregate or easily break apart. Drying microalgae can cause, due to oxidation, a loss of PUFAs [108], which are essential components for larval growth [87]; the poor performance reported for dried microalgae was associated chiefly with the difficulty to keep cells in suspension without disintegrating them, so as to avoid said oxidation [13]. Moreover, when cell walls are broken, a high fraction of water-soluble components cannot be ingested by the organism, and may consequently interfere with the water quality of the aquaculture [109]. Therefore, pathogenic bacterial proliferation may occur, and cause costly production losses. Similar difficulties arise when using microalga paste, because the preparation procedures (i.e. centrifugation, flocculation or filtration) and/or preservation techniques (i.e. additives or freezing) must ensure that cell wall integrity is essentially preserved.

Products other than alive microalgae must obviously be free of bacterial contamination and devoid of toxicity. Consequently, the use of alive bacteria as a food source in hatcheries seems somehow inappropriate, since physical and chemical treatments are often used to limit bacterial contamination that would otherwise be responsible for drastic larval mortality [110]. Oyster larvae fed with alive microalga diets underwent improved growth via addition of some bacterial isolates [111,112], but this advantage may obviously not be possible in a treated microalgal product. However, in alive microalgae, the natural bacterial flora was proven to enhance the health of molluscs. Langdon and Bolton [88] showed that antibiotic suppression of the bacterial flora in artificial feed of juvenile oysters reduced their growth.

In conclusion, mitigated or unsuccessful results when using nonliving microalgae have turned alive microalgae into the first choice in aquaculture feeding. Only partial replacement thereof has been possible in studies encompassing preserved non-living algae [113], microencapsulated diets [88] or spray-dried algae [114]; but no whole replacement can be recommended, despite intensive research efforts in that direction [107]. Consequently, novel solutions to totally replace microalgae in aquaculture diets cannot at present be widely adopted [4,24,81].

6. Use of microalgae to enrich zooplankton

Microalgae have an important role in aquaculture, also as a means to enrich zooplankton for feeding fish and other larvae afterwards. In addition to providing proteins (that contain essential amino acids) and energy, they carry such other key nutrients as vitamins, essential PUFAs, pigments and sterols – which are transferred up through the food chain. For instance, PUFA-rich microalgae, such as *Pavlova* sp. and *Isochrysis* sp., have been successfully fed to zooplankton to enrich them in DHA [115]. However, when the level of enrichment attained is not sufficient, commercial oil-emulsions are often used. Recently, such products as dried preparations of *Schizochytrium* sp. (which contain 5-15% of their DW as DHA) have been utilized, which produce levels of DHA enrichment in zooplankton comparable to use of commercial oils [116] – and also produce DHA to EPA ratios of 1–2, which are considered favorable for fish larval nutrition [117].

Brown, Skabo & Wilkinson [118] described that rotifers fed with microalgae (e.g. *Isochrysis* sp. and *N. oculata*) become rapidly enriched with ascorbic acid (AsA), whereas rotifers fed on baker's yeast (which itself is deficient in AsA) contained only residual amounts of AsA.; after 16 h of starving, rotifers lost ca. 10% of their AsA, while retaining ca. 50% of the total AsA ingested. Similarly, the concentration of AsA in *Artemia* sp. may be increased by feeding with microalgae [119]. However, little information is available on the transfer of other vitamins from microalgae to fish larvae.

Rønnestad, Helland & Lie [120] demonstrated that microalgal pigments transferred to zooplankton may add to their nutritional value; recall that the dominant pigments in the copepod *Temora* sp. are lutein and astaxanthin, whereas in *Artemia* it is canthaxanthin. When these microalgae were fed to copepods and then to halibut larvae, adequate amounts of vitamin A were found, but not when halibut was fed on *Artemia*; this was attributed to the ability of larvae to convert lutein and/or astaxanthin, but not canthaxanthin to vitamin A. They accordingly recommended that *Artemia* should routinely be enriched with astaxanthin

and lutein (the latter pigment is common in "green" microalgae, e.g. *Tetraselmis* sp.) to improve their nutritional value.

A common procedure during culture of both larval fish and prawns is to add microalgae (i.e. "green water") to intensive culture systems, together with the zooplankton prey [121]. The most popular microalga species used for this purpose are *N. oculata* and *T. suecica*. Addition of microalgae to larval tanks can also improve the production of larvae, but their exact mechanism of action remains unclear. Light attenuation (i.e. shading effects) may have a beneficial effect on larvae; however, maintenance of nutritional quality of the zooplankton, excretion of vitamins or other growth-promoting substances by the microalgae, and probiotic effects of the microalgae have also been hypothesized. Maintenance of NH_3- and O_2-balances has also been proposed, but this assumption failed to be supported by experimental evidence [121]. More research is still needed on the application of other microalgae – especially those species rich in DHA, to green water systems. Green water may also be applied to extensive outdoor production facilities, by fertilizing ponds in attempts to stimulate microalgal growth, and consequently zooplankton production.

7. Avenues for future research on microalgae

The high production costs of microalgae remain a constraint to many hatcheries. Despite efforts developed over the latest decades toward cost-effective artificial diets to replace microalgae, on-site microalgal production still remains a critical element for operation of most marine hatcheries. Improvements in alternative diets will surely continue, but production costs of microalgae will also likely decrease – so it is not expected that microalgae will be replaced in full, at least on the medium run. A wide selection of microalgal species is already available to support aquaculture activities. However, specific applications in industrial subsectors demand novel species with improved nutritional quality or growth characteristics, which are compatible with attempts to improve hatchery efficiency and yield.

Appart from improvements in cost-efficiency of on-site microalgal production, an alternative is centralizing microalga production in dedicated mass-culture facilities, using heterotrophic methods or nonconventional photobioreactors. These technologies may be coupled with post-harvest processing (e.g. spray-drying) or concentration (e.g. centrifugation or flocculation) to develop off-the-shelf microalgal biomass for ready distribution to hatcheries.

On the other hand, antifouling activity of extracts from some microalgae has been observed in microalga culture tanks, which are better (and less toxic) than common biocides. Those natural compounds could therefore be considered as good substitutes of commercial biocides in antifouling paints. Furthermore, as paint coatings remain the predominant preventative technique of marine biofouling, coatings adapted to the needs of aquaculture apparatuses – containing an active product from microalgae and able to inhibit the major microorganisms causing trouble in cultivation, are a potentially good solution to fight fouling. The exact substances that exhibit antifouling activities in microalgae are not yet known, so this type of study is warranted – to purify and identify the active compounds involved.

The need to reduce water consumption in aquaculture has long been recognized, so a great deal of effort has been directed toward development of recirculating systems. Unfortunately, current research and development encompassing aquaculture water re-use is largely devoted to bacteria-based systems – and the possibility of using microalga-based water re-use has been essentially neglected. The bacterial component in a water re-use system dedicates itself in full to excessive nutrient removal; conversely, a microalga-based water re-use system produces microalgae that can be used to produce a second crop, such as bivalve seed or *Artemia* – which may thus be sold to generate extra income. The main difficulty faced in development of microalga-based water re-use systems is the inability to maintain the desired microalgal species in an open system. A breakthrough in marine diatom production technology may allow one to focus on development of water re-use systems where the 'effluent' becomes itself a valuable resource: an integrated shrimp/microalga/oyster production system reduces water consumption, and turns effluent 'waste' into a profitable item – while taking advantage of the antibacterial properties of the marine diatom to control diseases, and thus reduce susceptibility of the shrimp to viral infections.

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9. References

[1] G. Oron, L. R. Wildschut, D. Porath, "Wastewater recycling by duckweed for protein production and effluent renovation," *Water Science and Technology*, vol. 17, pp. 803-818, 1985.

[2] O. Hammouda, A. Gaber, N. Abdel-Raouf, "Microalgae and wastewater treatment," *Ecotoxicology and Environmental Safety*, vol. 31, pp. 205-210, 1995.

[3] W. J. Oswald, "Algal Pond Systems–Habitats," in *Proceedings of a Seminar held at Murdoch University*, Western Australia, 1991, pp. 61-69.

[4] A. Muller-Feuga, "The role of microalgae in aquaculture: situation and trends," *Journal of Applied Phycology*, vol. 12, pp. 527-534, 2000.

[5] C. T. Enright, G. F. Newkirk, J. S. Craigie, J. D. Castell, "Evaluation of phytoplankton as diets for juvenile *Ostrea edulis* L.," *Journal of Experimental Marine Biology and Ecology*, vol. 96, pp. 1-13, 1986.

[6] M. R. Brown, S. W. Jeffrey, J. K. Volkman, G. A. Dunstan, "Nutritional properties of microalgae for mariculture," *Aquaculture,* vol. 151, pp. 315–331, 1997.

[7] K. Yamaguchi, "Recent advances in microalgal bioscience in Japan, with special reference to utilization of biomass and metabolites: a review," *Journal of Applied Phycology*, vol. 8, pp. 487–502, 1997.

[8] W. Becker, "Microalgae for aquaculture. The nutritional value of microalgae for aquaculture." In Richmond, A. (ed.), *Handbook of Microalgal Culture*. Blackwell, Oxford, pp. 380–391, 2004.

[9] C. T. Enright, G. F. Newkirk, J. S. Craigie, J. D. Castell, "Growth of juvenile *Ostrea edulis* L. fed *Chaetoceros gracilis* Schütt of varied chemical composition," *Journal of Experimental Marine Biology and Ecology,* vol. 96, pp. 15-26, 1986.

[10] P. A. Thompson, M. Guo, P. J. Harrison, "The influence of irradiance on the biochemical composition of three phytoplankton species and their nutritional value for larvae of the Pacific oyster (*Crassostrea gigas*)," *Marine Biology*, vol. 117, pp. 259-268, 1993.

[11] L. R. Marínez-Córdova, E. Peña-Messina, "Biotic communities and feeding habits of *Litopenaeus vannamei* (Boone 1931) and *Litopenaeus stylirostris* (Stimpson 1974) in monoculture and polyculture semi-intensive ponds," *Aquaculture Research*, vol. 36, pp. 1075-1084, 2005.

[12] M. Kent, C. L. Browdy, J. W. Leffler, "Consumption and digestion of suspended microbes by juvenile Pacific white shrimp *Litopenaeus vannamei,*" *Aquaculture,* doi: 10.1016/j.aquaculture.2011.06.048, 2011.

[13] P. Coutteau, P. Sorgeloos, "The use of algal substitutes and the requirement for live algae in the hatchery and nursery rearing of bivalve molluscs: an international survey," *Journal of Shellfish* Research, vol.11, pp. 467-476, 1992.

[14] C. E. Epifanio, "Growth in bivalve molluscs: nutritional effects of two or more species of algae in diets fed to the American oyster *Crassostrea virginica* (Gmelin) and the hard clam *Mercenaria mercenaria* (L.)," *Aquaculture,* vol. 18, pp. 1-12, 1979.

[15] D. A. Kreeger, C. J. Langdon, "Effect of dietary protein content on growth of juvenile mussels, *Mytilus trossulus* (Gould 1850)," *Biological Bulletin,* vol. 185, pp. 123-139, 1993.

[16] J. K. Volkman, S. W. Jeffrey, P. D. Nichols, G. I. Rogers, C. D. Garland, "Fatty acid and lipid composition of 10 species of microalgae used in mariculture," *Journal of Experimental Marine Biology and Ecology*, vol. 128, pp. 219-240, 1989.

[17] G. A. Dunstan, J. K. Volkman, S. M. Barrett, J.-M. Leroi, S. W. Jeffrey, "Essential polyunsaturated fatty acids from 14 species of diatom (Bacillariophyceae)," *Phytochemistry,* vol. 35, pp. 155-161, 1994.

[18] R. M. Knuckey, M. R. Brown, S. M. Barrett, G. M. Hallegraeff, "Isolation of new nanoplanktonic diatom strains and their evaluation as diets for juvenile Pacific oysters (*Crassostrea gigas*)," *Aquaculture,* vol. 211, pp. 253-274, 2002.

[19] D. Chuntapa, S. Powtongsook, P. Menasveta, "Water quality control using *Spirulina platensis* in shrimp culture tanks," *Aquaculture*, vol. 220, pp. 355-366, 2003.

[20] G. D. Lio-Po, E. M. Leaño, M. M. D. Peñaranda, A. U. Villa- Franco, C. D. Sombito, N. G. Guanzon, "Antiluminous *Vibrio* factors associated with the 'green water' growout culture of the tiger shrimp *Penaeus monodon*," *Aquaculture*, vol. 250, pp. 1-7, 2005.

[21] L. Rodolfi, G. C. Zittelli, L. Barsanti, G. Rosati, M. R. Tredici, "Growth medium recycling in *Nannochloropsis* sp. mass cultivation," *Biomolecular Engineering*, vol. 20, pp. 243-248, 2003.

[22] A. Muller-Feuga, "Microalgae for aquaculture. The current global situation and future trends." In Richmond, A. (ed.), *Handbook of Microalgal Culture*. Blackwell, Oxford, 2004, pp. 352-364.

[23] A. Irianto, B. Austin, "Probiotics in aquaculture," *Journal of Fish Diseases*, vol. 25, pp. 633-642, 2002.

[24] P. Spolaore, C. Joannis-Cassan, E. Duran, A. Isambert, "Review: commercial applications of microalgae," *Journal of Bioscience and Bioengineering,* vol. 101, pp. 87-96, 2006.

[25] D. S. Nichols, "Prokaryotes and the input of polyunsaturated fatty acids to the marine food web," *FEMS Microbiology Letters*, vol. 219, pp. 1-7, 2003.

[26] C. Aragão, L. E. C. Conceição, M. T. Dinis, H.-J. Fyhn," Amino acid pools of rotifers and *Artemia* under different conditions: nutritional implications for fish larvae," *Aquaculture*, vol. 234, pp. 429-445, 2004.

[27] K. E. Apt, P. W. Behrens, "Commercial developments in microalgal biotechnology," *Journal of Phycology*, vol. 35, pp. 215-226, 1999.

[28] L. Waldenstedt, J. Inborr, I. Hansson, K. Elwinger, "Effects of astaxanthin-rich algal meal (*Haematococcus pluvalis*) on growth performance, faecal campylobacter and clostridial counts and tissue astaxanthin concentration of broiler chickens," *Animal Feed Science and Technology*, vol. 108, pp. 119-132, 2003.

[29] M. R. Brown, "The amino acid and sugar composition of 16 species of microalgae used in mariculture," *Journal of Experimental Marine Biology and Ecology*, vol. 145, pp. 79-99, 1991.

[30] M. R. Brown, G. A. Dunstan, S. W. Jeffrey, J. K., Volkman, S. M. Barrett, J. M. Leroi, "The influence of irradiance on the biochemical composition of the prymnesiophyte *Isochrysis* sp. (clone T-ISO)," *Journal of Phycology*, vol. 29, pp. 601-612, 1993.

[31] M. R. Brown, C. D. Garland, S. W. Jeffrey, I. D. Jameson, J. M. Leroi, "The gross and amino acid compositions of batch and semi-continuous cultures of *Isochrysis* sp. (clone T.ISO), *Pavlova lutheri* and *Nannochloropsis oculata*," *Journal of Applied Phycology*, vol. 5, pp. 285-296, 1993.

[32] J. Knauer, S. M. Barrett, J. K. Volkman, P. C. Southgate, "Assimilation of dietary phytosterols by Pacific oyster *Crassostrea gigas* spat," *Aquaculture Nutrition*, vol. 5, pp. 257-266, 1999.

[33] J. Fabregas, C. Herrero, "Marine microalgae as a potential source of minerals in fish diets," *Aquaculture*, vol. 51, pp. 237-243, 1986.

[34] S. M. Renaud, L. V. Thinh, D. L. Parry, "The gross composition and fatty acid composition of 18 species of tropical Australia microalgae for possible use in mariculture," *Aquaculture*, vol. 170, pp. 147-159, 1999.

[35] P. J. Harrison, P. A. Thompson, G. S. Calderwood, "Effects of nutrient and light limitation on the biochemical composition of phytoplankton," *Journal of Applied Phycology*, vol. 2, pp. 45-56, 1990.

[36] J. N. C. Whyte, N. Bourne, C. A. Hodgson, "Influence of algal diets on biochemical composition and energy reserves in *Patinopecten yessoensis* (Jay) larvae," *Aquaculture*, vol. 78, pp. 333-347, 1989.

[37] J. A. Mendiola, C. F. Torres, P. J. Martín-Alvarez, S. Santoyo, A. Toré, B. O. Arredondo, F. J. Señoráns, A. Cifuentes, E. Ibáñez, "Use of supercritical CO_2 to obtain extracts with antimicrobial activity from *Chaetoceros muelleri* microalga. A correlation with their lipidic content," *European Food Research and Technology,* vol. 224, pp. 505-510, 2007.

[38] K. Benkendorff, A. R. Davis, C. N. Rogers, J. B. Bremner, "Free fatty acids and sterols in the benthic spawn of aquatic molluscs, and their associated antimicrobial

properties," *Journal of Experimental Marine Biology and Ecology,* vol. 316, pp. 29-44, 2005.

[39] V. J. Smith, A. P. Desbois, E. A. Dyrynda, "Conventional and unconventional antimicrobials from fish, marine invertebrates and micro-algae," *Marine Drugs*, vol. 8, pp. 1213-1262, 2010.

[40] F. Jüttner, "Liberation of 5,8,11,14,17-eicosapentaenoic acid and other polyunsaturated fatty acids from lipids as a grazer defense reaction in epiphithic diatom biofilms," *Journal of Phycology,* vol. 37, pp. 744-755, 2001.

[41] G. d'Ippolito, S. Tucci, A. Cutignano, G. Romano, G. Cimino, A. Miralto, A. Fontana, "The role of complex lipids in the synthesis of bioactive aldehydes of the marine diatom *Skeletonema costatum*," *Biochimica et Biophysica Acta,* vol. 1686, pp. 100-107, 2004.

[42] M. Naviner, J.-P. Bergé, P. Durand, H. le Bris, "Antibacterial activity of the marine diatom *Skeletonema costatum* against aquacultural pathogens," *Aquaculture*, vol. 174, pp. 15-24, 1999.

[43] K. Das, J. Pradhan, P. Pattnaik, B. R. Samantaray, S. K. Samal, "Production of antibacterials from the freshwater alga *Euglena viridis* (Ehren)," *World Journal of Microbiology and Biotechnology,* vol. 21, pp. 45-50, 2005.

[44] M. A. Borowitzka, "Commercial production of microalgae: ponds, tanks, tubes and fermenters," *Journal of Biotechnology*, vol. 70, pp. 313–321, 1999.

[45] H. M. Amaro, A. C. Guedes, F. X. Malcata, "Advances and perspectives in using microalgae to produce biodiesel," *Applied Energy*, vol. 88, pp. 3402-3410, 2011.

[46] S. A. Scott, M. P. Davey, J. S. Dennis, I. Horst, C. J. Howe, D. J. Lea-Smith, "Biodiesel from algae: challenges and prospects," *Current Opinion in Biotechnology,* vol. 21, pp. 277-286, 2010.

[47] P. Soong, "Production and development of *Chlorella* and *Spirulina* in Taiwan." In: Shelef, G., Soeder, C. J. (Eds.), *Algae Biomass*. Elsevier, Amsterdam, 1980, pp. 97-113.

[48] L. J. Borowitzka, M. A. Borowitzka, "Industrial production: methods and economics." In: Cresswell, R. C., Rees, T. A. V., Shah, N. (Eds.), *Algal and Cyanobacterial Biotechnology*. Longman Scientific, London, 1989, pp. 294–316.

[49] A. Belay, "Mass culture of *Spirulina* outdoors—the Earthrise Farms experience". In: Vonshak, A. (Ed.), *Spirulina platensis* (*Arthrospira*): *Physiology, Cell-biology and Biotechnology.* Taylor & Francis, London, 1997, pp. 131-158.

[50] I. Setlík, S. Veladimir, I. Malek, "Dual purpose open circulation units for large scale culture of algae in temperate zones. I. Basic design considerations and scheme of pilot plant," *Algology Studies (Trebon)*, vol. 1, pp. 11, 1970.

[51] K. Kawaguchi, "Microalgae production systems in Asia." In: Shelef, G., Soeder, C. J. (Eds.), *Algae Biomass Production and Use*. Elsevier, Amsterdam, 1980, pp. 25-33.

[52] J. Kyle, R. M. Gladue, "Eicosapentaenoic acids and methods for their production," *World Patent,* vol. 9, pp. 114,427, 1991.

[53] J. Kyle, S. E. Reeb, V. J. Sicotte, "Dinoflagellate biomass, methods for its production, and compositions containing the same," *USA Patent* 5,711,983, 1998.

[54] A. P. Carvalho, L. A. Meireles, F. X. Malcata, "Microalgal reactors: a review of enclosed system designs and performances," *Biotechnology Progress,* vol. 22, pp. 1490-1506, 2006.

[55] C. Jiménez, B. R. Cossío, D. Labella, N. F. Xavier, "The feasibility of industrial production of *Spirulina* (*Arthrospira*) in southern Spain," *Aquaculture,* vol. 217, pp. 179-190, 2003.

[56] D. Chaumont, "Biotechnology of algal biomass production: a review of systems for outdoor mass culture," *Journal of Applied Phycology*, vol. 5, pp. 593-604, 1993.

[57] L. Terry, L. P. Raymond, "System design for the autotrophic production of microalgae," *Enzyme and Microbial Technology,* vol. 7, pp. 474-487, 1985.

[58] Y. Chisti, "Biodiesel from microalgae beats bioethanol," *Trends in Biotechnology,* vol. 26, pp. 126-131, 2008.

[59] L. Rodolfi, G. C. Zittelli, N. Bassi, G. Padovani, N. Biondi, G. Bonini, "Microalgae for oil: strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor," *Biotechnology and Bioengineering,* vol. 102, pp. 100-112, 2008.

[60] U. Ugwu, H. Aoyagi, H. Uchiyama, "Photobioreactors for mass cultivation of algae," *Bioresource Technology*, vol. 99, pp. 4021-4028, 2008.

[61] Y. Chisti, "Biodiesel from microalgae," *Biotechnology Advances*, vol. 25, pp. 294-306, 2007.

[62] O. Pulz, "Photobioreactors: production systems for phototrophic microorganisms," *Applied Microbiology and Biotechnology,* vol. 57, pp. 287-293, 2001.

[63] B. Metting, "Biodiversity and application of microalgae," *Journal of Industrial Microbiology,* vol. 17, pp. 477-489, 1996.

[64] E. Molina-Grima, "Microalgae, mass culture methods." In M. C. Flickinger, & S. W. Drew (Eds.), *Encyclopedia of Bioprocess Technology*: *Fermentation*, *Biocatalysis and Bioseparation*. New York: Wiley, 1999, vol. 3, pp. 1753-1769.

[65] R. Tredici, "Bioreactors, photo." In Flickinger, M. C. & Drew, S. W. (Eds.), *Encyclopedia of Bioprocess Technology*: *Fermentation*, *Biocatalysis and Bioseparation*, vol. 1. New York, Wiley, 1999, pp. 395-419.

[66] G. Acién-Fernández, F. García-Camacho, J. A. Sánchez-Pérez, J. M. Fernández-Sevilla, E. Molina-Grima, "Modelling of biomass productivity in tubular photobioreactors for microalgal cultures: effects of dilution rate, tube diameter and solar irradiance," *Biotechnology and Bioengineering*, vol. 58, pp. 605-616, 1998.

[67] S. Aiba, "Growth kinetics of photosynthetic microorganisms," *Advances in Biochemical Engineering*, vol. 23, pp. 85-156, 1982.

[68] L. E. Erickson, H. Y. Lee, "Process analysis and design of algal growth system." In: Barclay, W. R. and McIntosh, R. P. (Eds), *Algal Biomass Technologies: an Interdisciplinary Perspective*. J. Cramer, Berlin-Stuttgart, 1986, pp. 197–206.

[69] S. L. Pirt, Y. K. Lee, M. R. Walach, M. W. Pirt, H. H. Balyuzi, M. J. Bazin, "A tubular bioreactor for photosynthetic production of biomass from carbon dioxide: design and performance," *Journal of Chemical Technology and Biotechnology*, vol. 33B, pp. 35-58, 1983.

[70] L. Rorrer, R. K. Mullikin, "Modeling and simulation of a tubular recycle photobioreactor for macroalgal cell suspension cultures," *Chemical Engineering Science*, vol. 54, pp. 3153-3162, 1999.

[71] C. Posten, "Design principles of photo-bioreactors for cultivation of microalgae," *Engineering in Life Sciences,* vol. 9, pp. 165-177, 2009.

[72] A. A. Tsygankov, "Laboratory scale photobioreactors," *Applied Biochemistry and Microbiology,* vol. 37, pp. 333-341, 2001.

[73] A. Borowitzka, "Microalgae as sources of pharmaceuticals and other biologically active compounds," *Journal* of *Applied Phycology*, vol. 7, pp. 3-15, 1995.
[74] A. Vonshak, "Tubular photobioreactors for algal mass production; prospects and achievements," *Israeli Journal of Aquaculture (Bamidgeh),* vol. 44, pp. 151, 1992.
[75] M. Olaizola, "Commercial development of microalgal biotechnology: from the test tube to the marketplace," *Biomolecular Engineering,* vol. 20, pp. 459-466, 2003.
[76] E. Molina-Grima, F. G. Acién-Fernández, F. García-Camacho, F. Camacho-Rubio, Y. Chisti, "Scale-up of tubular photobioreactors," *Journal of Applied Phycology,* vol. 12, pp. 355-368, 2000.
[77] N. de Pauw, G. Persoone, "Micro-algae for aquaculture." In: Borowitzka, M. A., Borowitzka, L. J. (Eds.), *Micro-Algal Biotechnology*. Cambridge University Press, Cambridge, 1988, pp. 197-221.
[78] R. Benemann, W. J. Oswald, "Systems and economic analysis of microalgae ponds for conversion of CO_2 to biomass," *Final Report, Subcontract XK 4-04136- 06*, Pittsburgh Energy Technology Center Grant No. DE-FG22-93PC93204, p. 260, 1996.
[79] FAO, 2000. *Aquaculture Production 1992–1998*. FAO, Rome.
[80] I. Tzovenis, G. Triantaphyllidis, X. Naihong, E. Chatzinikolaou, K. Papadopoulou, G. Xouri, T. Tafas, "Cryopreservation of marine microalgae and potential toxicity of cryoprotectants to the primary steps of the aquacultural food chain," *Aquaculture*, vol. 230, pp. 457-473, 2004.
[81] M. A. Borowitzka, "Microalgae for aquaculture: opportunities and constraints," *Journal of Applied Phycology*, vol. 9, pp. 393-401, 1997.
[82] J. Knauer, P. C. Southgate, "A review of the nutritional requirements of bivalves and the development of alternative and artificial diets for bivalve aquaculture," *Reviews in Fisheries Science*, vol. 7, pp. 241–280, 1999.
[83] R. Robert, P. Trintignac, "Substitutes for live microalgae in mariculture: a review," *Aquatic Living Resources*, vol. 10, pp. 315–327, 1997.
[84] G. Chini-Zittelli, F. Lavista, A. Bastianini, L. Rodolfi, M. Vincenzini, M. R. Tredici, "Production of eicosapentaenoic acid by *Nannochloropsis* sp. cultures in outdoor tubular photobioreactors," *Journal of Biotechnology*, vol. 70, pp. 299-312, 1999.
[85] B. Spolaore-Robinson, T. M. Samocha, J. M. Fox, R. L. Gandy, D. A. McKee, "The use of inert artificial commercial food sources as replacements of traditional live food items in the culture of larval shrimp, *Farfantepenaeus aztecus*," *Aquaculture*, vol. 245, pp. 135-147, 2005.
[86] C. Seguineau, A. Laschi-Loquerie, J. Moral, J.-F. Samain, "Vitamin requirements in great scallop larvae," *Aquaculture International*, vol. 4, pp. 315-324, 1996.
[87] R. Brown, S. M. Barret, J. K. Volkman, S. P. Nearhos, J. Nell, G. L. Allan, "Biochemical composition of new yeasts and bacteria evaluated as food for bivalve aquaculture," *Aquaculture*, vol. 143, pp. 341-360, 1996.
[88] J. Langdon, E. T. Bolton, "A microparticulate diet for suspension-feeding bivalve mollusc, *Crassostrea virginica* (Gmelin)," *Journal of Experimental Marine Biology and Ecology,* vol. 89, pp. 239-258, 1984.
[89] P. Coutteau, M. Dravers, P. Leger, P. Sorgeloos, "Manipulated yeast diets and dried algae as a partial substitute for live algae in the juvenile rearing of the Manila clam *Tapes philippinarum* and the Pacific oyster *Crassostrea gigas*," *Special Publication of the European Aquatic Society Ghent*, Belgium, vol. 18, pp. 523-531, 1993.

[90] J. A. Nell, W. A. O'Connor, "The evaluation of fresh algae and stored algal concentrates as a food source for Sydney rock oyster, *Saccostrea commercialis* (Iredale and Roughley) larvae," *Aquaculture*, vol. 99, pp. 277-284, 1991.

[91] A. McCausland, M. R. Brown, S. M. Barrett, J. A. Diemar, M. P. Heasman, "Evaluation of live and pasted microalgae as supplementary food for juvenile Pacific oysters (*Crassostrea gigas*)," *Aquaculture and Research*, vol. 174, pp. 323-342, 1999.

[92] M. Heasman, J. Diemar, W. O'Connor, T. Sushames, L. Foulkes, "Development of extended shelf-life micro-algae concentrate diets harvested by centrifugation for bivalve molluscs—a summary," *Aquaculture and Research*, vol. 31, pp. 637–659, 2000.

[93] A. Muller-Feuga, R. Robert, C. Cahu, J. Robin, P. Divemach, "Use of microalgae in aquaculture." In: Støttrup, J. A., McEvoy, L. A., (Eds), *Live Feeds in Marine Aquaculture*. Blackwell, Oxford, 2003, pp. 253-299.

[94] E. Montaini, G. C. Zittelli, M. R. Tredici, E. M. Grima, J. M. F. Sevilla, J. A. S. Perez, "Long-term preservation of *Tetraselmis suecica*: influence of storage on viability and fatty acid profile," *Aquaculture*, vol. 134, pp. 81-90, 1995.

[95] A. Csordas, J.-K. Wang, "An integrated photobioreactor and foam fractionation unit for the growth and harvest of *Chaetoceros* spp. in open systems," *Aquacultural Engineering*, vol. 30, pp. 15-30, 2004.

[96] M. Millamena, E. J. Aujero, I. G. Borlongan, "Techniques on algae harvesting and preservation for use in culture as larval food," *Aquaculture Engineering*, vol. 9, pp. 295-304, 1990.

[97] E. Poelman, N. de Pauw, B. Jeurissen, "Potential of electrolytic flocculation for recovery of micro-algae," *Resources*, *Conservation* and *Recycling*, vol. 19, pp. 1-10, 1997.

[98] N. Rossingol, L. Vandanjon, P. Jaouen, F. Quéméneur, "Membrane technology for the continuous separation microalgae/culture medium: compared performances of cross-flow microfiltration and ultrafiltration," *Aquaculture Engineering*, vol. 20, pp. 191-208, 1999.

[99] E. Sandbank, "The utilization of microalgae as feed for fish," *Ergeb Limnology*, vol. 11, pp. 108-120, 1978.

[100] R. M. Knuckey, M. R. Brown, R. Robert, D. M. F. Frampton, "Production of microalgal concentrates by flocculation and their assessment as aquaculture feeds," *Aquacultural Engineering*, vol. 35, pp. 300-313, 2006.

[101] J. O. M. Karlson, M. Toner, "Long-term storage of tissues by cryopreservation: critical issues," *Biomaterials*, vol. 17, pp. 243-256, 1996.

[102] P. Mazur, "Freezing of living cells: mechanisms and implications," *American Journal of Physiology*, vol. 247, pp. 125-142, 1984.

[103] M. Fahy, "The relevance of cryoprotectant 'toxicity' to cryobiology," *Cryobiology*, vol. 23, pp. 1-13, 1986.

[104] M. Fahy, T. H. Lilley, H. Lindsell, M. J. Douglas, H. T. Meryman, "Cryoprotectant toxicity and cryoprotectant toxicity reduction: in search of molecular mechanisms," *Cryobiology*, vol. 27, pp. 247- 268, 1990.

[105] R. Taylor, R. L. Fletcher, "Cryopreservation of eukaryotic algae—a review of methodologies," *Journal of Applied Phycology*, vol. 10, pp. 481–501, 1999.

[106] W. Barclay, S. Zeller, "Nutritional enhancement of n-3 and n-6 fatty acids in rotifers and *Artemia nauplii* by feeding spray-dried *Schizochytrium* sp.," *Journal of the World Aquaculture Society*, vol. 27, pp. 314-322, 1996.

[107] C. Langdon, E. Önal, "Replacement of living microalgae with spray-dried diets for the marine mussel *Mytilus galloprovincialis,*" *Aquaculture*, vol. 180, pp. 283-294, 1999.

[108] A. Dunstan, J. K. Volkman, S. W. Jeffrey, S. M. Barret, "Biochemical composition of microalgae from the green algal classes Chlorophyceae and Prasinophyceae," *Journal of Experimental Marine Biology and Ecology,* vol. 161, pp. 115-134, 1992.

[109] J. Dhont, G. van Stappen, *Live Feeds in Marine Aquaculture*, Blackwell Science, pp. 65-121. 2003.

[110] A. Elston, "Mollusc diseases, guide for the shellfish farmer," *Washington Sea Grant Program*, Seattle, WA, p. 73, 1990.

[111] P. Douillet, C. J. Langdon, "Effects of marine bacteria on the culture of axenic oyster *Crassostrea gigas* (Thunberg) larvae," *Biological Bulletin,* vol. 184, pp. 36-51, 1993.

[112] P. Douillet, C. J. Langdon, "Use of a probiotic for the culture of larvae of the Pacific oyster (*Crassostrea gigas* Thunberg)," *Aquaculture*, vol. 119, pp. 25-40, 1994.

[113] J. Donaldson, *Proceedings of US-Asia Workshop*, Honolulu, HA, January 28-31, 1991, The Oceanic Institute, HA, pp. 229-236.

[114] B. Zhou, W. Liu, W. Qu, C. K. Tseng, "Application of *Spirulina* mixed feed in the breeding of bay scallop,"*Bioresource Technology*, vol. 38, pp. 229-232, 1991.

[115] P. D. Nichols, D. G. Holdsworth, J. K. Volkman, M. Daintith, S. Allanson, "High incorporation of essential fatty acids by the rotifer *Brachionus plicatilis* fed on the prymnesiophyte alga *Pavlova lutheri*," *Australian Journal of Marine and Freshwater Research,* vol. 40, pp. 645-655, 1989.

[116] B. Gara, R. J. Shields, L. McEvoy, "Feeding strategies to achieve correct metamorphosis of Atlantic halibut, *Hippoglossus hippoglossus* L., using enriched *Artemia*," *Aquaculture Research*, vol. 29, pp. 935-948, 1998.

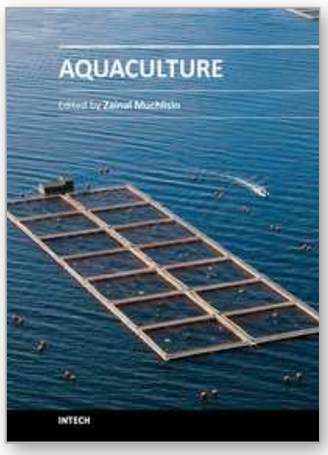
[117] C. Rodríguez, J. A. Pérez, P. Badía, M. S. Izquierdo, H. Fernández-Palacios, A. Lorenzo Hernández, "The n-3 highly unsaturated fatty acid requirements of gilthead seabream (*Sparus aurata* L.) larvae when using an appropriate DHA/EPA ratio in the diet," *Aquaculture,* vol. 169, pp. 9-23, 1998.

[118] M. R. Brown, S. Skabo, B. Wilkinson, "The enrichment and retention of ascorbic acid in rotifers fed with microalgal diets," *Aquaculture Nutrition,* vol. 4, pp. 151-156, 1998.

[119] G. Merchie, P. Lavens, P. Dhert, M. Dehasque, H. Nelis, A. de Leenheer, P. Sorgeloos, "Variation of ascorbic acid content in different live food organisms," *Aquaculture*, vol. 134, pp. 325-337, 1995.

[120] I. Rønnestad, S. Helland, Ø. Lie, "Feeding *Artemia* to larvae of Atlantic halibut (*Hippoglossus hippoglossus* L.) results in lower larval vitamin A content compared with feeding copepods," *Aquaculture*, vol. 165, pp. 159-164, 1998.

[121] C. S. Tamaru, R. Murashige, C.-S. Lee, "The paradox of using background phytoplankton during the larval culture of striped mullet, *Mugil cephalus* L.," *Aquaculture*, vol. 119, pp. 167-174, 1994.



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This book provides an understanding on a large variety of aquaculture related topics. The book is organized in four sections. The first section discusses fish nutrition second section is considers the application of genetic in aquaculture; section three takes a look at current techniques for controlling lipid oxidation and melanosis in Aquaculture products. The last section is focused on culture techniques and management, ,which is the larger part of the book. The book chapters are written by leading experts in their respective areas. Therefore, I am quite confident that this book will be equally useful for students and professionals in aquaculture and biotechnology.

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