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REVIEW

The commercial potential of *Aphanizomenon flos-aquae*, a nitrogen-fixing edible cyanobacterium

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Abstract

Aphanizomenon flos-aquae (AFA) is a Gram-negative nitrogen-fixing freshwater filamentous cyanobacterium with a rich nutritional profile that is approved for human consumption by both the Food and Drug Administration and the European Food and Safety Authorities. It has a high protein content (60-70%) and contains numerous vitamins, minerals and trace elements together with several high-value compounds with nutraceutical properties, such as C-phycocyanin and β-phenylethylamine. 500-1000 t of *AFA* dry biomass are currently wild harvested from natural blooms that occur seasonally in Klamath Lake, Oregon, USA, and distributed as a nutritional supplement worldwide. The requirements and unreliability of wild harvesting, owing to the dependence of *AFA* growth on environmental conditions and potential contamination by microcystin toxins, threaten the availability of biomass supply and restricts commercial expansion. In this review we propose *AFA* cultivation in open ponds or closed photobioreactors to obtain a reliable production of unialgal biomass to resolve the supply issue and enhance *AFA* as a feedstock for specific high-value by-products. Furthermore, we explore potential strategies for maximising overall yield and seasonal robustness by using a synergistic combination of cultivation and wild harvesting. Controlled *AFA* cultivation would also facilitate the use of genetic manipulation to generate bespoke strains with improved commercial applications, such as increasing the cyanobacterium's nitrogen-fixation rate to enhance its value as a biofertiliser. Ultimately, realising the untapped biotechnological potential of *AFA* requires a better understanding of its fundamental biology, robust methodologies for laboratory and large-scale cultivation, and the development of *AFA*-specific genetic engineering techniques.

Keywords *Aphanizomenon flos-aquae* (*AFA*) · Phycocyanin · Nitrogen Fixing · Mass Cultivation · Filamentous cyanobacterium · Photobioreactor (PBR)

Introduction

Cyanobacteria, also known as blue-green algae, are some of the oldest and most abundant photosynthetic microorganisms on our planet and contributed to the so-called "oxygenic revolution" 2.4 billion years ago (Zahra et al. 2020). With the increasing demand for sustainable and environmentally friendly production of high-value compounds, cyanobacteria have emerged as attractive candidates for biotechnological applications (Zahra et al. 2020; Sproles et al. 2021). Cyanobacteria are capable of producing multiple secondary metabolites and can be considered as sunlight-driven microcellular factories (Al-Haj et al. 2016). Their relatively simple growth requirements allow inexpensive maintenance and cultivation, and modern genome analysis and genetic engineering tools make possible the creation of bespoke strains for the production of high-value products (Sreenikethanam et al. 2022). Nevertheless, the biotechnological exploitation of cyanobacteria is currently limited to a few model species such as *Synechococcus* sp. PCC 7002 (CP000951) or *Anabaena* sp. PCC 7120 (BA000019) (Zahra et al. 2020). The majority of cyanobacterial species have either not been studied, or are unable to adapt to laboratory growth conditions and be genetically manipulated (Lea-Smith et al. 2021). Cyanobacteria are also well-known for their high nutritional value and protein content, which have made them important players in the food and supplement markets. Among these, we find several species of filamentous cyanobacteria, such as *Arthrospira* spp., which are commercially marketed

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as Spirulina, and colonial species such as *Nostoc*, with a notable presence in Asian markets (Li and Guo, 2018, Zhu et al., 2023, Gentscheva et al. 2023). Currently, Spirulina dominates the cyanobacterial food market, together with the green algae from the genus *Chlorella* (Andrade et al. 2018). However, there exist other cyanobacteria that have an undiscovered and untapped biotechnological potential. One such example is *Aphanizomenon flos-aquae* (AFA).

Aphanizomenon flos-aquae Ralfs ex Bornet & Flahault is a filamentous nitrogen-fixing obligate phototroph that was first discovered towards the end of the 19th century (Cirés and Ballot 2016). It belongs to the Nostocacae family and is the type species for the genus *Aphanizomenon* (Cirés and Ballot 2016). Its most distinguishing feature is its ability to form fascicles: bundles of filaments that can reach lengths of 2 cm and can be seen with the naked eye (Carmichael et al. 2000). *AFA* is widely distributed in eutrophic freshwater ecosystems at high latitudes where it has developed traits that are unique to the Nostocacae (Cirés and Ballot 2016). These include nitrogen fixation, tolerance to varying temperatures and pH levels, buoyancy-dependent adjustments in the water column and the ability to regulate the expression of light-absorbing pigments (Komárek and Komárková, 2006; Komárek 2013). Furthermore, the vegetative cells of *AFA* can specialise into akinetes: dormant cells that form under unfavourable conditions, and which have enabled *AFA*'s survival across the millennia (Wildman et al. 1975).

AFA has attracted commercial interest owing to its high nutritional value. It boasts a high protein content (60-70%). It is a rich source of minerals and vitamins and is characterised by a high concentration of omega-3 polyunsaturated fatty acids (PUFAs) (Sandgruber et al. 2021). Furthermore, *AFA* produces a number of bioactive compounds, the most impactful being C-phycocyanin (C-PC), a pigment with potent anti-inflammatory and antioxidant effects, and β-phenylethylamine (PEA), an organic and endogenous neurostimulator (Benedetti et al. 2006; Nuzzo et al. 2018; Billy et al 2023). *AFA* is approved for human consumption by both the Food and Drug Administration (FDA) and the European Food and Safety Authorities (EFSA). Currently, its biomass is marketed worldwide as a nutritional supplement (Schaeffer et al. 1999; Carmichael et al. 2000).

The *AFA* biomass used for commercial products is exclusively harvested from the wild; specifically from Klamath Lake in Oregon, USA (Fig. 1). Here, *AFA* forms seasonal blooms with dense mats of the cyanobacterium accumulating

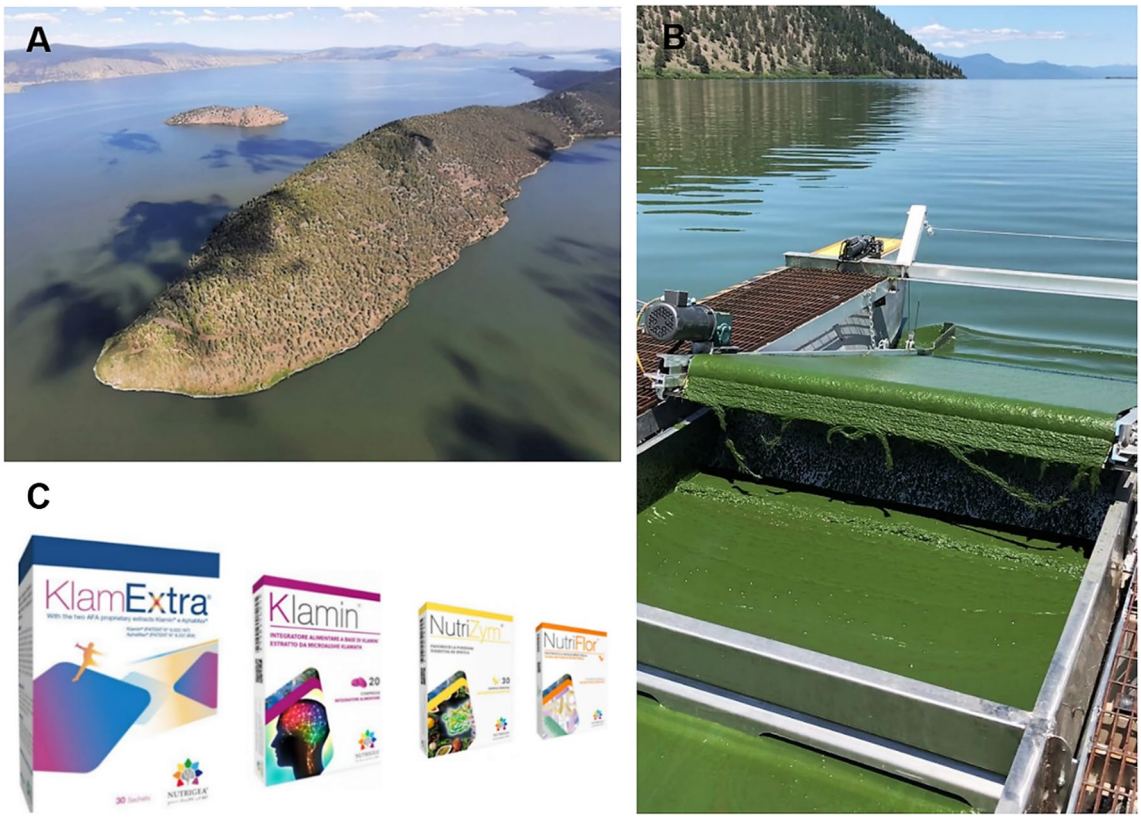

Fig. 1 Illustration of *Aphanizomenon flos-aquae (AFA)* harvesting in Klamath Lake, Oregon, and the derived products obtained. A) aerial photograph of Upper Klamath Lake (UKL) taken days before the explosion of an *AFA* bloom, B) photograph of *AFA* harvesting during a summer bloom taken from Online Resource 1, and C) *AFA*-based supplements sold by Nutrigea s.r.l.

at the lake water surface. These blooms are harvested *en masse* (see: Online Resource 1), processed, and further commercialised (Carmichael et al. 2000). *AFA* is the second most consumed cyanobacterial nutritional supplement following Spirulina (Grewe and Pulz 2012). However, due to the limitations of wild harvesting, *AFA*'s market has not been able to expand within the last 20 years. *AFA* blooms are seasonal and dependent on specific natural conditions, making *AFA* harvesting viable only during the warmer months (Carmichael et al. 2000). Furthermore, Klamath Lake also harbours *Microcystis aeruginosa,* a cyanobacterium known to produce a class of hepatotoxins, termed microcystins (Carmichael et al. 2000; Sivonen 2009). As a result, companies harvesting from Klamath Lake must ensure that any recovered *AFA* biomass has a microcystin concentration that is below the threshold considered safe for human consumption, which further reduces the number of harvestable days per annum (WHO, 1999, Saker et al. 2005, EPA, 2016, Lyon-Colbert et al. 2018). Overall, *AFA*'s commercial expansion has been restricted by these harvesting requirements of quantity and quality. However, these restrictions could be overcome through cultivation under carefully controlled conditions using optimised media in photobioreactors (PBRs).

Advances in the controlled, large-scale cultivation of microalgae and cyanobacteria are enabling the industrialisation of previously non-commercialisable microalgae, including strains of *Chlorella*, *Arthrospira*, and *Nannochloropsis* (Belay 2013; Safi et al. 2014; Al-Hoqani et al. 2016). Notably, the cultivation of Spirulina and *Chlorella* has resulted in a rapid increase in their global production, leading them to dominate the microalgal- and cyanobacterial-based industry (Araújo et al. 2021; Show 2022). Additionally, recent improvements in PBR design have facilitated successful large-scale cultivation of diverse microalgae and cyanobacteria with unique properties, allowing for enhanced utilisation of their high-value products (Deprá et al. 2019; Legrand et al. 2021). For instance, the mass cultivation of the green alga *Haematococcus pluvialis* in tubular PBRs for the extraction of the valuable astaxanthin pigment has been highly successful (Li et al., 2020). Considering these factors, the cultivation of *AFA* holds significant commercial potential, particularly due to its established market presence (Carmichael et al. 2000). Controlled growth of *AFA* in PBRs has the potential to not only address the issue of limited biomass supply but also opens avenues for expanding *AFA*-based products into new markets, such as animal feed, biofertilisers and pharmaceuticals (Kumar et al. 2019).

Knowledge of the optimal growth requirements for *AFA* remains limited. There are only a handful of studies that have investigated laboratory cultivation of *AFA*, and, to date, its mass cultivation has never been achieved or even attempted (Gerloff et al. 1950, Gentile and Maloney 1969, O'Flaherty and Phinney, 2008, Debella 2007). Therefore, the greatest risk associated with the deployment of a commercial facility for mass cultivation is technological feasibility (Kumar et al. 2021). Furthermore, the capital and operating costs associated with cyanobacterial mass cultivation would not be able to compete with the cost-effectiveness of Klamath Lake harvesting unless the PBR-based products carried a significant premium (Zhu et al. 2018; Clippinger and Davis 2019). Therefore, a more attractive strategy involves a combination and integration of both *AFA* cultivation in PBRs and wild harvesting from Klamath Lake, leveraging the synergistic benefits of both approaches. By cultivating *AFA* in PBRs the overall annual yield of *AFA* can be increased, providing a more reliable baseline production, while the bulk *AFA* biomass from wild harvesting could help off-set the overall costs associated with PBR cultivation.

Another advantage of successful cultivation of *AFA* in the laboratory is the opportunity to investigate strain improvements by developing molecular tools for genetic manipulation (Santos-Merino et al. 2019). Cyanobacteria such as *Synechococcus* and *Anabaena* have been successfully genetically engineered with varying degrees of development of the molecular toolkits and technologies for strain improvement (Kumar et al. 2020; Sproles et al. 2021; Grama et al. 2022). The establishment of DNA transformation technology for *AFA* would allow the generation of optimised strains for a range of biotechnological purposes. For example, as *AFA* is one of only a few safe and commercially distributed cyanobacterial species that is a diazotroph – i.e. able to convert atmospheric nitrogen into bioavailable forms of nitrogen – the enhancement of *AFA*'s nitrogen fixation rates would greatly promote its performance as a biofertiliser (Chaurasia and Apte 2011; Grewe and Pulz 2012). Nevertheless, genetic modification of filamentous cyanobacteria presents numerous challenges. For instance, a high concentration of extracellular polymeric substances can impede DNA entry into cells, while a substantial number of endogenous restriction endonuclease systems within the cell may degrade incoming DNA. Some filamentous species, such as *Anabaena* sp. 90 (CP003284), have proven recalcitrant to genetic modification, even after extensive characterisation. Nevertheless, as no transformation attempts have been made with *AFA* thus far, the feasiblity of its genetic modification has yet to be determined.

The primary objective of this review is to highlight *AFA*'s untapped biotechnological potential. We examine the biology and nutritional benefits of *AFA* and address the existing knowledge gaps that may impede a broader understanding of its possible application in the field of biotechnology. For example, we examine whether *AFA* represents a natural source of vitamin B_{12} (cyanocobalamin) or merely a source of pseudocobalamin, an analogue that lacks bioactivity in humans (Miyamoto et al. 2006; Helliwell et al. 2016). We also detail the potential DNA transformation methods

employed for other cyanobacteria which may be applicable to *AFA*. Finally, we present a strategic plan for the establishment of an integrated *AFA* wild harvesting and cultivation workflow at Klamath Lake, aiming to facilitate further translational research on this promising microorganism.

Aphanizomenon flos-aquae Characterisation

Morphology, physiology and life cycle

Aphanizomenon flos-aquae is composed of long trichomes (= filaments) that aggregate into 1.5 – 2 cm long fascicles, which can be described as bundles of trichomes (Fig. 2) (Carmichael et al. 2000). It is believed that the formation of these fascicles in *AFA* serves as a defence mechanism against grazing. This hypothesis is based on the fact that *Daphnia pulex*, a common water flea, is unable to graze *AFA* filaments longer than 1.5 cm (Dawidowicz 1990). Each trichome in *AFA* consists of intercalary cylindrical vegetative cells, typically straight but occasionally bent. The ends of each trichome are composed of a short series of terminal cells, which are also called hyaline apical cells (Komarek 2006; Komárek 2013). Each vegetative cell is enwrapped in a peptidoglycan cell wall, while each trichome is subsequently enveloped in a polysaccharide cellulose-like sheath (Cirés and Ballot 2016). The dimensions of the different cell types are detailed in Table 1.

Importantly, *AFA* forms heterocysts and akinetes, both of which are specialised vegetative cells (Fig. 3). The former, which is characterised by the presence of an active nitrogenase enzyme, fixes dinitrogen (N_2) from the air into ammonia (NH_3) which is then used in the production of other nitrogenous compounds including nitrites and nitrates. These bioavailable compounds are used to build key cellular components such as amino acids and nucleotides (Haselkorn 1978). Akinetes, instead, can be thought of as "*AFA* spores". These are dormant cells which form under unfavourable conditions such as during the winter season, and germinate to form new vegetative cells when favourable conditions return (Wildman et al. 1975). They develop from vegetative cells under stress, which can occur, for example, under conditions of low temperature, inadequate irradiance, and low phosphorus concentration. Akinetes are a crucial part of *AFA*'s life cycle, as seen in Fig. 4, and have been reported to survive for up to 66 years (Kaplan-Levy et al. 2010).

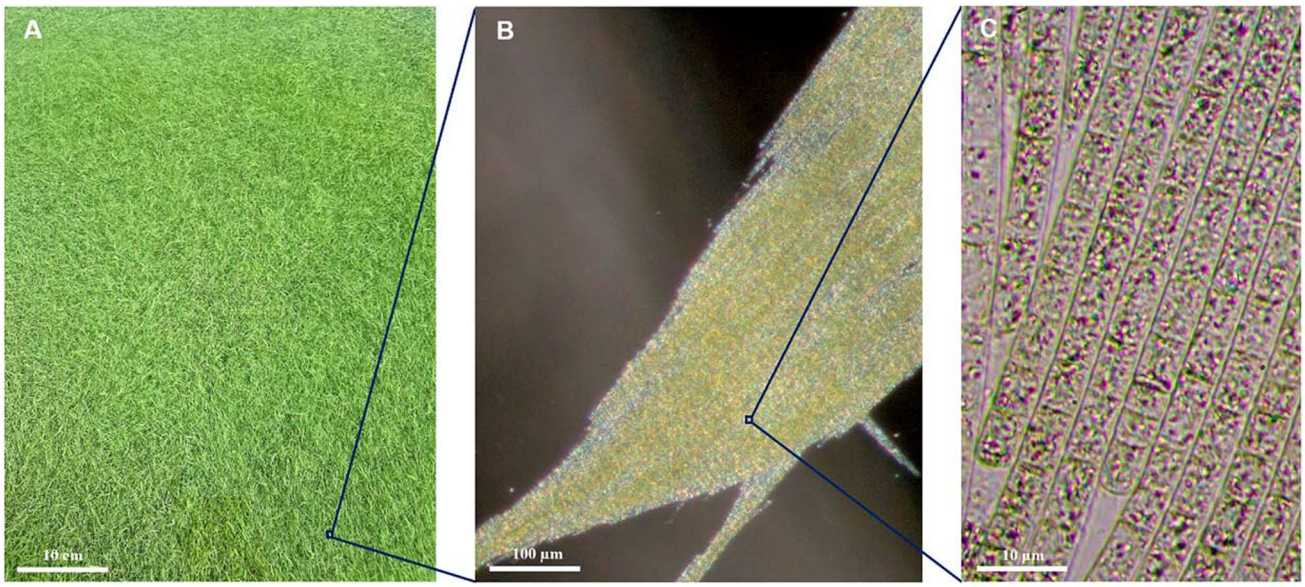

Fig. 2 Micrographs of Klamath Lake *Aphanizomenon flos-aquae (AFA)*. A), B) and C) show Klamath *AFA* fascicles and filaments at 1x, 10x and 100x magnification, respectively.

Table 1 Description of the length and width of the different *Aphanizomenon flos-aquae* -type cells.

Vegetative cells		Terminal cells		Heterocysts		Akinetes	
Length	Width	Length	Width	Length	Width	Length	Width
4-12.1 µm	4.4-8 µm	≤24.3 µm	4.4-8 µm	10-18 µm	5-8.5 µm	40-220 µm	6-10.8 µm

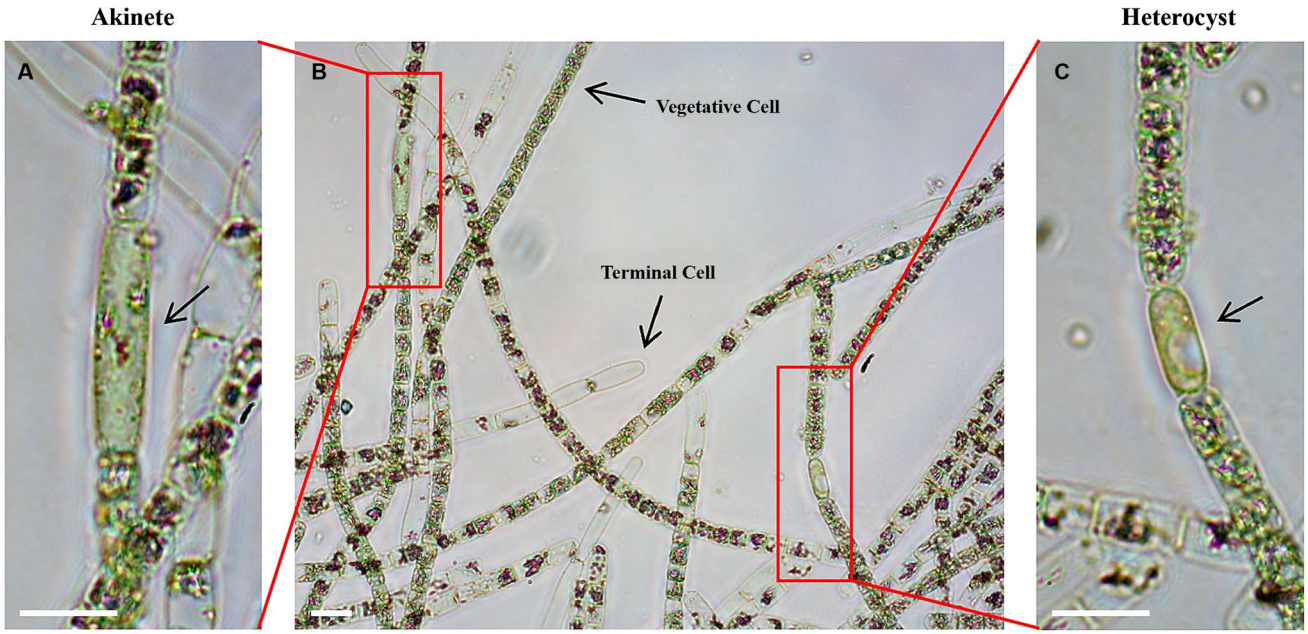

Fig. 3 *AFA* cell type morphology. B) micrograph of *Aphanizomenon flos-aquae* NIES 4052 trichomes at 40x magnification showing its vegetative cells, terminal cells, heterocyst (shown in A) at 100x magnification, and akinete (shown in C) at 100x magnification. Scale bar: 10 µm

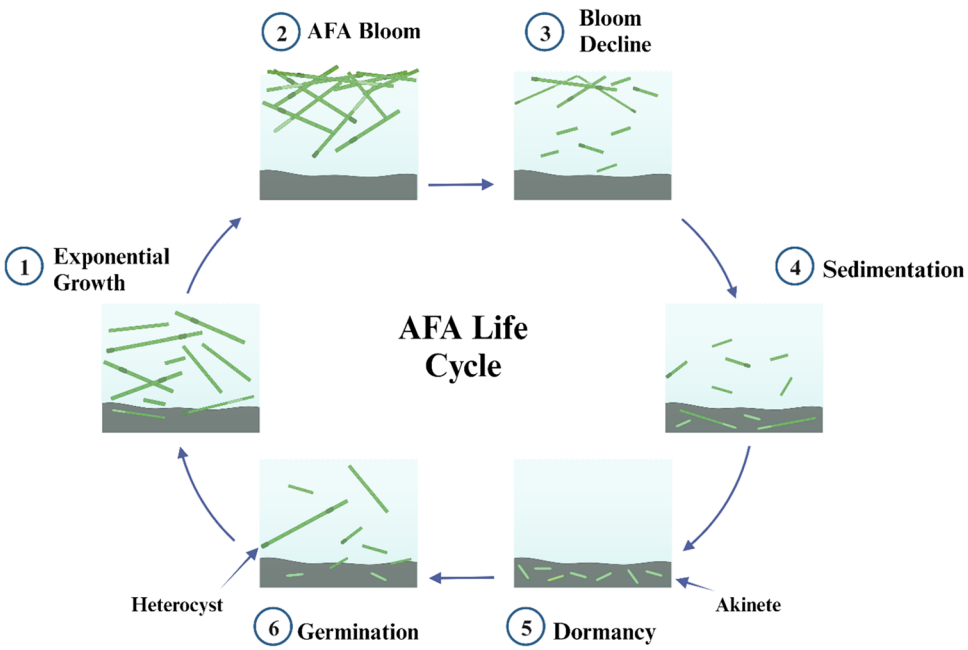

Fig. 4 Illustration of *Aphanizomenon flos-aquae* (AFA) life cycle within freshwater lakes, with its bloom occurring in the summer and autumn seasons and its dormancy in the winter seasons. Following akinite germination, vegetative cells divide exponentially via binary fission allowing for the formation of AFA blooms. Adapted from (Yamamoto and Nakahara 2009a)

AFA discovery, ecology and distribution

Aphanizomenon flos-aquae was first isolated in 1883 from Lake Tetonka, Minnesota, USA (Cirés and Ballot 2016). Since then, 122 different *AFA* strains have been reported based on morphological and phylogenetic analyses, and have been isolated from sites around the world (Suppl. Table 1). *AFA* is found in North America, Asia, and all across Europe, from Portugal and Spain to the Baltic Sea and Scandinavia (Cirés and Ballot 2016). Currently, there are 40 lakes and 17 countries worldwide that are known to harbour *AFA* (Fig. 5). *AFA* is predominantly found in freshwater lakes at high latitudes (temperate zones) as it prefers temperatures between 23°C and 29°C (Tsujimura et al. 2001; Yamamoto and Nakahara 2009b). However, *AFA* is highly adaptable: a notable example is its ability to grow in the brackish waters of the Baltic Sea (Cirés and Ballot 2016). The cyanobacterium can endure temperatures up to approximately 30°C,

Fig. 5 Wild distribution of *Aphanizomenon flos-aquae* (*AFA*) across the globe. Each pin represents a specific lake from which AFA has been collected. All *AFA* strains have been confirmed morphologically and phylogenetically. All locations are listed in Suppl. Table 1. *AFA* strains FACHB 1200, 1208, 1209, 1249, 1259, 1260, 1265, 1287, 1290, 1040, 1168, 1169, 1170, 1171, Zayi, SAG 31.87 and 0HA35S1 are not listed as their exact locations have not been determined. Figure made via Google Maps. To view the interactive map and their associated Google Earth location use this URL: https://www.google.com/maps/d/viewer?mid=1gsHAkbBdW-BF0tiqCrcit6JdEA5ZLZk&ll

and *AFA* trichomes are known to survive at 10°C (Yamamoto and Nakahara 2005; Reinl et al. 2023). One study from Lake Stechlin even describes "winter blooms" of *AFA* at 2°C (Üveges et al. 2012). Similarly, *AFA* has a high resistance to alkalinity, growing at a pH of 10 (Yamamoto and Nakahara 2005). Cyanobacterial blooms have actually been shown to directly increase lake pH levels as dissolved CO_2 is consumed during photosynthesis (Wood et al., 1996, Newton 2015, Zepernick et al. 2021). Like most cyanobacteria, *AFA* prefers low light intensities, around 40-60 µmol photons m^{-2} s^{-1} (Park et al. 2018). *AFA*'s ability to control its position within the water column by the production of gas vesicles allows it to regulate the amount of irradiance it receives and, thus, to fine-tune and optimise photosynthesis (Yamamoto 2009).

Aphanizomenon flos-aquae typically cohabits with other cyanobacteria, most commonly *Microcystis* species: mainly *M. aeruginosa*, *Dolichospermum/Anabaena flos-aquae* and *Gloeotrichia echinulata* (Aavad 1994; Eldridge et al. 2012) (Fig. 6). However, the underlying factors governing their ecological interactions within their relative ecosystems are poorly understood. Recent research has highlighted some key factors which may explain the seasonal dynamics between *AFA* and *Microcystis.* The latter has a preference for higher temperatures and light intensities, unlike *AFA*, favouring *Microcystis* blooms in the warmer months (Wu et al. 2010a; Wen et al. 2022). Relative concentrations in the lakes are also greatly affected by nutrient availability: *Microcystis* growth is favoured under low phosphorus conditions as it has a more effective phosphorus uptake and storage system; while *AFA*, being a nitrogen-fixer (unlike *Microcystis*), is greatly favoured under low nitrogen conditions (Wu et al. 2010a; Eldridge et al. 2012). *Microcystis* blooms generally occur following the decline of an *AFA* bloom, which most likely reflects a release of bioavailable nitrogen into the environment as *AFA* cells break down (Yamamoto and Nakahara 2009b). This hypothesis is supported by a study conducted in Lake Dianchi, China, where coexisting *AFA* and *M. aeruginosa* were observed. In 2010, the nitrogen levels in the lake increased significantly following industrial wastewater

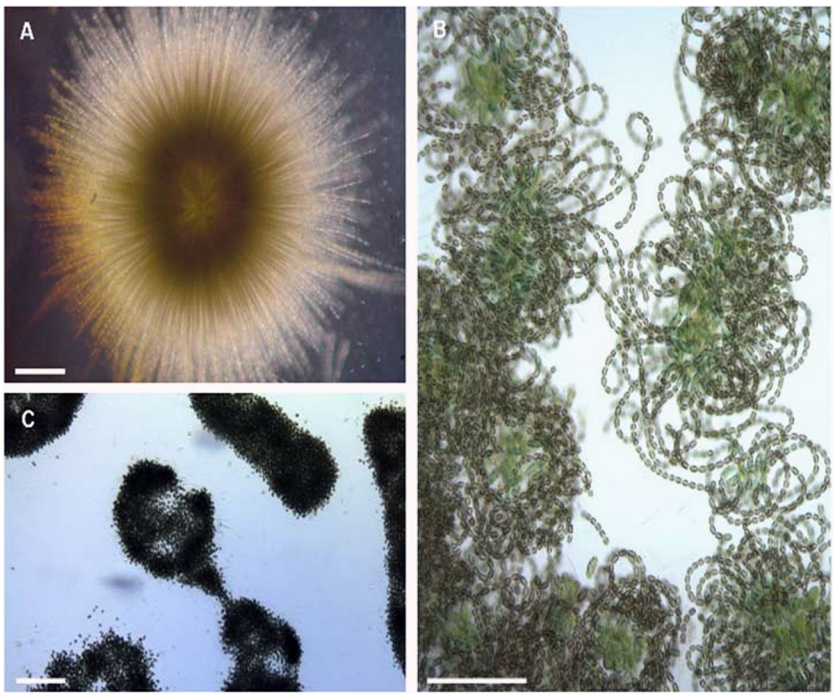

Fig. 6 Micrographs of cyanobacterial isolates from Upper Klamath Lake. a) *Gloeotrichia echinulata,* b) *Anabaena flos-aquae*, c) *Microcystis aeruginosa*. Scale bars: 100 µm

discharges, leading to a gradual decline in *AFA* blooms and a rapid take over by *M. aeruginosa*, ultimately resulting in the complete disappearance of *AFA* blooms in 2012 (Wu et al. 2016). Nitrogen may therefore serve as a limiting factor for the growth of *Microcystis*. However, it is unclear whether external inputs such as temperature cause *AFA* blooms to decline naturally and favour those of *Microcystis*, implying a facilitative relationship, or whether *Microcystis* strains may directly influence the decline of the *AFA* bloom, making their relationship competitive (Eldridge et al. 2012). Laboratory experiments point towards the latter hypothesis: *Microcystis* growth is directly correlated with the growth inhibition of *AFA* cells (Ma et al. 2015) (Wen et al. 2022). It is thought that *Microcystis* may release a compound which suppresses *AFA* proliferation. However, this has yet to be confirmed (Ma et al. 2015). The interplay between *AFA* and *Anabaena/Dolichospermum* strains is much less understood, as they have similar nutrient and growth condition preferences (Yamamoto 2009).

Taxonomy, phylogeny and potential toxicity

The genus *Aphanizomenon* was first established in 1886 (Bornet and Flahault 1886). However, the taxonomy of the genus has been the subject of several revisions, which, until the advent of molecular tools, were based purely on morphology (Komarek 2006). The most well-defined and accepted morphological criteria were established by Komarek in 1989 (Komárek 1989): it subdivided the genus into four subgroups depending on their fascicle formation, cell size, heterocyst frequency, trichome symmetry, and the shape of the terminal cells (Li et al. 2000; Komárek and Komárková, 2006; Komárek 2013). While these criteria did provide an accurate taxonomic framework for the majority of *Aphanizomenon* species, it did not for some. Species within different sub-groups were found to have overlapping characteristics, especially between *A. flos-aquae* and *Aphanizomenon gracile* strains, which have been often misidentified as one another due to their trichome similarity (Cirés and Ballot 2016). An example of this is observed with the taxonomic categorisation of *Aphanizomenon* sp. NH-5 (AY196086) over the years: it was initially identified as an *AFA* strain, later as an "atypical non-fasciculated strain", and ultimately as *A. gracile* strain (Li et al. 2000). The sole morphological attribute that makes *AFA* obviously distinguishable, especially in comparison with *A. gracile,* is its ability to form fascicles.

With the advent of molecular phylogenetic analysis, the taxonomic arrangement of the *Aphanizomenon* genus underwent a complete revision. Investigation into conserved DNA sequences – e.g. genes for 16S rRNA, phycocyanin, ribulose-bisphosphate carboxylase and the sequence of rRNA internal transcribed spacers, found that several strains were only distantly related to each other (Wu et al. 2010b; Komárek 2013; Cirés and Ballot 2016). This led to the re-classification of several species, such as *A. gracile* or *Aphanizomenon issatchenkoi*, into different or novel genera (Rajaniemi et al. 2005). The genus *Aphanizomenon* itself was restricted to *AFA* and seven of its morphospecies: *A. klebahnii, A. yezoense, A. paraflexuosum, A. flexuosum, A.*

slovenicum, A. platense and *A. hungaricum* (Cirés and Ballot 2016). However, *AFA* and *A. klebahnii* are the only strains within the genus that have had their genomes or highly conserved genomic regions sequenced and made available. As a result, morphology, with special attention to heterocyst and akinete size, is the sole basis for intra-genera identification (Cirés and Ballot 2016). As such, there is an urgent need for further phylogenetic studies that include all eight morphospecies to validate the legitimacy of this genus.

Despite the establishment of the genus *Aphanizomenon*, there remains considerable disagreement concerning the taxonomic classification of species that share genetic similarity with *AFA* but differ in morphology (Dreher et al. 2021a). Examples of this are observed with *Anabaena* and *Dolichospermum* species: these have been shown to cluster with *AFA* in phylogenetic analyses, indicating that they are not monophyletic (Cirés and Ballot 2016; Driscoll et al. 2018; Dreher et al. 2021b). The three genera, in fact, are collectively referred to as the "*Anabaena*, *Dolichospermum* and *Aphanizomenon* (ADA) clade". Recent papers have proposed that the ADA clade become a single genus (Wacklin et al. 2009; Dreher et al. 2021a; Dreher et al. 2021b). However, the morphology of *Anabaena* and *Dolichospermum*, defined by oval/globular and elongate-barrel shaped vegetative cells and heterocysts, differs from the cylindrical structure of *AFA* (Prasanna et al. 2006). This further underscores the significant misalignment between morphological and phylogenetic taxonomical categorisations and highlights the necessity of employing a combined approach for a more comprehensive and accurate identification process.

The taxonomic uncertainties may have been further exacerbated by the misidentification of filamentous diazotrophic strains during the 20th century, as observed with *A. gracile* NH-5 (AY196086), and the submission of unverified DNA and protein sequences to official databases, without being associated with a peer-reviewed published paper (Li et al. 2000; Palinska and Surosz 2014). Deposition of incorrectly identified strains to culture collections, which may subsequently feature in phylogenetic trees and be erroneously used as a proxy for an genus of interest, has also potentially skewed our understanding of various cyanobacterial genera, including *Aphanizomenon* (Komárek and Komárková, 2006). For example, studies have highlighted the ability of certain *Aphanizomenon* species to produce cyanotoxins: specifically, paralytic shellfish poisons such as saxitoxins, anatoxin-α, and cylindrospermin (Mahmood and Carmichael 1986; Li et al. 2000; Pereira et al. 2000; Ferreira et al. 2001; Li et al. 2003; Liu et al. 2006; Preußel et al. 2006; Zhang et al. 2013). However, a recent review by Cires and Ballot (2016) demonstrated that the majority of the investigated strains had been misidentified as *AFA* rather than morphologically similar cyanobacteria such as *A. gracile* and *A. issatchenkoi*, which are known to be important cyanotoxin producers. The few remaining cyanotoxin-producing species that have been investigated have not been confirmed as *AFA* due to a lack of available phylogenetic data making their taxonomic assignment uncertain (Cirés and Ballot 2016). To date, *AFA* is yet to be confirmed as a cyanotoxin producer and Klamath Lake *AFA* is, in fact, approved as edible by both the FDA and EFSA (Schaeffer et al. 1999; Lähteenmäki-Uutela et al. 2021). Nevertheless, further research is required to confirm the safety of the genus *Aphanizomenon*, especially due to the morphological or phylogenetic similarity to other cyanotoxin-producing nitrogen-fixing filamentous cyanobacteria. One initial approach would be to investigate the presence of genes for biochemical pathways associated with cyanotoxin production within the available *AFA* genome sequences accessible through the NCBI database.

Industrial role of *AFA*

Benefits

Klamath *AFA,* the only *AFA* strain to be characterised from a nutritional and nutraceutical perspective, is one of the richest foods on the planet and is considered a "superfood". It has been reported to have average protein levels of 60-70% (Grewe and Pulz 2012) and contains a high concentration of essential amino acids (Sandgruber, 2021). In addition, Klamath *AFA* contains mycosporine-like amino acids (MAAs), specifically shinorine and porphyra, which are known to confer antioxidant properties and DNA damage protection (Righi et al. 2016). Furthermore, Klamath *AFA* is a source of all 14 vitamins (although, see the discussion on vitamin B_{12} below), and 73 trace minerals (Sandgruber et al. 2021). More specifically, 1 g of dried Klamath AFA biomass contains Recommended Daily Allowance (RDA)-relevant amounts of vitamins A, K, B_1, B_3, B_5 and B_9, and the minerals, iron, molybdenum and iodine (Tables 2 and 3) (Kushak et al. 2001; McCommon et al. 2013). Klamath *AFA* is also an important producer of carotenoids with high levels of three xanthophylls: lutein, echinenone and canthaxanthin, and two carotenes: lycopene and β-carotene (Hertzberg and Jensen 1966; Krajewska et al. 2019). Moreover, Klamath *AFA* produces a number of polyphenols, specifically caffeic, vanillic and hydroxytyrosol acid (Righi et al. 2016). These are thought to contribute to the cyanobacteria's nutraceutical effects by conferring anti-cancer, anti-inflammatory, antioxidant, cell growth promotion, and wound healing properties (Cory et al. 2018; Peng et al. 2023).

Klamath *AFA* is also a rich source of polyunsaturated fatty acids (PUFAs). It produces 12-14 mg g^{-1} of the anti-inflammatory omega-3 fatty acid, alpha-linoleic acid and this accounts for approximately 25% of its total lipid biomass (Sandgruber et al. 2021). The balance between omega-6 and

Table 2 Table summarising and comparing the macromolecules and vitamins of commercial Klamath *AFA*, Spirulina (*Arthrospira*) and *Chlorella* products. The lowest and highest values for each compound were chosen from the available literature, independent of the specific species and strains of Spirulina or *Chlorella*. Vitamin A only considers carotenes. All Klamath *AFA* information was obtained from the literature (McCommon et al. 2013; Sandgruber et al. 2021), and from unpublished data provided by Nutrigea s.r.l, a distributor of Klamath *AFA* (Suppl. Table 2). Spirulina references: Ali and Saleh, 2012, Soni, 2017, Andrade et al. 2018, Sangruber et al., 2021, *Chlorella* references: Safi et al. 2014, Andrade et al. 2018, Bito et al. 2020, Sandgruber et al. 2021

	Klamath *AFA*	**Spirulina**	***Chlorella***
Macromolecules - % Biomass			
Protein	60-70 %	50-70 %	55-65 %
Carbohydrates	20-30 %	13-36 %	12-25 %
Fat	3-5 %	4.2-5.6 %	6-13 %
PUFAs	30-37 %	35-45 %	35-47 %
Omega-6s	20-25 %	>99.99 %	65-98 %
Omega-3s	75-80 %	<0.01 %	2-35 %
Omega-6/-3	0.2-0.3	<200	2-5
Ash	5-7%	5-8%	5-8%
Vitamins - mg g^{-1} (% RDA)			
Vitamin A	0.30 (33.3%)	0.21-0.30 (24.0-33.3%)	0.33-0.38 (36.6-42.2%)
Vitamin B1	0.25 (17%)	0.012-0.025 (0.8-1.7%)	0.017-0.023 (1.2-1.7%)
Vitamin B2	0.038 (2.3%)	0.033-0.043 (2.0-2.7%)	0.020-0.20 (3.6-12%)
Vitamin B3	4.0 (22.0%)	0.10-0.43 (0.6-2.3%)	0.15-0.25 (0.8-1.4%)
Vitamin B5	1.6 (25%)	0.01-0.03 (0.25-0.5%)	0.02-0.19 (0.3-3.2%)
Vitamin B6	0.028 (1.5%)	0.01 (1.5%)	1.9-25 (0.1-1.3%)
Vitamin B9	0.040 (20%)	≤0.001-0.005 (0.2-2.5%)	0.01-0.10 (1.7-16.7%)
Vitamin C	2.0 (3.3%)	0.1 (0.2%)	0.1 (0.2%)
Vitamin D	0.007 (<0.01%)	0-0.003 (<0.01%)	0.8-1.3 (5%-8%)
Vitamin E	0.28 (7%)	0.01-2.0 (<0.01-50%)	0.1-2.5 (4%-63%)
Vitamin K	33.3 (55.6%)	0.3-10.0 (1.7-16.7%)	0-0.7 (0-3.3%)

Table 3 Table summarising and comparing the mineral, essential metals & bioactive compounds of commercial Klamath *AFA*, Spirulina *(Arthrospira)* and *Chlorella* products. The lowest and highest values for each compound were chosen from the available literature, independent of the specific species and strains of Spirulina or *Chlorella*. All Klamath *AFA* information was obtained from the literature, and from unpublished data provided by Nutrigea s.r.l, a distributor of Klamath *AFA* (Suppl. Table 2)

	Klamath *AFA*	**Spirulina**	***Chlorella***
Minerals & Essential Metals - mg g^{-1} (% RDA)			
Calcium	10.0-15.0 (1-1.7%)	1.2-15.0 (0.1-1.7%)	5.0-8.3 (0.5-0.8%)
Magnesium	3.0 (1%)	2.0-4.0 (0.7-1.3%)	3.0-4.0 (1-1.3%)
Phosphorus	16.0 (2%)	5.0-14.0 (0.7-1.8%)	15.0-16.7 (1.8-2.2%)
Iron	1.7-4.0 (11.7-28.3%)	0.33-1.70 (2.3-11.7%)	0.33-2.67 (2.3-18.3%)
Potassium	N/A	10.0-16.7 (≤0.01%)	3.3-13.3 (≤0.01%)
Manganese	0.030 (3%)	0.017-0.033 (1.7-3.3%)	0.020-0.050 (2-5%)
Molybdenum	0.005 (10%)	0.005 (10%)	N/A
Zinc	0.02 (0.3%)	0.02 (0.1%)	0.01-0.02 (0.1%)
Copper	0.008 (0.7%)	0.015-0.027 (1.3-2.3%)	0.003-0.010 (0.3-1%)
Selenium	0.001 (1.7%)	<0.001 (<0.01%)	<0.001 (≤1%)
Iodine	0.054 (12%)	5.0-6.7 (3.3-4.4%)	1.67-0.33 (1.2-2.2%)
Fluoride	0.8 (21%)	13.3-23.3 (<0.01%)	N/A
Boron	0.011 (1.1%)	0.001-0.030 (0.1-1%)	N/A
Vanadium	0.003 (30%)	N/A	N/A
Nickel	1.8 (1.7%)	≤0.001 (≤1%)	≤0.001 (≤1%)
Chromium	0.001 (2%)	0.003-0.004 (7.3-8.0%)	≤0.001 (≤1%)
Bioactive compounds - mg g^{-1}			
Total Carotenoids	6.5-8	4-5	6.5-8
Chlorophyll	0.6-4	0.7-1.5	1.5-4
Phycocyanin	13-17	13-20	0
β-Phenylethylamine	2-3	0	0

omega-3 fatty acids plays a crucial role in regulating bodily inflammation (Simopoulos 2002). Omega-6 fatty acids tend to promote the production of pro-inflammatory mediators, while omega-3 fatty acids favour anti-inflammatory mediators (Simopoulos 2002, DiNicolantonio and O'Keefe, 2018). The ideal omega-6 to omega-3 ratio is believed to be between 1:1 and 4:1. The average omega-6/omega-3 ratio in the modern Western diet is between 10:1 and 20:1, largely due to the increased consumption of processed and fried foods within the past few decades (Simopoulos 2008, Di Nicolantonio and O'Keefe, 2018). This imbalance is known to cause chronic inflammation and obesity, which are important risk factors for many chronic diseases, such as heart disease, arthritis, and cancer. Regular consumption of Klamath *AFA* would help reduce bodily inflammation and the risk of developing chronic illnesses owing to its high concentration of omega-3 PUFAs (Sandgruber et al. 2021). In contrast, *Arthrospira* produces, for the most part, only omega-6s, whilst there is a wide and unknown variability between different *Chlorella* products, making it unreliable for the end-user. Even so, while Klamath *AFA* has a lower omega-6/3 ratio, some *Chlorella* strains actually have a higher amount of omega-3 PUFA due to their high lipid content (Table 2 and Fig. 7A) (Sandgruber et al. 2021).

Moreover, the pigments found in Klamath *AFA*, specifically chlorophyll and phycocyanin, possess well-known nutraceutical properties (Benedetti et al. 2006). Chlorophyll, characterised by a bitter taste, reduces inflammation, aids in weight loss, and acts as a prophylactic against certain cancers. Cyanobacterial phycocyanin (C-PC) is a powerful antioxidant, anti-inflammatory, anti-cholesterol and anti-cancer agent, with important promoting properties for the immune system and wound healing (Fig. 8A) (Jensen et al. 2000, Reddy et al. 2000, Romay et al. 2003, Madhyastha et al. 2008, Kuriakose and Kurup 2010, Xue et al., 2015, Kefayat et al. 2019, Zahiri et al. 2020, Blas-Valdivia et al. 2022, Liu et al. 2022). The inhibitory effect of C-PC on cycloxygenase-2 (COX-2), a pivotal mediator of inflammation, is analogous to the non-steroidal anti-inflammatory drug Celecoxib (Romay et al. 2003; Gong et al. 2012). Klamath *AFA* and *Arthrospira* both produce similar concentrations of C-PC, around 15% of the total biomass, while *Chlorella* as a green microalga does not produce any (Table 3). However, *Chlorella* does have higher levels of chlorophyll (Fig. 7B). Klamath *AFA* C-PC extracts, such as AphaMax®, also concentrate the related pigment phycoerythrocyanin (PEC) that is absent in *Arthrospira*. PEC is identical to phycocyanin in structure except in that it contains a phycoviobilin chromophore, giving it a deep violet colour (Bryant 1982; Galizzi et al. 2023). Further research is required to investigate the biological properties of PEC. Overall, the physiological effects of C-PC extracts from Klamath *AFA* and *Arthrospira* are noteworthy, and their use as a prophylactic agent against

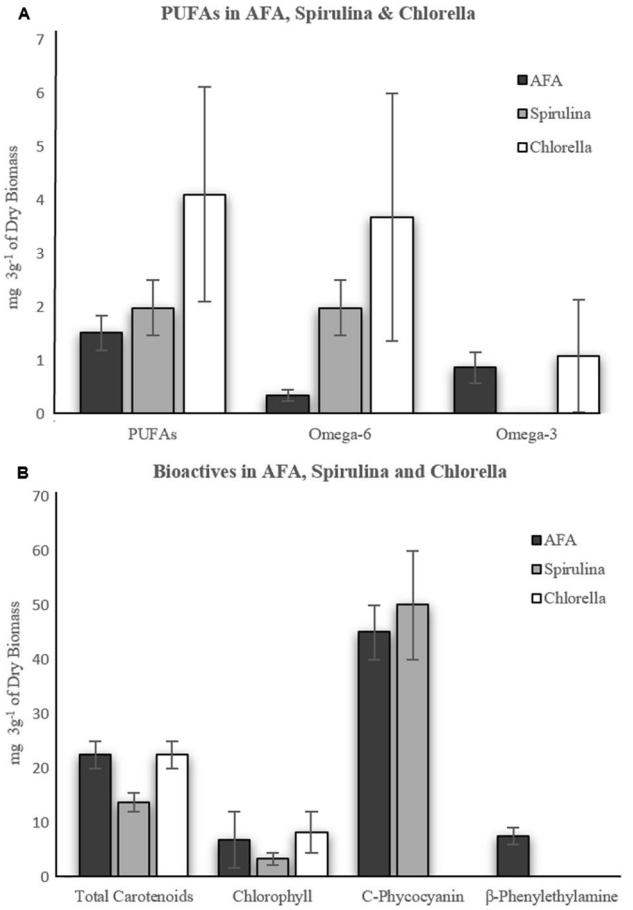

Fig. 7 Bar charts illustrating the mean concentration of PUFAs (A) and bioactive compounds (B) in Klamath *AFA*, Spirulina (*Arthrospira*) and *Chlorella* supplements based on the minima and maxima reported amongst commercially sold products. The error bars detail the minima and maxima. For example, the maximum reported Omega-3 content in *Chlorella* products is 0.7 mg g^{-1} of dry biomass, while the minima approach 0

specific diseases – from inflammatory-based ones to cancers – is garnering increasing interest. *Arthrospira* C-PC extracts are in fact being considered as a potential drug candidate for the treatment of cancer (Braune et al. 2021).

Another key compound that has gained notoriety in recent years is β-phenylethylamine (PEA), an endogenous phenolic compound primarily produced in humans during exercise. Amongst commercially available microalgal and cyanobacterial supplements, PEA is present only in Klamath *AFA* (Irsfeld et al. 2013; Nuzzo et al. 2018). PEA has important psychoactive properties which make it a powerful anti-anxiety and anti-depressant agent, natural painkiller, libido enhancer, hunger suppressant, and immune modulator (Barroso and Rodriguez 1996; Hart et al. 2007; Ohta et al. 2017). Klamath *AFA* PEA extracts, approved and sold alongside Klamath *AFA* biomass, contain approximately 1.4

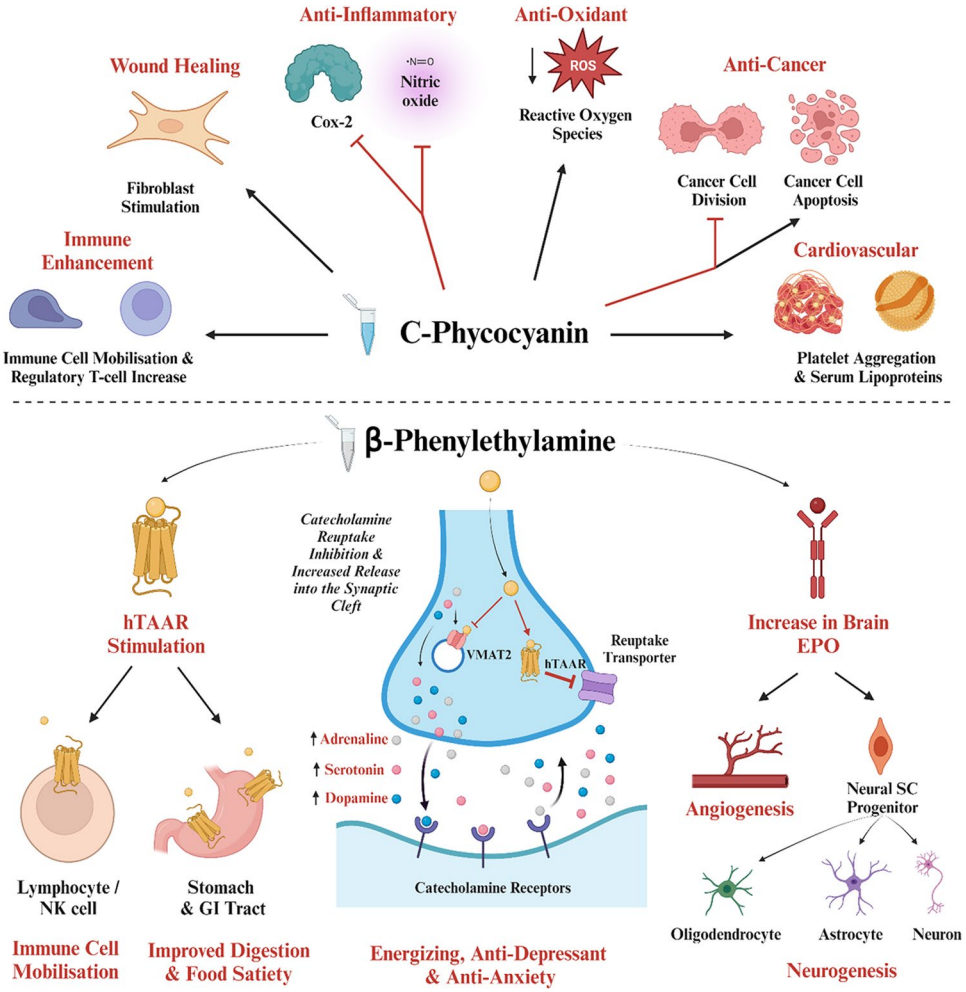

Fig. 8 Mechanism of action of C-PC (A) and PEA (B). a) C-PC specifically inhibits the COX-2 enzyme, nitric oxide production, and cancer cell proliferation while also promoting the mobilisation of immune cells b) PEA's nutraceutical effects are through its stimulation of the hTAAR receptor: hTAAR brain stimulation causes an increase in brain EPO, leading to angiogenesis and neurogenesis, catecholamine reuptake and VMAT2 receptor inhibition, leading to increased catecholamine concentrations. Red arrows indicate inhibition, while black ones indicate stimulation. Abbr.: COX-2: Cyclooxygenase 2; VMAT2: vesicular monoamine transporter 2. hTAAR: Human trace amine-associated receptor: SC: Stem Cell; NK: Natural Killer. Figure generated using Biorender

mg g^{-1} of PEA (McCarthy et al. 2022). PEA's half-life when orally ingested is around 5-10 min as it is rapidly broken down by monoamine oxidases (MAO), specifically MAO-B enzymes (Irsfeld et al. 2013). However, Klamath *AFA* PEA extracts also contain potent MAO-B inhibitors in the form of MAAs (Scoglio et al. 2014). These have similar potency to the synthetic MAO-B inhibitor, Deprenyl, and can cross the blood-brain barrier, thereby greatly extending PEA's overall half-life and allowing it to exert its mechanism of action over an extended period (Fig. 8B) (Magyar and Szende 2004; Scoglio et al. 2014). The Klamath *AFA* PEA extract (commercially marketed as Klamin®) has been shown to significantly improve the general well-being and self-esteem in individuals with post-menopause-induced depression (Scoglio et al. 2009; Genazzani et al. 2010). The extract has also been shown to improve the overall condition of children suffering from ADHD, both in terms of attention deficit and hyperactivity (Chen et al. 2007; Wang et al. 2012; Cremonte et al. 2017). In addition, the underlying mechanism of PEA's neuronal and brain tissue regeneration/survival properties may also play a prophylactic role against the development of neurodegenerative diseases such as Alzheimer's (Shytle et al. 2010; Borhani-Haghighi et al. 2012; Nuzzo et al. 2018; Galizzi et al. 2023). Klamath *AFA* PEA extracts hold substantial potential within the psychoactive space and the health benefits merit further in-depth research.

Overall, Klamath *AFA*'s nutritional profile and nutraceutical properties, though comparable to *Arthrospira* and *Chlorella*, demonstrate more potent and comprehensive prophylactic and therapeutic effects, especially when considering PEA's psychoactive properties (Sandgruber et al. 2021) (Fig. 8B). The nutritional value of Klamath *AFA* is only slightly diminished, as stated by Sandgruber et al., by its elevated levels of saturated fatty acids (SFAs), particularly C16:0, which are associated with heightened inflammation and cholesterol levels (Sandgruber et al. 2021). Finally, it is important to note that while many strains and species of *Arthrospira* and *Chlorella* have been characterised for nutritional content, Klamath AFA is the only *Aphanizomenon* species to be characterised (Sangruber et al., 2021). It would be worthwhile to characterise other *AFA* strains as they may possess different nutritional and nutraceutical properties and

allow the expansion of *AFA*'s biotechnological opportunity and market.

Vitamin B_{12}

One market that has been receiving attention recently is vitamin B_{12} supply for the vegetarian and vegan industries as there is now an important consumer demand for non-animal sources of vitamin B_{12} supplements (Curtain and Grafenauer 2019). As green algae such as *Chlorella* are eukaryotesthey are not able to synthesise B_{12}. However, *Chlorella* is often grown in co-culture with B_{12}-producing bacteria and its cells are able to actively import the B_{12} from the medium, making *Chlorella* and other edible green algae potential sources of B_{12} (Bito et al. 2020). Nonetheless, a recent analysis by Bito et al. (2016) shows that only four out of thirteen commercially sold *Chlorella* tablets contained B_{12}, most likely due to the differences in their associated bacterial populations. Furthermore, the amount of B_{12} in the *Chlorella* products is highly variable, with values ranging between 0.2 to 5 μg g^{-1}, complicating matters for potential customers (Bito et al. 2020). Cyanobacteria are also unable to synthesise the vitamin, but instead produce a B_{12} analogue termed pseudocobalamin or pseudo-B_{12} (Fig. 9) (Baroni et al. 2009; Helliwell et al. 2016). *Arthrospira* and Klamath *AFA* produce 1-2 μg g^{-1} and 6-8 μg g^{-1} of pseudo-B_{12}, respectively. Pseudo-B_{12} is poorly absorbed by the body due to its lower binding affinity to the Intrinsic Factor protein which allows for B_{12} absorption in the stomach. As a result, pseudo-B_{12} should not be used for supplementation purposes (Wienhausen et al. 2022).

Nevertheless, two spectroscopy studies have shown that *Arthrospira* and Klamath *AFA* also contain human-absorbable B_{12}, despite the lack of genes for its biosynthesis in these cyanobacteria. More specifically, Klamath *AFA* has been found to have 0.32-0.42 μg g^{-1} of B_{12} such that a few grams of dry Klamath *AFA* biomass per day would satisfy the current RDA for male adults (2.4 μg g^{-1}) (Miyamoto et al. 2006). Once again, the B_{12} is thought to derive from bacterial communities present within Klamath *AFA* blooms with released B_{12} present in the water being taken up by *AFA* cells (Watanabe et al. 1999; Miyamoto et al. 2006; Helliwell et al. 2016). For Klamath *AFA*, these bacteria are likely represented by a subset of the gammaproteobacteria (Driscoll 2016). An *in vivo* study by Baroni et al. (2009) carried out using vegan individuals provided evidence that Klamath *AFA* supplements are a reliable source of B_{12}. However, it was not established whether the B_{12} is imported into the Klamath *AFA* cells, as with *Chlorella*, or whether the gammaproteobacteria are harvested from the wild alongside the Klamath *AFA* and are therefore a component of the end product. Recent research suggests that cyanobacteria possess a B_{12} import system known as the *btu* import system (Pérez et al. 2016). The presence of this import system in *AFA* followed by experimental validation of B_{12} translocation from the exogenous space into the *AFA* cell is required to confirm that the harvested Klamath Lake *AFA* biomass is the source of B_{12}, rather than the associated commensal bacteria. Confirmation of this would signify that fluctuations in the amount of bacterial contamination within the supplements would not affect the amount of bioavailable B_{12}, thereby making it a reliable source of the vitamin, so long as it is being produced by bacterial populations within Klamath Lake.

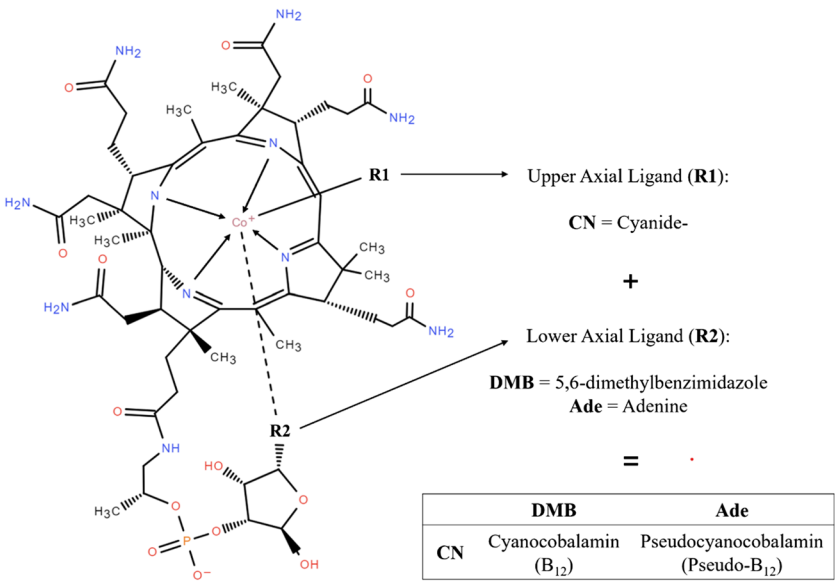

Fig. 9 Structure differences between B_{12} (cyanocobalamin) and pseudo-B_{12} (pseudocyanocobalamin). In general, the term cobalamin refers to any cobalt-containing corrin structure. Pseudo-B_{12}, produced by cyanobacteria, distinguishes itself from B_{12} by featuring adenine in its lower axial ligand position (R2) instead of DMB.

Klamath Lake *AFA* harvesting

Klamath *AFA* biomass has been harvested from Klamath Lake in Oregon, USA since the early 1980s (Fig. 1) (Carmichael et al. 2000). The cyanobacterium is harvested from the lake's surface during the bloom seasons via rolling screens on self-powered barges. Each year 500-1000 t dry weight is harvested and distributed around the globe, with a particular focus on USA and European markets (Carmichael et al. 2000). The biomass is sold either as a whole-cell superfood supplement or as preparations of its bioactive compounds such as C-PC and PEA, which are sold as high-value nutraceuticals. Nevertheless, the global Klamath *AFA* market has remained fixed at around £100 millon and has not been able to expand since the early 2000s, unlike that of Spirulina and *Chlorella* (Carmichael et al. 2000; Pulz and Gross 2004; Grewe and Pulz 2012). The underlying reason is the lack of a continuous and reliable stream of Klamath *AFA* biomass supply owing to the dependence on wild harvesting. Firstly, the *AFA* blooms are dependent on nature and its seasons (Carmichael et al. 2000). These blooms predominantly occur, although sporadically, between the end of June and the beginning of July and, again, between the beginning of September and Mid-November (Eldridge et al. 2012). Secondly, harvesting companies must ensure that their *AFA* product is not contaminated with microcystins. The Federal State of Oregon set a stringent limit in the early 2000s of 1 μg g^{-1} of microcystins in Klamath *AFA*-based nutritional supplements based on the World Health Organisation limit of 1 μg L^{-1} of microcystin in water, thereby forcing harvesting companies to hold off any harvesting if *M. aeruginosa* is present and discard any Klamath *AFA* biomass that contains microcystins above the WHO limit (Schaeffer et al. 1999; Saker et al. 2005; Heussner et al. 2012; Vichi et al. 2012; Lyon-Colbert et al. 2018). *Microcystis aeruginosa* blooms do not coincide with those of Klamath *AFA*, as they occur between late July and the end of August, but they can certainly overlap or be present in low concentrations (Carmichael et al. 2000). These factors have limited the scope and range of *AFA*'s downstream applications. Klamath *AFA* is currently the only commercial cyanobacteria that is not grown in a controlled environment, and technologies for its cultivation in open ponds or PBRs would offer an important opportunity to resolve many of the issues associated with wild harvesting.

Future prospects

AFA cultivation parameters

Published studies on the cultivation of *AFA* in laboratory and photobioreactor (PBR) settings is limited and its optimal growth requirements have yet to be fully characterised. *AFA* prefers low light intensities (40-60 μmol photons m^{-2} s^{-1}) but can tolerate up to a maximum of 115 μmol photons m^{-2} s^{-1}, beyond which *AFA* experiences photoinhibition leading to reduced growth rates and eventually death (Gentile and Maloney 1969). Prolonged exposure to lower irradiances, above 60 μmol photons m^{-2} s^{-1}, can also cause loss of buoyancy owing to the accumulation of polysaccharides within the cells, resulting in sedimentation and cell death (Konopka et al. 1987). To avoid these effects, most cultivation regimes advise a daily light-dark cycle between 12h:12h and 16h:8h (Debella 2007, Xue et al., 2015). *AFA*'s growth is relatively unaffected by pH between 7.1 and 11, with its optimal at ~7.5 (Yamamoto and Nakahara 2005). *AFA* can also tolerate a range of temperatures but demonstrates faster growth rates when exposed to temperatures between 26°C and 28°C (Gentile and Maloney 1969; Debella 2007). The addition of CO_2 to the culture increases *AFA* growth and biomass yield, although the exact CO_2 levels required are not well-defined (Debella 2007). Various different cyanobacterial media have been recommended for *AFA* cultivation including BG11, CT, ASM, and JM media (Watanabe 1977, Jaworski et al. 1981, Castenholz 1988, Carmichael et al. 2000, Tsujimura et al. 2001, Debella 2007, O'Flaherty and Phinney, 2008).

These parameters allow for laboratory maintenance and cultivation of *AFA*. However, these parameters may not translate to the upscaling process and may require further optimisation (Acien et al. 2013). If one growth parameter was suboptimal in a PBR this would markedly affect overall productivity as all parameters are interdependent. For example, an inexact mass transfer of CO_2/O_2 could lead to a decreased growth rate and biomass density thereby altering the light exposure of *AFA* cells and potentially increasing photo-saturation/-oxidation (Acien et al. 2017). One suboptimal parameter could generate a productivity-lowering positive feedback loop which may essentially impede *AFA* cultivation (Acien et al. 2017). One major concern, for example, is the mixing regime. As *AFA* is filamentous it is relatively fragile and susceptible to shear stress (Wang and Lan 2018). In Klamath Lake, *AFA* is generally mixed by small currents, methane springs and wind-induced waves. Reliance on continuous bubbling, mechanical stirring, or aeration mixing may compromise the culture (Wang and Lan 2018). Overall, the technological feasibility of *AFA* mass cultivation poses the greatest barrier to its realisation. Therefore, laboratory and pilot scale optimisation studies are required prior to *AFA* mass culture deployment. Low-cost proxies, such as vertical plastic hanging bags could be used for lab scale investigation of *AFA* cultivation feasibility and growth at commercial scale (Cui et al. 2022). Furthermore, while Klamath *AFA* is the obvious strain to cultivate,

exploring the cultivation potential of other *AFA* strains is warranted. Such strains might possess different growth properties that lend themselves well to cultivation.

The selection of a suitable commercial cultivation system is a critical factor in the mass cultivation of microalgae and cyanobacteria. Currently, two main types of systems are employed: open and closed systems (Zittelli et al. 2013). The predominant open system is the raceway pond system (Acien et al. 2017). Drawbacks associated with this system include contamination, poor gas/liquid mass transfer, poor temperature control and light utilisation efficiency (Zittelli et al. 2013; Carney and Lane 2014). Contamination can be minimised by employing media conditions such as alkaline pH, thereby minimising fouling from other microorganisms, and worldwide Spirulina production occurs predominantly (<90%) in raceway ponds owing to its ability to tolerate high pH levels, similar to *AFA* (Acien et al. 2013). Raceway ponds would provide the best system to mimic Klamath Lake conditions and potentially obtain successful *AFA* mass cultivation.

Closed systems include tubular photobioreactors (tPBRs), flat panels and columns (Singh and Sharma 2012). These PBR systems provide accurate control of culture conditions and transfer of nutrients and gas between the inside and outside environment, thereby minimising any culture contamination and maximising production of high-value compounds (Acien et al. 2013; Acien et al. 2017). PBRs typically possess a biomass productivity rate ~5-10x higher than raceways. However, they are much more capital intensive: their deployment costs can be ~20x more expensive and operating costs as much as 15x that of a raceway pond (Lizzul 2016, Narala et al., 2016, Acien et al. 2017). PBR use is typically restricted to high-value products from microalgae or cyanobacteria, such as astaxanthin or phycocyanin (Ranjbar et al. 2008; Yuan et al. 2011). The most employed PBR system is a serpentine tubular PBR (stPBR) as it is easily cleanable, extremely reliable and automated. These stPBRs are composed of straight tubes interconnected by U-bends to create a flat loop that may either be arranged vertically or horizontally (Zittelli et al. 2013). Nevertheless, stPBRs can reach linear liquid velocities of 20-50 m s^{-1}, potentially causing unsuitable turbulent conditions for *AFA* (Singh and Sharma 2012). Another potential option for *AFA* cultivation are flat panel PBRs: cuboidal-shaped reactors with a width ranging between 0.07-5 cm which provides a minimal light path (Singh and Sharma 2012; Banerjee and Ramaswamy 2019). This latter characteristic allows high final biomasses as the light is able to reach cells even at the centre of a dense culture, namely the "aphotic zone", which would otherwise be unable to receive light (Masojídek et al. 2015). *Arthrospira* grown in flat panels has reached biomass productivities of up to 12 g L^{-1} day^{-1}, the highest reported of any PBR (Singh and Sharma 2012). The flat panel's simple design, adaptable mixing rate and minimal light path could provide the optimal platform for maximising *AFA* production for high value bioactives and macromolecules.

Nutrigea s.r.l., a European distributor of Klamath *AFA*, have reported that 1 t of *AFA* currently generate approximately US$1 million in overall revenue. These sales figures are based on 1 t of dry AFA biomass being sold as a nutritional supplement for human consumption, together with C-PC extract sales that has been extracted from 60% of the biomass. Typical raceway pond yields of *Arthrospira* range from 10 to 15 t ha^{-1} $year^{-1}$, while PBR yields average approximately 40 to 50 t ha^{-1} $year^{-1}$ (Acien et al. 2013). Based on these results, the estimated maximum revenues obtainable from *AFA* cultivation range from US$10 –15 million for raceway ponds and US$32 – 40 million for PBRs. While these estimates highlight the commercial potential of growing *AFA* within PBRs, they may not be directly applicable to this cyanobacterium: unsuccessful cultivation, low productivities and/or low final biomass may not justify the high capital and operating costs of these cultivation platforms, especially when compared to the cost-effectiveness of *AFA* wild harvesting from Klamath Lake (Carmichael et al. 2000).

Towards Klamath Lake *AFA* cultivation

Klamath Lake *AFA* only requires sunlight and lake nutrients to bloom, both of which are provided by nature (Carmichael et al. 2000). According to Nutrigea s.r.l., the concentration of Klamath Lake *AFA*'s blooms range between 5-7% of total lake water content, or, in other words, 50-70 g L^{-1}. In comparison, typical final biomass *Arthrospira* concentrations obtained from open cultivation systems are 1-3 g L^{-1} and, from closed cultivation systems, equal or above 10 g L^{-1} (Costa et al. 2014). Klamath Lake *AFA* wild harvesting is more cost-efficient than any current PBR system. The only operating costs for wild harvesting are the energy to power the lake harvester and the workforce salaries (Carmichael et al. 2000). The density of the obtained biomass also lowers downstream processing costs compared to cultivated biomass, as the amount of dewatering required is less (Fasaei et al. 2018). In contrast, *AFA* cultivation would require supplementation with inorganic nutrients, provision of light emitting diodes (LEDs) to optimise light availability and regular PBR maintenance (Blanken et al. 2013). Nevertheless, cultivation would provide three main advantages over Klamath Lake harvesting: 1) the control and monitoring of *AFA*'s growth parameters; 2) a continuous yield of *AFA,* unaffected by seasonality; and 3) unialgal and microcystin-free biomass.

The optimisation of *AFA* growth parameters within PBRs such as flat panels, has the potential to increase the production of high-value compounds such as C-PC, carotenoids,

and lipids compared to Klamath Lake *AFA* biomass (Sierra et al. 2008). Studies have shown that lower irradiances and wavelengths of 660 nm can increase C-PC production in cyanobacteria and could potentially raise *AFA*'s C-PC concentrations above 15% of its whole cell dry biomass, a phenomenon already observed in *Arthrospira* cultivation (Takano et al. 1995; Rivera et al. 2021). Similar techniques could be applied to enhance the production of carotenoids, which prefer wavelengths between 500-600 nm (Maltsev et al. 2021). Temperature modifications can also be employed to favour the production of lipids, with lower temperatures known to increase the amount of PUFAs (Balakrishnan and Shanmugam 2021). Overall, controlled growth would allow a boost to final product pricing and enable better targeting of specific markets. For instance, *AFA* biomass with an elevated omega-3 content could be sold as an aquaculture product for fish oil applications (Remize et al. 2021). However, modification of cultivation parameters for the maximisation of secondary products could have knock-on effects on the growth rate and final biomass, thereby reducing any potential profit. Therefore, it will be important to focus on those products such as C-PC with already established markets that would yield the best net margins while ensuring its overall feasibility (Takano et al. 1995; Rivera et al. 2021). Most importantly, *AFA* cultivation would yield unialgal microcystin-free biomass all-year-round, providing a marketing and supply advantage compared to wild harvesting (Tan et al. 2020). In addition, the know-how underlying successful *AFA* mass cultivation would be a failsafe against any potential impacts of climate change on *AFA* blooms in Klamath Lake in the future. Currently, cultivated *AFA* products may not be able to compete with wild harvested *AFA*-based ones in terms of overall profitability, and it might be that a combination of controlled cultivation and wild harvesting would maximise the advantages of both methods (Table 4).

The productivity of Klamath Lake harvesting could be greatly increased by blending the wild harvested *AFA* biomass with that of microcystin-free cultivated *AFA*. This would significantly reduce the microcystin concentration within the wild harvested biomass, depending on the employed degree of dilution (Carmichael et al. 2000). In this scenario, Klamath Lake *AFA* could be harvested even with microcystins present, thereby increasing the amount of total harvestable days per annum and *AFA* supply, at minimal extra cost. Similarly, cultivated and wild-harvested *AFA* could be integrated according to the market need to ensure *AFA* biomass supply availability throughout the year. For example, if 10 t of dry *AFA* biomass were required to satisfy the estimated market demand and only 5 t of Klamath Lake *AFA* had been harvested, another 5 t of cultivated *AFA* could be blended in throughout the year , thereby also reducing the microcystin concentration by 50 %.

The successful integration of wild harvesting and cultivation would necessitate the deployment of *AFA* cultivation platforms close to the shore of Klamath Lake. This would save on capital investment for new equipment, as the machinery for downstream processing could be shared; save on overall transport costs, due to the proximity of cultivated and wild-harvested *AFA* biomass; and, most importantly, allow access to Klamath Lake's natural resources. The lake water, which has the required nutrients for *AFA* growth, could be directly filtered and used as the employed PBR medium, thereby eliminating a large proportion of the cost for nutrient supply (Ganesh Saratale et al. 2022). It would be however necessary that correct filtering of all biological material is ensured, including bacteriophage that might target the *AFA* or commensal bacteria. Specific key nutrients such as phosphorus may need to be added to the lake water to improve the growth rate within PBRs (Yaakob et al. 2021). After biomass dewatering, the water could be returned to the lake, or, to a centralised treatment system, depending on the water quality, effluent, and discharge regulations. The changes in the lake's nutrient concentrations throughout the year suggest that the nutrients needed for *AFA* growth are, for the most part, present at the optimal

Table 4 SWOT (Strengths, Weaknesses, Opportunities and Threats) analysis of integration and combination of Klamath Lake *AFA* harvesting and mass cultivation

Strengths • Increased number of Klamath Lake *AFA* harvestable days. • Increased all year-round supply of *AFA*. Production of unialgal microcystin-free *AFA* biomass. • Reduced *AFA* cultivation operating costs, like dewatering.	Weaknesses • Increase in overall capital and operating costs (especially with closed-system cultivation). • Skill workforce and downstream processing optimisation required, especially between harvested and cultivated biomass. • Dependence on transport and vicinity to Klamath Lake, restricting *AFA* mass cultivation to lands bordering the lake.
Opportunities • Controlled growth & increased production of high-value compounds, like C-PC (especially with closed-system cultivation) • Increased *AFA* supply and the ability to tailor biomass composition would allow expansion into novel markets, from biofertilisers to high-value products.	Threats • *AFA* mass cultivation feasibility is unknown, and it may not be possible to realise. • *AFA* mass cultivation productivity may not justify the overall costs.

concentrations during the bloom seasons (Wood et al., 1996, Eldridge et al. 2012, Walker et al. 2012). To address this, lake water collected during *AFA* blooms could be stored in large silos and subsequently combined with filtered lake water extracted at other times with specific key nutrients such as CO_2, phosphorus and nitrogen added to enhance growth (Debella 2007). The mineral and vitamins provided by Klamath Lake water would further ensure that the final biomass would have the same nutritional benefits as that harvested directly from the lake surface (Fig 10).

As *AFA* is a diazotroph it may not necessarily require supplementation with nitrogen compounds such as ammonium or nitrate salts, unlike most other commercially grown microorganisms including heterotrophic species such as *E. coli* and yeast, and phototrophic species such as *Chlorella* and *Arthrospira* (De Nobel et al. 1997; Yaakob et al. 2021). Nitrogen is a primary element for cyanobacterial growth (together with carbon, phosphorus, iron, etc.) and the supply of bioavailable forms of nitrogen is one of the major costs in cultivation of microalgae and cyanobacteria (Peccia et al. 2013). For example, Zarrouk medium used for *Arthrospira* cultivation contains 2.5 g L^{-1} nitrates (Madkour et al. 2012). However, the process of nitrogen fixation by diazotrophs is energy intensive and nitrate supplementation might result in faster *AFA* growth rates compared to cultivation in nitrate-free media (Touloupakis et al. 2022). Further research is required to verify this.

Another advantage of locating AFA cultivation close to Klamath Lake is the amount of sunlight it receives – circa 300 days annually (Nuzzo et al. 2018). This would facilitate the set-up of both open and closed cultivation systems. Open ponds could be completely sunlight-driven and employed during the summer months to generate *AFA* biomass (Slegers et al. 2013). As shown in Figure 10, open ponds could be used to maximise *AFA* supply and minimise the negative outcomes of a poor harvest from the lake. Closed systems could be reserved for the production of high-value compounds such as C-PC (Acien et al. 2017). The excess biomass could then be blended with the *AFA* from the lake or from the open ponds. Sunlight could also be captured using solar panels and the energy employed to power the majority of the associated closed-system operations, such as LED illumination and CO_2 bubbling (Nwoba et al. 2020). Importantly, the PBRs could also be repurposed to increase overall *AFA* biomass, especially during the winter months, in order to meet any *AFA* biomass yield targets. This would provide some harvesting and supply flexibility (Fig 10). Finally, Klamath Lake *AFA* may be used as the inoculum

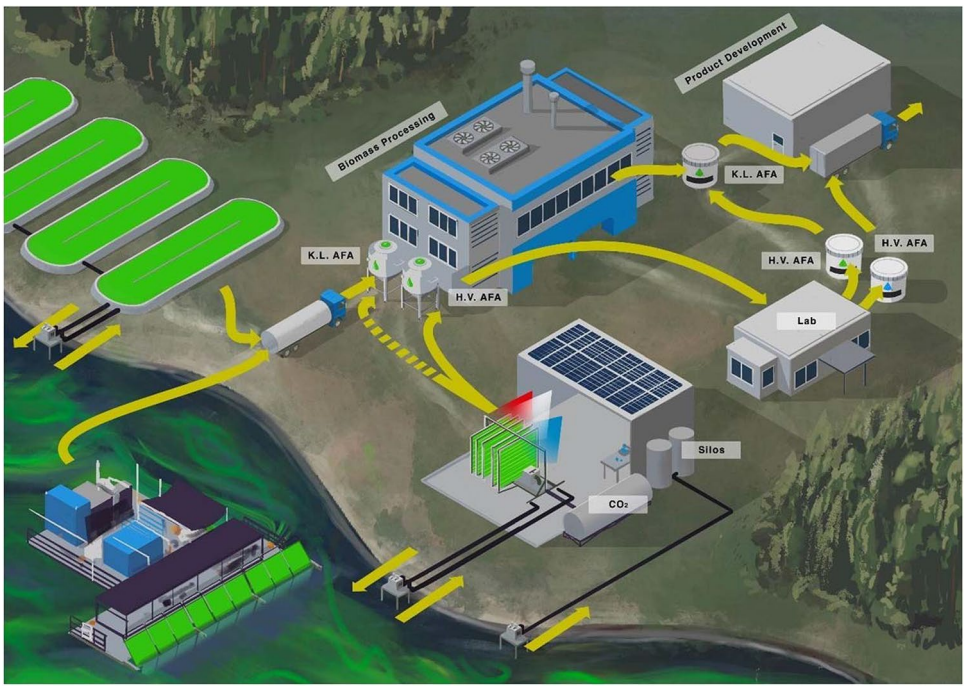

Fig. 10 Model for the integration of Klamath Lake *AFA* harvesting and cultivation. The biomass harvested from Klamath Lake and open ponds is combined and stored in the K.L. (Klamath Lake) *AFA* unit. Biomass derived from closed system photobioreactors (flat panels here), specifically enriched for particular products, is stored in the H.V. (High value) *AFA* unit. Importantly, the latter biomass could also be combined into the K.L. *AFA* storage unit to tackle any supply problems. The biomass processing facility then dewaters and dries all harvested biomass into a powder. The H.V. powder is subsequently transferred to a lab for extraction of high-value products such as C-PC, and the remaining *AFA* powder is further blended into the K.L. *AFA* powder. Finally, the whole-cell *AFA* biomass powder and the extracted high-value product go through product development and are distributed worldwide

for cultivation, although adaptation of the cyanobacterium to a PBR environment might be necessary (Srivastava et al. 2013).

Overall, this model would increase the total harvestable *AFA* biomass from Klamath Lake, while ensuring a steady supply throughout the year. Additionally, closed-system cultivation would provide the opportunity to expand within high-value markets, as observed with the nutraceutical industry, and novel ones, including pharmaceuticals, sunscreens, and dyes or colourants (Rahman 2020; Stromsnes et al. 2021). C-PC extracts could be investigated for the treatment of cancer and/or inflammatory diseases, and PEA extracts for mood disorders and/or neurodegeneration (Nuzzo et al. 2018; De Caro et al. 2019; Braune et al. 2021; Galizzi et al. 2023). However, the drug development process is a lengthy and costly process which may threaten overall profitability (Mohs and Greig 2017). C-PC extracts could also be utilised as colourants within the food and drinks industry, or as fluorophores/markers in the biomedical industry (Kannaujiya et al. 2019). Another potential application is the use of *AFA* MAAs for the production of natural sunscreens as MAAs are low molecular weight compounds (<400 Da), which can dissipate light energy in the form of heat without generating ROS (Fig. 11) (Kumar et al. 2019). There are number of other potential compounds produced by *AFA* which may also hold important biotechnological potential, such as exopolysaccharide extracts for bioremediation treatments or lutein extracts for the prevention of eye diseases (De Philippis et al. 2011; Kumar et al. 2019).

Fig. 11 *Aphanizomenon flos-aquae* (*AFA*) biotechnological opportunities. Illustration of *AFA*'s potential market penetration following its successful mass cultivation. PC: Phycocyanin; PE: Phycoerythrin

Furthermore, an augmented supply of *AFA* would facilitate the entry of the cyanobacterium into the foods, animal feed and aquaculture markets. *AFA*'s nutritional properties could be delivered via its incorporation into everyday foods, such as pasta or baked goods. *AFA* PEA extracts have been shown to be heat-resistant and to survive the cooking process, a major challenge in terms of nutrient delivery (Caporgno and Mathys 2018; Nuzzo et al. 2019). The high protein, vitamin, and mineral content of *AFA* is likely to improve the growth, quality, and immune system of farmed fish and animals, as observed with *Anabaena* and *Arthrospira* species, although *AFA*-based animal studies are necessary to confirm this (Khatoon et al. 2010, Sudaporn et al. 2010, Fadl et al. 2020, Nagarajan et al. 2021, Altmann and Rosenau, 2022). Moreover, *AFA*'s ability to fix nitrogen could make its whole cell biomass, or even its wet biomass, an excellent natural biofertiliser. *AFA*-based biofertilisers might provide an environmentally friendly alternative to chemical nitrogen fertilisers, which are known to cause water quality problems through fertiliser run-off (Righini et al., 2022, Alvarez et al., 2021, Pathak et al., 2018, Chittora et al. 2020).

Towards genetic engineering of *AFA*

The establishment of a cultivation system for *AFA* would enable the application of genetic engineering strategies that could potentially be used to create bespoke strains with improved traits. Whilst the number of transformable microalgal and cyanobacterial species is growing, the development of more advanced genetic engineering technologies is still limited to a handful of model eukaryotes such as *Chlamydomonas reinhardtii*, *Nannochloropsis* sp., and the cyanobacterial models *Synechocystis* and *Synechococcus* (Doron et al. 2016; Poliner et al. 2018; Santos-Merino et al. 2019; Sebesta et al. 2019; Jackson et al. 2021; Baldia et al. 2023). A summary of studies reporting the successful transformation of filamentous cyanobacteria is given in Table 5. The most commonly adopted method of DNA delivery in these systems is electroporation, in which an electrical field is applied to cells to increase the permeability of the cell membrane to plasmid DNA. In systems such as *AFA* where transgenic DNA integration into the genome would be expected to occur in a targeted manner via homologous recombination, it is necessary to identify a 'neutral site' where genomic integration would not disrupt or affect the expression of essential genes (Chaurasia and Apte 2011). Another critical prerequisite for DNA transformation is the availability of a suitable selectable marker together with regulatory DNA elements to drive its expression. The identification of neutral sites and endogenous elements is highly dependent on the availability of a sequenced and well-annotated genome for the host organism, which for Klamath *AFA* has already been made available (NCBI entry: ASM159382v2) (Driscoll 2016).

Achieving successful transformation of filamentous cyanobacteria is technically challenging owing to the difficulties associated with quantifying cell numbers during the culturing and transformation process. Two possible methods to overcome this are the use of sonication to break the filaments into single cells to enable counting, or the use of optical density to estimate cell numbers (Toyomizu et al. 2001). Typically, individual colonies are sub-cultured following transformation to ensure the isolation of a clonal cell line, however, this is often not possible when transforming filamentous strains (Geada et al. 2018). It is also unclear how a filamentous structure affects the dynamics of transgenic DNA propagation and the ability to reach homoplasmy whereby all copies of the genome within a polyploid system contain the transgenic DNA. One study has reported the use of sonication to break filaments of *Arthrospra* into single cells prior to transformation in an attempt to generate discrete transformant colonies, although this was shown to be unsuccessful due to the gliding mobility of the strain (Geada et al. 2018). *Arthrospira* and *Nostoc* spp. have a high

Table 5 A summary of DNA deliverytr methods and related studies on the transformation and genetic engineering of filamentous cyanobacteria. Chlor: Chloramphenicol; GFP: green fluorescent protein; CAT: chloramphenicol acetyltransferase

Strain	Accession Number	Method	Reference
Anabaena PCC 7120	BA000019	Electroporation	Chaurasia and Apte 2011
Anabaena PCC 7120	BA000019	Electroporation	Chaurasia et al. 2008
Leptolyngbya KC45	MN414277	Electroporation	Mahanil et al. 2022
Anabaena M131	M24855	Electroporation / Conjugation	Thiel and Poo 1989
Nostoc punctiforme	CP001037	Electroporation	Argueta et al. 2004
Anabaena PCC 7120	BA000019	Conjugation	Yoon and Golden 1998
Anabaena . 90	CP003284	Electroporation	Rouhiainen et al. 2000
Anabaena PCC 7120	BA000019	Conjugation	Halfmann et al. 2014
Nostoc PCC 7121	MG461362	Electroporation / Conjugation	Moser et al. 1993
Arthrospira platensis NIES-39	AP026945	Electroporation	Toyomizu et al. 2001

prevalence of restriction endonucleases within their cells, and these are likely to be present also in *AFA*. Such endonucleases are known to inhibit transformation by degrading transgenic DNA within the cell before it can integrate stably into the genome (Moser et al. 1993; Walker et al. 2005). However, the use of endonuclease inhibitors has been shown to significantly improve transformation efficiencies in systems with a high endonuclease activity (Jeamton et al. 2017).

One other potential approach involves natural transformation, a process whereby prokaryotes actively take up extracellular DNA into the cytoplasm and integrate the acquired DNA into their genome via homologous recombination (Jester et al. 2022). Naturally competent prokaryotes can also take up whole self-replicating plasmids and express them as their own: this has been successfully employed to transform the filamentous heterocystous cyanobacterium *Chlorogloeopsis fritschii* PCC 6912 (AJLN00000000) (Nies et al. 2022). Natural competency is an evolutionary mechanism that enhances species survival by providing genetic diversity. This process depends on the presence of competence proteins, particularly those associated with type IV pili (Wendt and Pakrasi 2019).

Natural uptake of exogenous DNA requires the competence proteins ComEA, ComEC, and ComF. Subsequent integration of the acquired DNA into the recipient genome requires proteins that bind to single-stranded DNA, such as the DNA processing protein DprA and the DNA recombination and repair protein RecA (Wendt and Pakrasi 2019; Jester et al. 2022). Cyanobacteria that possess genes (termed competence genes) for all these proteins, have been experimentally proven to be naturally transformable. A recent bioinformatic analysis of 345 cyanobacterial genomes revealed that approximately 70% of all genomes possess the genes required for natural competency, including *AFA* strain 2012/KM1/D3 (Wendt and Pakrasi 2019). Natural competency has also been proven experimentally in *Nostoc muscorum* and *A. platensis* (Jester et al. 2022; Nies et al. 2022). Therefore, it is plausible that *Aphanizomenon* is naturally competent. However, further investigation is needed to confirm this hypothesis.

Genetically engineered strains of *AFA* could be generated for the enhanced accumulation of high-value products such as C-PC. Alternatively, metabolic pathways could be introduced or modified to synthesise novel metabolites or increase flux, for example increasing the nitrogen fixation rate for biofertiliser purposes. The latter option, for example, has already been demonstrated in *Anabaena* sp. 7120 by targeting the HetR gene responsible for heterocyst differentiation (Chaurasia and Apte 2011). The engineered line showed a heterocyst frequency of 12-18% in cells of *Anabaena* filaments compared to the 6-8% in the wild-type strain, resulting in a 1.5 to 2-fold higher nitrogenase activity. This was obtained via insertion into the genome of an additional copy of the *hetR* gene driven by the high-expression light-inducible promoter from *psbA1* (Chaurasia and Apte 2011). Importantly, *Nostoc* and *Anabaena* cultures are already known to fix a high amount of nitrogen, 20 to 30 kg N ha^{-1} $season^{-1}$ (Chittora et al. 2020). It is therefore possible that similar *AFA* strains over-expressing *hetR* could potentially fix up to 60 kg N ha^{-1} $season^{-1}$whilst being a natural, safe and sustainable option (Chaurasia and Apte 2011). Similarly, the introduction of another copy of the operon encoding C-PC, or genes for one of its precursors such as the haem oxygenase or uroporphyrinogen III gene could lead to increased cellular accumulation of C-PC, thereby increasing the biotechnological potential of such a strain (Manir AFAsha et al., 2018). Recent cyanobacterial research has also highlighted the potential of fine-tuning the synthesis of pseudocobalamin to produce bioactive vitamin B_{12}. More specifically, cyanobacteria lack the BluB enzyme required to make DMB from riboflavin and the CobT, CobC and CobS enzymes required for inclusion of DMB into the corrin ring (Helliwell et al. 2016). The expression of these four bacterial genes within cyanobacteria could offer a route to low-cost, light-driven production of B_{12}. This has recently been attempted in *A. platensis* and warrants an investigation as to whether this could be achieved in *AFA* as well (Morente 2021). Finally, *AFA*'s edible status offers the possibility of bespoke *AFA* strains being used for the oral delivery of high-value bioactive compounds and therapeutic proteins such as vaccines within the aquaculture and animal feed industry (Yan et al. 2016).

Conclusion

The commercial and biotechnological potential of the nitrogen-fixing filamentous cyanobacterium *Aphanizomenon flos-aquae* is largely untapped. Its comprehensive nutritional profile and high-value products such as C-PC and PEA, make it a promising candidate for markets beyond the nutritional supplement industry, including the animal feed, aquaculture and biofertiliser markets. Currently, *AFA* harvesting is limited to the natural occurrence of blooms in Klamath Lake, and this brings with it issues of seasonal fluctuations and the risk of contamination with microcystin toxins. The integration of artificial cultivation systems such as open ponds and closed photobioreactor systems with natural harvesting could help maximise and stabilise production. The blending of microcystin-free cultivated *AFA* and wild-harvested *AFA* biomass would increase the number of Klamath *AFA* harvestable days per annum and help resolve the current supply predicament. Furthermore, closed-system *AFA* cultivation would yield *AFA* biomass enriched for specific products, thereby facilitating the expansion into novel high-value industries, such as the dyes and colourants, sun cream, fish oil, and pharmaceutical

sectors. Genetic manipulation of *AFA* could generate bespoke strains with increased commercial potential that could further expand its market. Nevertheless, *AFA* is a poorly studied cyanobacterium, and its controlled cultivation and genetic engineering potential remains largely unexplored. It therefore remains a possibility that *AFA* strains are unable to be domesticated, grown at scale or genetically manipulated. Therefore, it is crucial to undertake further fundamental research into growth parameters and genetic manipulation prior to attempting to cultivate this cyanobacterium at industrial scale.

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Data availability All data generated or analysed during this study are included in this published article and its supplementary information.

Declarations

Competing interests G.D.S. is directly involved with Nutrigea s.r.l. operations: the Scoglio family is the owner of Nutrigea s.r.l. S.P. and H.O.J. have no competing interests.

References

Aavad JB (1994) Bloom formation of *Gloeotrichia echinulata* and *Aphanizomenon flos-aquae* in a shallow, eutrophic, Danish lake. Hydrobiologia 289:193–197

Acien G, Fernandez-Sevilla JM, Molina-Grima E (2013) Photobioreactors for the production of microalgae. Rev Env Sci Bio/Technol 12:131–151

Acien G, Molina-Grima E, Reis A, Torzillo G, Chini Zittelli G, Claudia S, Masojídek J (2017) Photobioreactors for the production of microalgae. In: Gonzalez-Fernandez C, Muñoz R (eds) Microalgae-Based Biofuels and Bioproducts. Woodhead Publishing, Oxford, pp 1–44

Al-Haj L, Lui YT, Abed RM, Gomaa MA, Purton S (2016) Cyanobacteria as chassis for industrial biotechnology: progress and prospects. Life (Basel) 6:42

Al-Hoqani U, Young R, Purton S (2016) The biotechnological potential of *Nannochloropsis*. Perspect Phycol 4:1–15

Ali SK, Shaleh AM (2012) *Spirulina* - an overview. Int J Pharm Pharmaceut Sci 4:9–15

Altmann BA, Rosenau S (2022) *Spirulina* as animal feed: opportunities and challenges. Foods 11:965

Alvarez AL, Weyers SL, Goemann HM, Peyton BM, Gardne, RD (2021) Microalgae soil and plants: A critical review of microalgae as renewable resources for agriculture. Algal Res 54:102200

Andrade L, Andrade C, Dias M, Nascimento C, Mendes M (2018) *Chlorella* and *spirulina* microalgae as sources of functional foods, nutraceuticals, and food supplements; an overview. MOJ Food Process Technol 6:45–58

Araújo R, Vázquez Calderón F, Sánchez López J, Azevedo IC, Bruhn A, Fluch S, Garcia Tasende M, Ghaderiardakani F, Ilmjärv T, Laurans M, Mac Monagail M, Mangini S, Peteiro C, Rebours C, Stefansson T, Ullmann J (2021) Current status of the algae production industry in Europe: an emerging sector of the blue bioeconomy. Front Mar Sci 7:626389

Argueta C, Yuksek K, Summers M (2004) Construction and use of GFP reporter vectors for analysis of cell-type-specific gene expression in *Nostoc punctiforme*. J Microbiol Meth 59:181–188

Balakrishnan J, Shanmugam K (2021) Lowering the culture medium temperature improves the omega-3 fatty acid production in marine microalga *Isochrysis* sp. CASA CC 101. Prep Biochem Biotech 51:511–518

Baldia A, Rajput D, Kumar A, Pandey A, Dubey KK (2023) Engineering microalgae as the next-generation food. Systems Microbiol Biomanufact 3:166–178

Banerjee S, Ramaswamy S (2019) Comparison of productivity and economic analysis of microalgae cultivation in open raceways and flat panel photobioreactor. Bioresour Technol Rep 8:100328

Baroni L, Scoglio S, Benedetti S, Bonetto C, Pagliarani S, Benedetti Y, Rocchi M, Canestrari F (2009) Effect of a Klamath algae product ("*AFA*-B12") on blood levels of vitamin B_{12} and homocysteine in vegan subjects: a pilot study. Int J Vitamin Nutr Res 79:117–123

Barroso N, Rodriguez M (1996) Action of β-phenylethylamine and related amines on nigrostriatal dopamine neurotransmission. Eur J Pharmacol 297:195–203

Belay A (2013) Biology and industrial production of *Arthrospira* (*Spirulina*). In: Richmond A, Hu Q (eds) Handbook of microalgal culture: Applied phycology and biotechnology. Blackwell, Oxford, pp 339–358

Benedetti S, Rinalducci S, Benvenuti F, Francogli S, Pagliarani S, Giorgi L, Micheloni M, D'Amici GM, Zolla L, Canestrari F (2006) Purification and characterization of phycocyanin from the blue-green alga *Aphanizomenon flos-aquae*. J Chromatogr B 833:12–18

Billy J, Pruvost J, Lépine O, Drouin D, Gonçalves O (2023) Development of a spectrophotometric method for quantification of C-phycocyanin in the cyanobacterium *Aphanizomenon flos-aquae*. J Appl Phycol 35:1715–1726

Bito T, Bito M, Asai Y, Takenaka S, Yabuta Y, Tago K, Ohnishi M, Mizoguchi T, Watanabe F (2016) Characterization and quantitation of Vitamin B_{12} compounds in various *Chlorella* supplements. J Ag Food Chem 64:8516–8524

Bito T, Okumura E, Fujishima M, Watanabe F (2020) Potential of *Chlorella* as a dietary supplement to promote human health. Nutrients 12:2524

Blanken W, Cuaresma M, Wijffels RH, Janssen M (2013) Cultivation of microalgae on artificial light comes at a cost. Algal Res 2:333–340

Blas-Valdivia V, Moran-Dorantes DN, Rojas-Franco P, Franco-Colin M, Mirhosseini N, Davarnejad R, Halajisani A, Tavakoli O, Cano-Europa E (2022) C-Phycocyanin prevents acute myocardial infarction-induced oxidative stress, inflammation and cardiac damage. Pharmaceut Biol 60:755–763

Borhani-Haghighi A, Ghodsi M, Razeghinejad MR, Mardani S, Mardani M, Nikseresht AR, Safari A, Bagheri MH (2012) Erythropoietin for acute multiple sclerosis in patients with optic neuritis as a first demyelination event. Neurosciences (Riyadh) 17:151–5

Bornet É, Flahault C (1886) Revision des Nostocacées hétérocystées contenues dans les principaux herbiers de France (quatrième et dernier fragment). Ann Sci Nat, Bot, Septième Série 7:177–262

Braune S, Krüger-Genge A, Kammerer S, Jung F, Küpper J-H (2021) Phycocyanin from *Arthrospira platensis* as potential anti-cancer drug: review of in vitro and in vivo studies. Life 11:91

Bryant DA (1982) Phycoerythrocyanin and phycoerythrin: properties and occurrence in cyanobacteria. Microbiology 128:835–844

Caporgno MP, Mathys A (2018) Trends in microalgae incorporation into innovative food products with potential health benefits. Front Nutr 5:58

Carmichael WW, Drapeau C, Anderson DM (2000) Harvesting of *Aphanizomenon flos-aquae* Ralfs ex Born. & Flah. var. *flos-aquae* (Cyanobacteria) from Klamath Lake for human dietary use. J Appl Phycol 12:585–595

Carney LT, Lane TW (2014) Parasites in algae mass culture. Front Microbiol 5:278

Castenholz RW (1988) Culturing methods for cyanobacteria. Meth Enzymol 167:68–93

Chaurasia AK, Parasnis A, Apte SK (2008) An integrative expression vector for strain improvement and environmental applications of the nitrogen fixing cyanobacterium, *Anabaena* sp. strain PCC7120. J Microbiol Meth 73:133–141

Chaurasia AK, Apte SK (2011) Improved eco-friendly recombinant *Anabaena sp*. strain PCC7120 with enhanced nitrogen biofertilizer potential. Appl Environ Microbiol 77:395–399

Chen Z-Y, Asavaritikrai P, Prchal JT, Noguchi CT (2007) Endogenous erythropoietin signaling is required for normal neural progenitor cell proliferation. J Biol Chem 282:25875–25883

Chittora D, Meena M, Barupal T, Swapnil P, Sharma K (2020) Cyanobacteria as a source of biofertilizers for sustainable agriculture. Biochem Biophys Rep 22:100737

Cirés S, Ballot A (2016) A review of the phylogeny, ecology and toxin production of bloom-forming *Aphanizomenon* spp. and related species within the Nostocales (cyanobacteria). Harmful Algae 54:21–43

Clippinger JN, Davis RE (2019) Techno-economic analysis for the production of algal biomass via closed photobioreactors: future cost potential evaluated across a range of cultivation system designs. National Renewable Energy Lab (NREL), Golden, Colorado. NREL/TP-5100-72716 pp 1-42

Cory H, Passarelli S, Szeto J, Tamez M, Mattei J (2018) The role of polyphenols in human health and food systems: a mini-review. Front Nutr 5:87

Costa JA, Colla L, Filho PF (2014) *Spirulina platensis* growth in open raceway ponds using fresh water supplemented with carbon, nitrogen and metal ions. Z Naturforsch C 58:76–80

Cremonte M, Sisti D, Maraucci I, Giribone S, Colombo E, Rocchi MBL, Scoglio S (2017) The effect of experimental supplementation with the klamath algae extract klamin on attention-deficit/hyperactivity disorder. J Med Food 20:1233–1239

Cui J, Purton S, Baganz F (2022) Characterisation of a simple 'hanging bag' photobioreactor for low-cost cultivation of microalgae. J Chem Technol Biotech 97:608–619

Curtain F, Grafenauer S (2019) Plant-based meat substitutes in the flexitarian age: an audit of products on supermarket shelves. Nutrients 11:2603

Dawidowicz P (1990) The effect of *Daphnia* on filament length of blue-green algae. Hydrobiologia 191:265–268

De Caro V, Murgia D, Seidita F, Bologna E, Alotta G, Zingales M, Campisi G (2019) Enhanced in situ availability of *Aphanizomenon flos-Aquae* constituents entrapped in buccal films for the treatment of oxidative stress-related oral diseases: biomechanical characterization and in vitro/ex vivo evaluation. Pharmaceutics 11:35

De Nobel W, Huisman J, Snoep JL, Mur LR (1997) Competition for phosphorus between the nitrogen-fixing cyanobacteria *Anabaena* and *Aphanizomenon*. FEMS Microbiol Ecol 24:259–267

De Philippis R, Colica G, Micheletti E (2011) Exopolysaccharide-producing cyanobacteria in heavy metal removal from water: molecular basis and practical applicability of the biosorption process. Appl Microbiol Biotech 92:697–708

Debella HJ (2007) Mass culture of *Aphanizomenon flos-aquae* Ralfs ex Born. and Flah. *var. flos-aquae* (cyanobacteria) from Klamath Falls, Oregon, USA, in closed chamber bioreactors. Ethiop J Biol 4:135–145

Deprá MC, Mérida LGR, De Menezes CR, Zepka LQ, Jacob-Lopes E (2019) A new hybrid photobioreactor design for microalgae culture. Chem Eng Res Design 144:1–10

Dinicolantonio JJ, O'keefe, J. H. (2018) Importance of maintaining a low omega–6/omega–3 ratio for reducing inflammation. Open Heart 5:e000946

Doron L, Segal NA, Shapira M (2016) Transgene expression in microalgae—from tools to applications. Fronti Plant Sci 7:505

Dreher TW, Davis EW, Mueller RS (2021a) Complete genomes derived by directly sequencing freshwater bloom populations emphasize the significance of the genus level ADA clade within the Nostocales. Harmful Algae 103:102005

Dreher TW, Davis EW, Mueller RS, Otten TG (2021b) Comparative genomics of the ADA clade within the Nostocales. Harmful Algae 104:102037

Driscoll CB (2016). Comparative Genomics of Freshwater Bloom-Forming Cyanobacteria and Associated Organisms. Doctorate Thesis, Oregon State University. 194 pp

Driscoll CB, Meyer KA, Šulčius S, Brown NM, Dick GJ, Cao H, Gasiūnas G, Timinskas A, Yin Y, Landry ZC (2018) A closely-related clade of globally distributed bloom-forming cyanobacteria within the Nostocales. Harmful Algae 77:93–107

Eldridge SLC, Wood TM, Echols KR (2012) Spatial and temporal dynamics of cyanotoxins and their relation to other water quality variables in Upper Klamath Lake, Oregon, 2007-09. Scientific Investigations Report. Reston, Virginia. pp 1-44

Environment Protection Agency (EPA) (2016) Contaminant candidate list (ccl) and regulatory determination. The Government Publishing Office. Federal Register81: 222

Fadl SE, Elsadany AY, El-Shenawy AM, Sakr OA, El Gammal GA, Gad DM, Abo Norag MA, Eissa I (2020) Efficacy of cyanobacterium *Anabaena sp*. as a feed supplement on productive performance and immune status in cultured Nile tilapia. Aquacult Rep 17**:**100406

Fasaei F, Bitter JH, Slegers PM, Van Boxtel AJB (2018) Techno-economic evaluation of microalgae harvesting and dewatering systems. Algal Res 31:347–362

Ferreira FMB, Soler JMF, Fidalgo ML, Fernández-Vila P (2001) PSP toxins from *Aphanizomenon flos-aquae* (cyanobacteria) collected in the Crestuma-Lever reservoir (Douro River, northern Portugal). Toxicon 39:757–761

Galizzi G, Deidda I, Amato A, Calvi P, Terzo S, Caruana L, Scoglio S, Mulè F, Di Carlo M (2023) *Aphanizomenon flos-aquae* (*AFA*) extract prevents neurodegeneration in the HFD mouse model by modulating astrocytes and microglia activation. Int J Mol Sci 24:4731

Ganesh Saratale R, Ponnusamy VK, Jeyakumar RB, Sirohi R, Piechota G, Shobana S, Dharmaraja J, Lay CH, Dattatraya Saratale G, Seung Shin H, Ashokkumar V (2022) Microalgae cultivation strategies using cost–effective nutrient sources: recent updates

and progress towards biofuel production. Bioresour Technol 361:127691

Geada P, Rodrigues R, Loureiro L, Pereira R, Fernandes B, Teixeira JA, Vasconcelos V, Vicente AA (2018) Electrotechnologies applied to microalgal biotechnology – applications, techniques and future trends. Renew Sustain Energy Rev 94:656–668

Genazzani AD, Chierchia E, Lanzoni C, Santagni S, Veltri F, Ricchieri F, Rattighieri E, Nappi RE (2010) Effects of Klamath Algae extract on psychological disorders and depression in menopausal women: a pilot study. Minerva Ginecology 62:381–388

Gentile JH, Maloney TE (1969) Toxicity and environmental requirements of a strain of *Aphanizomenon flos-aquae* (L.) Ralfs. Can J Microbiol 15:165–173

Gentscheva G, Nikolova K, Panayotova V, Peycheva K, Makedonski L, Slavov P, Radusheva P, Petrova P, Yotkovska I (2023) Application of *Arthrospira platensis* for medicinal purposes and the food industry: a review of the literature. Life 13:845

Gerloff GC, Fitzgerald GP, Skoog F (1950) The isolation, purification, and culture of blue-green algae. Am J Bot 37:216–218

Gong L, Thorn CF, Bertagnolli MM, Grosser T, Altman RB, Klein TE (2012) Celecoxib pathways: pharmacokinetics and pharmacodynamics. Pharmacogenetics and Genomics 22:310–318

Grama SB, Liu Z, Li J (2022) Emerging trends in genetic engineering of microalgae for commercial applications. Mar Drugs 20:285

Grewe CB, Pulz O (2012) The biotechnology of cyanobacteria. In: Whitton BA (ed) Ecology of Cyanobacteria II: Their Diversity in Space and Time. Springer, Dordrecht, pp 707–739

Halfmann C, Gu L, Gibbons W, Zhou R (2014) Genetically engineering cyanobacteria to convert CO_2, water, and light into the long-chain hydrocarbon farnesene. Appl Microbiol Biotechnol 98:9869–9877

Hart AN, Zaske LA, Patterson KM, Drapeau C, Jensen GS (2007) Natural killer cell activation and modulation of chemokine receptor profile in vitro by an extract from the cyanophyta *Aphanizomenon flos-aquae*. J Med Food 10:435–441

Haselkorn R (1978) Heterocysts. Annu Rev Plant Physiol 29:319–344

Helliwell KE, Lawrence AD, Holzer A, Kudahl UJ, Sasso S, Kräutler B, Scanlan DJ, Warren MJ, Smith AG (2016) Cyanobacteria and eukaryotic algae use different chemical variants of vitamin B_{12}. Curr Biol 26:999–1008

Hertzberg S, Jensen SL (1966) The carotenoids of blue-green algae—II.: the carotenoids of *Aphanizomenon flos-aquae*. Phytochemistry 5:565–570

Heussner AH, Mazija L, Fastner J, Dietrich DR (2012) Toxin content and cytotoxicity of algal dietary supplements. Toxicol Appl Pharmacol 265:263–271

Irsfeld M, Spadafore M, Prüß BM (2013) β-Phenylethylamine, a small molecule with a large impact. Webmedcentral 4:4409

Jackson HO, Taunt HN, Mordaka PM, Smith AG, Purton S (2021) The algal chloroplast as a testbed for synthetic biology designs aimed at radically rewiring plant metabolism. Front Plant Sci 12:708370

Jaworski GHM, Talling JF, Heaney SI (1981) The influence of carbon dioxide-depletion on growth and sinking rate of two planktonic diatoms in culture. Br Phycol J 16:395–410

Jeamton W, Dulsawat S, Tanticharoen M, Vonshak A, Cheevadhanarak S (2017) Overcoming intrinsic restriction enzyme barriers enhances transformation efficiency in *Arthrospira platensis* C1. Plant Cell Physiol 58:822–830

Jensen G, Ginsberg D, Huerta P, Citton M, Drapeau C (2000) Consumption of *Aphanizomenon flos-aquae* has rapid effects on the circulation and function of immune cells in humans a novel approach to nutritional mobilization of the immune system. J Am Nutraceut Assoc 2:51–58

Jester BW, Zhao H, Gewe M, Adame T, Perruzza L et al (2022) Development of *Spirulina* for the manufacture and oral delivery of protein therapeutics. Nature Biotechnol 40:956–964

Kannaujiya VK, Kumar D, Pathak J, Sinha RP (2019) Phycobiliproteins and their commercial significance. In: Mishra AK, Tiwari DN, Rai AN (eds) Cyanobacteria. Academic Press, NY, pp 207–216

Kaplan-Levy RN, Hadas O, Summers ML, Rücker J, Sukenik A (2010) Akinetes: dormant cells of cyanobacteria. In: Lubzens E, Cerda J, Clark M (eds) Dormancy and resistance in harsh environments. Springer, Berlin, pp 5–27

Kefayat A, Ghahremani F, Safavi A, Hajiaghababa A, Moshtaghian J (2019) C-phycocyanin: a natural product with radiosensitizing property for enhancement of colon cancer radiation therapy efficacy through inhibition of COX-2 expression. Sci Rep 9:19161

Khatoon N, Sengupta P, Homechaudhuri S, Pal R (2010) Evaluation of algae based feed in goldfish (*Carassius auratus*) nutrition. Proc Zool Soc 63:109–114

Komarek, (2006) Cyanobacterial taxonomy: current problems and prospects for the integration of traditional and molecular approaches. Algae 21:349–375

Komárek J (1989) Trichome structure of four *Aphanizomenon* taxa (Cyanophyceae) from Czechoslovakia, with notes on the taxonomy and delimitation of the genus. Plant Systemat Evol 164:47–64

Komárek, J. (2013) Cyanoprokaryota: 3rd Part: Heterocystous Genera. In: Büdel B, Gärtner G, Krienitz L, Schagerl M (eds.) Süßwasserflora von Mitteleuropa, Bd. 19 (3). Springer Spektrum, Berlin, 1130 p

Komárek J, Komárková J (2006) Diversity of *Aphanizomenon*-like cyanobacteria. Fottea 6:1–32

Konopka A, Kromkamp J, Mur LR (1987) Regulation of gas vesicle content and buoyancy in light- or phosphate-limited cultures of *Aphanizomenon flos-aquae* (Cyanophyta). J Phycol 23:70–78

Krajewska M, Szymczak-Żyła M, Kobos J, Witak M, Kowalewska G (2019) Canthaxanthin in recent sediments as an indicator of heterocystous cyanobacteria in coastal waters. Oceanologia 61:78–88

Kumar BR, Mathimani T, Sudhakar MP, Rajendran K, Nizami A-S, Brindhadevi K, Pugazhendhi A (2021) A state of the art review on the cultivation of algae for energy and other valuable products: application, challenges, and opportunities. Renew Sustain Energy Rev 138:110649

Kumar G, Shekh A, Jakhu S, Sharma Y, Kapoor R, Sharma TR (2020) Bioengineering of microalgae: recent advances, perspectives, and regulatory challenges for industrial application. Front Bioeng Biotechnol 8:914

Kumar J, Singh D, Tyagi MB, Kumar A (2019) Cyanobacteria: applications in biotechnology. In: Mishra AK, Tiwari DN, Rai AN (eds) Cyanobacteria. Academic Press, London, pp 327–346

Kuriakose GC, Kurup MG (2010) Antioxidant and hepatoprotective activity of *Aphanizomenon flos-aquae* Linn against paracetamol intoxication in rats. Ind J Exp Biol 48:1123–1130

Kushak R, Drapeau C, Winter H (2001) The effect of blue-green algae *Aphanizomenon flos-aquae* on nutrient assimilation in rats. Oxidat Med Cell Longev 3:35–39

Lähteenmäki-Uutela A, Rahikainen M, Lonkila A, Yang B (2021) Alternative proteins and EU food law. Food Control 130:108336

Lea-Smith DJ, Summerfield TC, Ducat DC, Lu X, McCormick AJ, Purton S (2021) Editorial: Exploring the growing role of cyanobacteria in industrial biotechnology and sustainability. Front Microbiol 12:725128

Legrand J, Artu A, Pruvost J (2021) A review on photobioreactor design and modelling for microalgae production. Reaction Chem Eng 6:1134–1151

Li R, Carmichael WW, Liu Y, Watanabe MM (2000) Taxonomic re-evaluation of *Aphanizomenon flos-aquae* NH-5 based on morphology and 16S rRNA gene sequences. Hydrobiologia 438:99–105

Li R, Carmichael WW, Pereira P (2003) Morphological and 16s rRNA gene evidence for reclassification of the paralytic shellfish toxin producing *Aphanizomenon flos-aquae* LMECYA 31 as *Aphanizomenon issatschenkoi* (Cyanophyceae). J Phycol 39:814–818

Li X, Wang X, Duan C, Yi S, Gao Z, Xiao C, Agathos SN, Wang G, Li J (2020) Biotechnological production of astaxanthin from the microalga *Haematococcus pluvialis*. Biotech Adv 43:107602

Li Z, Guo M (2018) Healthy efficacy of *Nostoc commune vaucher*. Oncotarget 9:14669.

Liu R, Qin S, Li W (2022) Phycocyanin: anti-inflammatory effect and mechanism. Biomed Pharmacother 153:113362

Liu Y, Chen W, Li D, Shen Y, Li G, Liu Y (2006) First report of aphantoxins in China—waterblooms of toxigenic *Aphanizomenon flos-aquae* in Lake Dianchi. Ecotoxicol Env Safety 65:84–92

Lizzul, A. M. (2016). Integrated production of algal biomass. Doctorate Thesis, University College London, UK. 285 pp

Lyon-Colbert A, Su S, Cude C (2018) A systematic literature review for evidence of *Aphanizomenon flos-aquae* toxigenicity in recreational waters and toxicity of dietary supplements: 2000–2017. Toxins 10:254

Ma H, Wu Y, Gan N, Zheng L, Li T, Song L (2015) Growth inhibitory effect of *Microcystis* on *Aphanizomenon flos-aquae* isolated from cyanobacteria bloom in Lake Dianchi, China. Harmful Algae 42:43–51

Madhyastha HK, Radha KS, Nakajima Y, Omura S, Maruyama M (2008) uPA dependent and independent mechanisms of wound healing by C-phycocyanin. J Cell Molec Med 12:2691–703

Madkour FF, Kamil AE-W, Nasr HS (2012) Production and nutritive value of *Spirulina platensis* in reduced cost media. Egypt J Aquat Res 38:51–57

Magyar K, Szende B (2004) Deprenyl, a selective MAO-B inhibitor, with apoptotic and anti-apoptotic properties. Neurotoxicology 25:233–242

Mahanil K, Sattayawat P, Pekkoh J, Kameya M, Ishii M, Pumas C (2022) Simple transformation of the filamentous thermophilic cyanobacterium *Leptolyngbya* sp. KC45. Algal Res66**:**102758

Mahmood NA, Carmichael WW (1986) Paralytic shellfish poisons produced by the freshwater cyanobacterium *Aphanizomenon flos-aquae* NH-5. Toxicon 24:175–186

Maltsev Y, Maltseva K, Kulikovskiy M, Maltseva S (2021) Influence of light conditions on microalgae growth and content of lipids, carotenoids, and fatty acid composition. Biology 10:1060

Manir AFAsha E, Murwanashyaka T, Ndikubwimana T, Rashid Ahmed N, Liu J, Lu Y, Zeng X, Ling X, Jing K (2018) Enhancement of cell growth and phycocyanin production in *Arthrospira (Spirulina) platensis* by metabolic stress and nitrate fed-batch. Bioresour Technol 255:293–301

Masojídek J, Sergejevová M, Malapascua JR, Kopecký J (2015) Thin-layer systems for mass cultivation of microalgae: flat panels and sloping cascades. In: Prokop A, Bajpai RK, Zappi ME (eds) Algal Biorefineries: Volume 2: Products and Refinery Design. Springer, Cham, pp 237–261

McCarthy B, O'Neill G, Abu-Ghannam N (2022) Potential psychoactive effects of microalgal bioactive compounds for the case of sleep and mood regulation: opportunities and challenges. Mar Drugs 20:493

Mccommon GW, Lochner F Jr, A. C. B. & Young, H. E. (2013) Primitive stem cells are present in the blood of adult equines and increase with moderate exercise or ingestion of the cyanobacteria *Aphanizomenon flos-aquae*. Autocoids 2:103

Miyamoto E, Tanioka Y, Nakao T, Barla F, Inui H, Fujita T, Watanabe F, Nakano Y (2006) Purification and characterization of a corrinoid-compound in an edible cyanobacterium *Aphanizomenon flos-aquae* as a nutritional supplementary food. J Ag Food Chem 54:9604–9607

Mohs RC, Greig NH (2017) Drug discovery and development: r ole of basic biological research. Alzheimers Dementia 3:651–657

Morente DL (2021) A first study for the establishment of a source of Vitamin B_{12} as of the genetic modification of the cyanobacteria *Arthrospira platensis*. MSc Thesis. Pompeu Fabra, Barcelona. 51 pp

Moser DP, Zarka D, Kallas T (1993) Characterization of a restriction barrier and electrotransformation of the cyanobacterium *Nostoc* PCC 7121. Arch Microbiol 160:229–237

Nagarajan D, Varjani S, Lee D-J, Chang J-S (2021) Sustainable aquaculture and animal feed from microalgae – Nutritive value and techno-functional components. Renew Sustain Energy Rev 150:111549

Narala RR, Garg S, Sharma KK, Thomas-Hall SR, Deme M, Li Y, Schenk PM (2016) Comparison of microalgae cultivation in photobioreactor, open raceway pond, and a two-stage hybrid system. Front Energy Res 4.

Newton A (2015) Alkaline lakes of old. Nature Geosci 8:90–90

Nies F, Springstein Benjamin L, Hanke Dustin M, Dagan T (2022) Natural competence in the filamentous, heterocystous cyanobacterium *Chlorogloeopsis fritschii* PCC 6912. Am Soc Microbiol 7:e00997-21

Nuzzo D, Contardi M, Kossyvaki D, Picone P, Cristaldi L, Galizzi G, Bosco G, Scoglio S, Athanassiou A, Di Carlo M (2019) Heat-resistant *Aphanizomenon flos-aquae* (*AFA*) extract (klamin®) as a functional ingredient in food strategy for prevention of oxidative stress. Oxidat Med Cell Longev 2019:9481390

Nuzzo D, Presti G, Picone P, Galizzi G, Gulotta E, Giuliano S, Mannino C, Gambino V, Scoglio S, Di Carlo M (2018) Effects of the *Aphanizomenon flos-aquae* extract (klamin®) on a neurodegeneration cellular model. Oxidat Med Cell Longev 2018:9089016

Nwoba EG, Parlevliet DA, Laird DW, Alameh K, Louveau J, Pruvost J, Moheimani NR (2020) Energy efficiency analysis of outdoor standalone photovoltaic-powered photobioreactors coproducing lipid-rich algal biomass and electricity. Appl Energy 275:115403

O'flaherty, L. & Phinney, H. (2008) Requirements for the maintenance and growth of *Aphanizomenon flos-aquae* in culture. J Phycol 6:95–97

Ohta H, Takebe Y, Murakami Y, Takahama Y, Morimura S (2017) Tyramine and β-phenylethylamine, from fermented food products, as agonists for the human trace amine-associated receptor 1 (hTAAR1) in the stomach. Biosci Biotech Biochem 81:1002–1006

Palinska KA, Surosz W (2014) Taxonomy of cyanobacteria: a contribution to consensus approach. Hydrobiologia 740:1–11

Park H-K, Kwon M-A, Lee H-J, Oh J, Lee S-H, Kim I-S (2018) Molecular verification of bloom-forming *Aphanizomenon flos-aquae* and their secondary metabolites in the Nakdong River. Int J Environ Res Public Health 15:1739

Pathak J, Rajneesh, Pankaj KM, Shailendra PS, Häder DP, Rajeshwar PS (2018) Cyanobacterial farming for environment friendly sustainable agriculture practices: innovations and perspectives. Front Env Sci 6:7

Peccia J, Haznedaroglu B, Gutierrez J, Zimmerman JB (2013) Nitrogen supply is an important driver of sustainable microalgae biofuel production. Trends Biotechnol 31:134–138

Peng J, Guo F, Liu S, Fang H, Xu Z, Wang T (2023) Recent advances and future prospects of mycosporine-like amino acids. Molecules 28:5588

Pereira P, Onodera H, Andrinolo DO, Franca S, Araújo F, Lagos N, Oshima Y (2000) Paralytic shellfish toxins in the freshwater cyanobacterium *Aphanizomenon flos-aquae*, isolated from Montargil reservoir, Portugal. Toxicon 38:1689–1702

Pérez AA, Rodionov DA, Bryant DA (2016) Identification and regulation of genes for cobalamin transport in the cyanobacterium *Synechococcus* sp. strain PCC 7002. J Bacteriol 198:2753–2761

Poliner E, Farré EM, Benning C (2018) Advanced genetic tools enable synthetic biology in the oleaginous microalgae *Nannochloropsis sp*. Plant Cell Rep 37:1383–1399

Prasanna R, Kumar R, Sood A, Prasanna BM, Singh PK (2006) Morphological, physiochemical and molecular characterization of *Anabaena* strains. Microbiol Res 161:187–202

Preußel K, Stüken A, Wiedner C, Chorus I, Fastner J (2006) First report on cylindrospermopsin producing *Aphanizomenon flos-aquae* (Cyanobacteria) isolated from two German lakes. Toxicon 47:156–162

Pulz O, Gross W (2004) Valuable products from biotechnology of microalgae. App Microbiol Biotechnol 65:635–648

Rahman KM (2020) Food and high value products from microalgae: Market opportunities and challenges. In: Alam MA, Xu J-L, Wang Z (eds.) Microalgae Biotechnology for Food, Health and High Value Products. Springer, Singapore pp 3-27

Rajaniemi P, Hrouzek P, Kaštovska K, Willame R, Rantala A, Hoffmann L, Komárek J, Sivonen K (2005) Phylogenetic and morphological evaluation of the genera *Anabaena, Aphanizomenon, Trichormus* and *Nostoc* (Nostocales, Cyanobacteria). Int J Systemat Evol Microbiol 55:11–26

Ranjbar R, Inoue R, Katsuda T, Yamaji H, Katoh S (2008) High efficiency production of astaxanthin in an airlift photobioreactor. J Biosc Bioeng 106:204–207

Reddy CM, Bhat VB, Kiranmai G, Reddy MN, Reddanna P, Madyastha K (2000) Selective inhibition of cyclooxygenase-2 by C-phycocyanin, a biliprotein from *Spirulina platensis*. Biochem Biophys Res Comm 277:599–603

Reinl KL, Harris TD, North RL, Almela P, Berger SA, Bizic M, Burnet SH, Grossart H-P, Ibelings BW, Jakobsson E, Knoll LB, Lafrancois BM, Mcelarney Y, Morales-Williams AM, Obertegger U, Ogashawara I, Paule-Mercado MC, Peierls BL, Rusak JA, Sarkar S, Sharma S, Trout-Haney JV, Urrutia-Cordero P, Venkiteswaran JJ, Wain DJ, Warner K, Weyhenmeyer GA, Yokota K (2023) Blooms also like it cold. Limnol Oceanogr Lett 8:546–564

Remize M, Brunel Y, Silva JL, Berthon JY, Filaire E (2021) Microalgae n-3 PUFAs production and use in food and feed industries. Mar Drugs 19:113

Righi V, Parenti F, Schenetti L, Mucci A (2016) Mycosporine-like amino acids and other phytochemicals directly detected by high-resolution NMR on Klamath *(Aphanizomenon flos-aquae)* blue-green algae. J Ag Food Chem 64:6708–6715

Righini H, Francioso O, Martel Quintana A, Roberti R (2022) Cyanobacteria: a natural source for controlling agricultural plant diseases caused by fungi and oomycetes and improving plant growth. Horticulturae, 8:58

Rivera C, Niño L, Gelves G (2021) Modeling of phycocyanin production from *Spirulina* platensis using different light-emitting diodes. S Afr J Chem Eng 37:167–178

Romay C, Gonzalez R, Ledon N, Remirez D, Rimbau V (2003) C-Phycocyanin: a biliprotein with antioxidant, anti-inflammatory and neuroprotective effects. Curr Protein Peptide Sci 4:207–216

Rouhiainen L, Paulin L, Suomalainen S, Hyytiäinen H, Buikema W, Haselkorn R, Sivonen K (2000) Genes encoding synthetases of cyclic depsipeptides, anabaenopeptilides, in *Anabaena* strain 90. Molec Microbiol 37:156–167

Safi C, Zebib B, Merah O, Pontalier P-Y, Vaca-Garcia C (2014) Morphology, composition, production, processing and applications of *Chlorella vulgaris*: a review. Renew Sustain Energy Rev 35:265–278

Saker ML, Jungblut AD, Neilan BA, Rawn DFK, Vasconcelos VM (2005) Detection of microcystin synthetase genes in health food supplements containing the freshwater cyanobacterium *Aphanizomenon flos-aquae*. Toxicon 46:555–562

Sandgruber F, Gielsdorf A, Baur AC, Schenz B, Müller SM, Schwerdtle T, Stangl GI, Griehl C, Lorkowski S, Dawczynski C (2021) Variability in macro- and micronutrients of 15 commercially available microalgae powders. Mar Drugs 19:310

Santos-Merino M, Singh AK, Ducat DC (2019) New applications of synthetic biology tools for cyanobacterial metabolic engineering. Front Bioeng Biotechnol 7:33

Schaeffer DJ, Malpas PB, Barton LL (1999) Risk assessment of microcystin in dietary *Aphanizomenon flos-aquae*. Ecotoxicol Environ Safety 44:73–80

Scoglio S, Benedetti S, Canino C, Santagni S, Rattighieri E, Chierchia E, Canestrari F, Genazzani AD (2009) Effect of a 2-month treatment with Klamin®, a Klamath algae extract, on the general well-being, antioxidant profile and oxidative status of postmenopausal women. Gynecol Endocrinol 25:235–240

Scoglio S, Benedetti Y, Benvenuti F, Battistelli S, Canestrari F, Benedetti S (2014) Selective monoamine oxidase B inhibition by an *Aphanizomenon flos-aquae* extract and by its constitutive active principles phycocyanin and mycosporine-like amino acids. Phytomedicine 21:992–997

Sebesta J, Werner A, Peebles CAM (2019) Genetic engineering of cyanobacteria: Design, implementation, and characterization of recombinant *Synechocystis sp.* PCC 6803. Meth Molec Biol 1927:139–154

Show PL (2022) Global market and economic analysis of microalgae technology: status and perspectives. Bioresour Technol357:127329

Shytle DR, Tan J, Ehrhart J, Smith AJ, Sanberg CD, Sanberg PR, Anderson J, Bickford PC (2010) Effects of blue-green algae extracts on the proliferation of human adult stem cells in vitro: a preliminary study. Med Sci Monitor 16**:**BR1-BR5.

Sierra E, Acién FG, Fernández JM, García JL, González C, Molina E (2008) Characterization of a flat plate photobioreactor for the production of microalgae. Chem Eng J 138:136–147

Simopoulos AP (2002) The importance of the ratio of omega-6/omega-3 essential fatty acids. Biomed Pharmacother 56:365–379

Simopoulos AP (2008) The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. Exp Biol Med 233:674–688

Singh RN, Sharma S (2012) Development of suitable photobioreactor for algae production – a review. Renew Sustain Energy Rev 16:2347–2353

Sivonen, K. (2009). Cyanobacterial toxins. In*:* Schaechter M (Ed) Encyclopedia of Microbiology (Third Edition). Academic Press, Oxford, pp290-307

Slegers PM, Lösing MB, Wijffels RH, Van Straten G, Van Boxtel AJB (2013) Scenario evaluation of open pond microalgae production. Algal Res 2:358–368

Soni RA, Sudhakar K, Rana RS (2017) *Spirulina* – From growth to nutritional product: A review. Trends Food Sci Technol 69A:157–171

Sproles AE, Fields FJ, Smalley TN, Le CH, Badary A, Mayfield SP (2021) Recent advancements in the genetic engineering of microalgae. Algal Res 53:102158

Sreenikethanam A, Raj S, J RB, Gugulothu P, Bajhaiya AK(2022) Genetic engineering of microalgae for secondary metabolite production: recent developments, challenges, and future prospects. Front Bioeng Biotechnol 10:836056

Srivastava AK, Rai AN, Neilan BA (eds) (2013) Stress biology of cyanobacteria: molecular mechanisms to cellular responses. CRC Press, Boca Raton

Stromsnes K, Correas AG, Lehmann J, Gambini J, Olaso-Gonzalez G (2021) Anti-inflammatory properties of diet: role in healthy aging. Biomedicines 9:922

Sudaporn T, Kringsak M-A, Yuwadee P (2010) Effect of replacing fishmeal with *Spirulina* on growth, carcass composition and pigment of the Mekong Giant Catfish. Asian J Ag Sci 2:106–110

Takano H, Arai T, Hirano M, Matsunaga T (1995) Effects of intensity and quality of light on phycocyanin production by a marine cyanobacterium *Synechococcus* sp. NKBG 042902. Appl Microbiol Biotechnol 43:1014–1018

Tan JS, Lee SY, Chew KW, Lam MK, Lim JW, Ho S-H, Show PL (2020) A review on microalgae cultivation and harvesting, and their biomass extraction processing using ionic liquids. Bioengineered 11:116–129

Thiel T, Poo H (1989) Transformation of a filamentous cyanobacterium by electroporation. J Bacteriol 171:5743–5746

Touloupakis E, Zittelli GC, Benavides AMS, Torzillo G (2022) Growth and photosynthetic performance of *Nostoc linckia* (formerly *N. calcicola*) cells grown in BG11 and BG110 media. Photochem Photobiol Sci 22:795–807

Toyomizu M, Suzuki K, Kawata Y, Kojima H, Akiba Y (2001) Effective transformation of the cyanobacterium *Spirulina platensis* using electroporation. J Appl Phycol 13:209–214

Tsujimura S, Ishikawa K, Tsukada H (2001) Effect of temperature on growth of the cyanobacterium *Aphanizomenon flos-aquae* in Lake Biwa and Lake Yogo. Phycol Res 49:275–280

Üveges V, Tapolczai K, Krienitz L, Padisák J (2012) Photosynthetic characteristics and physiological plasticity of an *Aphanizomenon flos-aquae* (Cyanobacteria, Nostocaceae) winter bloom in a deep oligo-mesotrophic lake (Lake Stechlin, Germany). Hydrobiologia 698:263–272

Vichi S, Lavorini P, Funari E, Scardala S, Testai E (2012) Contamination by *Microcystis* and microcystins of blue-green algae food supplements (BGAS) on the Italian market and possible risk for the exposed population. Food Chem Toxicol 50:4493–4499

Wacklin P, Hoffmann L, Komárek J (2009) Nomenclatural validation of the genetically revised cyanobacterial genus *Dolichospermum* (Ralfs ex Bornet et Flahault) comb. nova. Fottea 9:59–64

Walker TL, Becker DK, Dale JL, Collet C (2005) Towards the development of a nuclear transformation system for *Dunaliella tertiolecta*. J Appl Phycol 17:363–368

Walker WW, Walker JD, Kann J (2012) Evaluation of water and nutrient balances for the Upper Klamath Lake Basin in water years 1992-2010. Technical Report to the Klamath Tribes Natural Resources Department, Chiloquin, Oregon. pp 50

Wang C, Lan CQ (2018) Effects of shear stress on microalgae - a review. Biotech Adv 36:986–1002

Wang Y, Cooke MJ, Morshead CM, Shoichet MS (2012) Hydrogel delivery of erythropoietin to the brain for endogenous stem cell stimulation after stroke injury. Biomaterials 33:2681–2692

Watanabe MM (1977) Fresh- and salt-water forms of *Spirulina platensis* in axenic cultures. Bull Jap Soc Phycol 25:371–377

Watanabe F, Katsura H, Takenaka S, Fujita T, Abe K, Tamura Y, Nakatsuka T, Nakano Y (1999) Pseudovitamin B_{12} is the predominant cobamide of an algal health food, *spirulina* tablets. J Ag Food Chem 47:4736–4741

Wen Q, Xiao P, Li H, Li W, Yu G, Li R (2022) Succession of *Aphanizomenon flos-aquae* and *Microcystis aeruginosa* in direct coculture experiments at different temperatures and biomasses. J Oceanol Limnol 40:1819–1828

Wendt KE, Pakrasi HB (2019) genomics approaches to deciphering natural transformation in cyanobacteria. Front Microbiol 10:1259

Wienhausen G, Dlugosch L, Jarling R, Wilkes H, Giebel H-A, Simon M (2022) Availability of vitamin B_{12} and its lower ligand intermediate α-ribazole impact prokaryotic and protist communities in oceanic systems. ISME J 16:2002–2014

Wildman RB, Loescher JH, Carol LW (1975) Development and germination of akinetes of *A phanizomenon flos-aquae*. J Phycol 11:96–104

Wood TM, Fuhrer GJ, Morace JL (1996) Relation between selected water-quality variables and lake level in Upper Klamath and Agency Lakes, Oregon. U.S. Geological Survey, Portland, Oregon. NREL/TP-96-4079 pp 1-64

World Health Organisation (WHO) (1999). Toxic Cyanobacteria in water: A guide to their public health consequences, monitoring and management. Chorus I, Bartram J (Eds.). E & FN Spon, London. pp 400.

Wu W, Li G, Li D, Liu Y (2010a) Temperature may be the dominating factor on the alternant succession of *Aphanizomenon flos-aquae* and *Microcystis aeruginosa* in Dianchi Lake. Fresenius Env Bull Adv Food Sci 19:846–853

Wu Y, Li L, Zheng L, Dai G, Ma H, Shan K, Wu H, Zhou Q, Song L (2016) Patterns of succession between bloom-forming cyanobacteria *Aphanizomenon flos-aquae* and *Microcystis* and related environmental factors in large, shallow Dianchi Lake, China. Hydrobiologia 765:1–13

Wu Z, Shi J, Lin S, Li R (2010b) Unraveling molecular diversity and phylogeny of *A phanizomenon* (Nostocales, Cyanobacteria) strains isolated from China. J Phycol 46:1048–1058

Xue X, Lv Y, Liu Q, Zhang X, Zhao Y, Zhang L, Xu S (2015) Extracellular polymeric substance from *Aphanizomenon flos-aquae* induces apoptosis via the mitochondrial pathway in A431 human epidermoid carcinoma cells. Exp Therapeut Med 10:927–932

Yaakob MA, Mohamed R, Al-Gheethi A, Aswathnarayana Gokare R, Ambati RR (2021) Influence of nitrogen and phosphorus on microalgal growth, biomass, lipid, and fatty acid production: an overview. Cells 10:393

Yamamoto Y (2009) Environmental factors that determine the occurrence and seasonal dynamics of *Aphanizomenon flos-aquae*. J Limnol 68:122

Yamamoto Y, Nakahara H (2005) The formation and degradation of cyanobacterium *Aphanizomenon flos-aquae* blooms: the importance of pH, water temperature, and day length. Limnology 6:1–6

Yamamoto Y, Nakahara H (2009a) Life cycle of cyanobacterium *Aphanizomenon flos-aquae*. Taiwania 54:113–117

Yamamoto Y, Nakahara H (2009b) Seasonal variations in the morphology of bloom-forming cyanobacteria in a eutrophic pond. Limnology 10:185–193

Yan N, Fan C, Chen Y, Hu Z (2016) The Potential for microalgae as bioreactors to produce pharmaceuticals. Int J Molec Sci 17:962

Yoon H-S, Golden JW (1998) Heterocyst pattern formation controlled by a diffusible peptide. Science 282:935–938

Yuan JP, Peng J, Yin K, Wang JH (2011) Potential health-promoting effects of astaxanthin: a high-value carotenoid mostly from microalgae. Molec Nut Food Res 55:150–165

Zahiri M, Pourkhalili K, Darvishi S, Heydari H, Akbari Z (2020) The effect of oral *Aphanizomenon flos-aquae* extract on excisional wound healing. Tehran Univ Med J 77:646–650

Zahra Z, Choo DH, Lee H, Parveen A (2020) Cyanobacteria: review of current potentials and applications. Environments 7:13

Zepernick BN, Gann ER, Martin RM, Pound HL, Krausfeldt LE, Chaffin JD, Wilhelm SW (2021) Elevated pH conditions associated with Microcystis spp. blooms decrease viability of the cultured diatom Fragilaria crotonensis and natural diatoms in Lake Erie. Front Microbiol 12:598736.

Zhang DL, Hu CX, Li DH, Liu YD (2013) Lipid peroxidation and antioxidant responses in zebrafish brain induced by *Aphanizomenon flos-aquae* DC-1 aphantoxins. Aquat Toxicol 144–145:250–256

Zhu Y, Jones SB, Anderson DB (2018) Algae farm cost model: considerations for photobioreactors. Pacific Northwest National Laboratory (PNNL), Richland, WA (United States).

Zittelli GC, Rodolfi L, Bassi N, Biondi N, Tredici MR (2013) Photobioreactors for microalgal biofuel production. In: Borowitzka MA, Moheimani NR (eds) Algae for biofuels and energy. Springer, Dordrecht, pp 115–131

Zhu S, Xu J, Adhikari Weiqiao B, Chen LH (2023) *Nostoc sphaeroides* cyanobacteria: a review of its nutritional characteristics and processing technologies. Crit Rev Food Sci Nutr 63:8975–8991