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Cyanobacteria in Scandinavian coastal waters — A potential source for biofuels and fatty acids?

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ABSTRACT

Since land-based biofuel production competes with conventional food production, a water-based biomass and biofuel production from cyanobacteria offers large potential. This study investigates the application potential of cyanobacteria for fuel production and by-products by mimicking nutrient depleted environmental conditions. Three Baltic cyanobacteria strains (*Aphanizomenon flos-aquae*, *Dolichospermum lemmermannii* and *Nodularia spumigena*) were inoculated in full nutrient levels, as well as phosphorus and nitrogen depleted medium, before being monitored for 14 days. For screening reasons, multiple parameters such as fatty acids, photosynthetic pigments including phycobilins, biovolume, photosynthetic activity, inorganic nutrients, particulate organic carbon, nitrogen and phosphorous were investigated every seven days. We observed a strong negative relationship between lipid content, growth and nutrient availability, resulting in high lipid and pigment production in combination with a limited growth rate in nutrient depleted treatments. Our results suggest that cultivation and harvest of bloom-forming cyanobacteria for fuel and by-product production are feasible in Scandinavia, but strongly depends on the desired compounds and biomass. Each cyanobacteria species originally has a species-specific chemical fingerprint that may be modified by rearing conditions and harvesting period to meet the needs of the consumer. This leads to important conclusions regarding future culturing conditions and biomass production of the desired compounds.

1. Introduction

The interest and the demand of biofuels derived from water-living organisms skyrocketed during the last decade. Since land-based biofuel production competes with conventional food production, a water-based biomass and biofuel production offers a large potential. However, the idea of using aquatic primary producers, e.g., algae, for production of various bio-chemicals such as lipids and antimicrobial substances, is not new. After the end of World War II, several working groups around the globe studied the "scientific and economic feasibility of the commercial production of algae in mass cultures" [1–4].

1.1. Biofuels

For the production of biodiesel, biomethane, bioethanol and biohydrogen, many potential biofuel sources have been identified so far (e.g. corn, switchgrass, sugarcane, wheat). Aquatic primary producers are known to have better solar-to-biomass energy conversion efficiencies (~2–10%) than current biofuels from land-based plants (~0.2–2% [5]) and therefore become increasingly more and more attractive as biofuel precursors [6]. Biodiesel production from microalgae via transesterification is regarded as one of the most efficient ways of generating biofuels and is to present knowledge the "only current renewable source of oil [lipids] that could meet the global demand for transport fuels" [7,8].

Although many lobbying groups have been established to govern green energy, such as the European Algae Biomass Association (EABA) or the Carbon Trust in the UK, the total energy content in biodiesel and bioethanol is still less than 1% of the world's energy consumption [9]. Consequently, the perseverative questions remain: Are these biofuels suitable for mass production? Can we grow, harvest and extract the required products in an appropriate and efficient way, considering both economic and sustainable factors? What are the impacts on the ecosystems now and in the future?

Cyanobacteria, sometimes called blue-green algae, have the advantage of carrying characteristics from both algae and bacteria. Their ability to perform photosynthesis is based on their association with algae, while the fixation of atmospheric nitrogen by several cyanobacteria species indicates their bacterial roots. Filamentous cyanobacteria are known to form massive blooms in the Baltic Proper during summer, resulting in greenish carpets of biomass in the upper water layer. The

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Table 1
Nutrient treatments [μM] of the experiment for *D. lemmermannii*, *A. flos-aquae* and *N. spumigena* for phosphorus depleted f/2 medium (−P), nitrogen depleted f/2 medium (−N) and full nutrient treatment (f/2 medium).

Species	Nutrients							
	Treatment	P		N nitrite + nitrate		Si		Nitrite	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Dlemmermanni-i	Control[a]	<2.00	–	144.9	6.5	26.8	0.8	<2.00	–
	−P	<2.00	–	1153.4	3.8	34.9	11.0	<2.00	–
	−N	44.9	0.4	129.2	1.3	34.5	0.8	<2.00	–
	f/2	44.8	0.5	1123.6	9.9	32.2	3.8	<2.00	–
A. flos-aquae	Control[a]	3.77	2.5	24.5	14.8	23.0	0.2	<2.00	–
	−P	2.4	0.2	1005.8	1.9	29.0	9.9	<2.00	–
	−N	45.2	1.0	10.8	1.4	26.5	0.2	<2.00	–
	f/2	47.7	1.2	1032.3	2.0	26.3	2.3	<2.00	–
N. spumigena	Control[a]	<2.00	–	52.9	2.5	27.0	9.8	<2.00	–
	−P	<2.00	–	949.3	3.8	25.0	5.4	<2.00	–
	−N	38.9	0.4	41.1	0.5	24.4	0.2	<2.00	–
	f/2	38.8	0.1	967.7	1.6	24.6	0.5	<2.00	–

The control shows the nutrient values before addition of artificial media. SD refers to standard deviation.
[a] Baltic seawater + respective species.

three dominating species are *Aphanizomenon* sp., *Dolichospermum* sp. and *Nodularia spumigena*. Regional and global climate change, as well as human-induced nutrient over-enrichment, may lead to an increase in growth rates, biomass and oxygen depletion. This could alter food webs and ecosystem structures [10,11] as well as harm tourism industries in the Baltic Sea [12]. To turn the threat [13] into gain, further research related to the application and harvest of cyanobacteria, as precursors for fuel production and by-products, is pressing [14].

Since some cyanobacteria species represent the only phototrophs capable of fixing atmospheric nitrogen, they prosper in low ratios of nitrogen:phosphorus supply. However, nutrient ratios and availabilities influence cell contents. In order to obtain optimal culture conditions, maximum biomass, or maximum output of certain lipids or by-products, nutrient availabilities have to be determined and carefully considered. In contrast to many other prokaryotes, cyanobacteria have a direct correlation between growth and secondary metabolite production [15–17]. Various types of chemical compounds and toxins are produced by cyanobacteria; Nagle et al. [18] classified 424 marine cyanobacterial natural products (Marin Lit database [16]) resulting in 40.2% lipopeptides (amino-acid derived fragment linked to a fatty-acid derived portion [19]), 9.4% amides, 5.6% with pure amino acid composition, 4.2% fatty acids (FA), 4.2% macrolides and 36.4% others (lactones, indoles, esters, pyrroles and undefined substances). Biological activities of the compounds were reported to be anticarcinogenic, cytotoxic, antibiotic, antifungal, and antiviral and some had either other or no activities [16]. Because polyunsaturated Ω-3 fatty acids have proven health benefits, demand for them is rising. Presently, these compounds are commonly extracted from natural fish and krill populations, pressing the global fish stocks. Accordingly, the search and the market for alternative sources are speeding up [20]. Cyanobacteria are known to be a source of several fuel types. Hydrogen, for example, can be produced by many strains, ethanol is produced from their carbohydrates, biogas (methane) via anaerobic digestion of their biomass, photanol, short-chained alcohols produced by combining phototrophy and chemotrophy in genetically engineered cyanobacteria [21] and diesel from their FA and hydrocarbons [22]. The demand for present and new industrial applications of cyanobacteria has set the frame for this study.

In this study we investigated three bloom-forming cyanobacteria strains of the Baltic Sea. Our aim was to study: 1) whether their FA content is suitable for a potential biofuel production; 2) whether nutrient enrichment and depletion under simulated natural radiation conditions can change and enrich total FA content or FA composition and 3) whether these cyanobacteria contain promising marine products, such as lipopeptidic compounds, of importance for future industrial use.

2. Material and methods

For the experiments, cultures of the Kalmar Algal Collection (KAC, Linnaeus University, Kalmar, Sweden) isolated from the Baltic Proper were used. The three cyanobacterial strains *Aphanizomenon flos-aquae* Ralfs ex Bornet & Flahault (KAC 15), *Dolichospermum lemmermannii* (P. Richter) Wacklin, Hoffmann et Komárek (syn: *Anabaena lemmermannii*; KAC 16) and *N. spumigena* Mertens (KAC 12) were inoculated for two weeks at full nutrient levels (f/2 according to [23]) and salinity 7 to obtain desired biovolumes. The cultures were aerated and grown at ~450 μmol photons m^2 s^{-1} photosynthetically active radiation (PAR 400–700 nm) similar to expected natural radiation intensities in the upper water layer of the Baltic Proper during summer. PAR was provided by six fluorescent tubes (Osram L 36W/72-965 Biolux, Osram, München, Germany) and logged continuously during the course of the experiment.

Before the start of the experiment, the number of cells L^{-1} and the biovolume in mm^3 L^{-1} were analyzed and adjusted to obtain comparable biovolumes [24] for all three species. Control samples for all parameters (FA, photosynthetic pigments including phycobilin pigments, biovolume, photosynthetic activity, inorganic nutrients, particulate organic carbon (POC), particulate organic nitrogen (PON) as well as particulate organic phosphorous (POP)) were taken in five replicates. Each bottle containing one cyanobacteria species was then divided into three additional bottles, before adding nutrient solutions (Table 1), creating three different nutrient treatments: 1. Nitrogen depletion (−N treatment, f/2 medium without NO_3^-), 2. Phosphorus depletion (−P treatment, f/2 medium without PO_4^{3-}) and 3. Full nutrient levels (f/2 treatment, f/2 medium). Nutrient samples were also taken in five replicates for each nutrient treatment and for each species. After this, 180 mL of the respective cyanobacteria and nutrient solutions were distributed into 250 mL Nunc-bottles (NUNC, Numbrecht, Germany). The bottles were subsequently placed in a thermoconstant room at 17 °C for two weeks. Nutrients were added after seven days to assure nutrient availabilities comparable to the initial values (Table 1) throughout the experimental period. Sampling of all parameters was done initially (Day 0) and repeated after 7 and 14 days (Day 7 and Day 14).

2.1. Fatty acid analysis

For each treatment, 20 mL from each of the five replicates was prepared for FA analysis by filtration on precombusted GF/C Filters (Whatman, Maidstone, UK), covered with dichloromethane/methanol (2:1 v/v, Merck, Darmstadt, Germany), frozen in liquid nitrogen and

Table 2
FA profiles, TFA, ratios of monounsaturated to polyunsaturated fatty acids (MUFA:PUFA) and saturated to monounsaturated fatty acids (SAFA:MUFA) [μg mm^{-3}] for *D. lemmermannii*, *A. flos-aquae* and *N. spumigena* at Day 0, Day 7 and Day 14 for phosphorus depleted f/2 medium (−P), nitrogen depleted f/2 medium (−N) and full nutrient treatment (f/2 medium). SD refers to standard deviation.

	D. lemmermannii														*A. flos-aquae*							
Day	0		7						14						0		7					
Treatment	Initial		−P		−N		f/2		−P		−N		f/2		Initial		−P		−N		f/2	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
14:0	0.3	0.1	17.4	6.7	12.6	6.6	5.2	2.1	2.0	1.2	6.5	3.0	11.3	9.5	0.2	0.2	2.4	3.4	1.8	0.8	4.2	0.4
i-15:0	1.1	0.8	37.7	24.3	16.8	10.1	8.5	5.5	10.3	2.9	11.6	4.8	22.4	19.2	0.0	0.0	1.3	1.4	1.4	0.9	1.7	0.5
a-15:0	0.6	0.3	18.7	12.7	6.6	4.1	3.3	2.4	5.8	1.6	4.5	1.9	8.3	6.9	0.0	0.0	0.4	0.5	0.5	0.3	1.1	0.4
15:0	0.0	0.0	5.7	3.6	2.0	1.2	2.8	1.9	1.6	0.6	1.1	0.5	2.0	1.6	0.0	0.0	0.4	0.3	0.4	0.4	0.6	0.0
16:0	3.1	0.7	109.9	45.9	60.9	30.8	29.3	16.3	31.1	8.4	38.7	18.5	65.7	59.3	2.6	1.2	12.1	6.3	15.7	8.1	21.6	1.5
16:1(n-7)	1.3	0.3	29.0	17.8	15.2	8.6	7.8	5.7	13.1	4.7	10.4	4.4	16.4	12.0	0.1	0.0	2.5	3.1	1.8	0.9	4.6	0.7
16:2(n-4)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	4.8	7.5	0.0	0.1	0.4	0.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
17:0	0.0	0.0	0.3	0.6	0.6	0.5	0.0	0.0	1.3	0.6	0.6	0.3	0.6	0.6	0.1	0.0	0.3	0.1	0.0	0.1	0.0	0.1
16:3(n-4)	0.2	0.1	0.4	0.8	0.0	0.0	1.1	1.4	0.1	0.3	0.4	1.0	0.5	1.1	0.3	0.1	0.4	0.9	0.7	0.6	0.2	0.3
16:4(n-1)	0.1	0.0	0.0	0.0	0.9	1.3	0.0	0.0	0.2	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.9	3.8
18:0	1.8	0.3	43.6	23.2	15.4	6.7	13.3	16.6	25.4	9.4	9.7	4.3	17.9	15.1	1.4	0.6	8.2	3.7	9.9	7.4	8.0	2.9
18:1(n-9).cis + trans	0.8	0.2	26.9	12.8	12.6	6.2	7.1	7.7	9.8	3.6	7.2	3.4	14.7	12.6	0.4	0.1	2.2	1.9	3.3	1.7	7.1	6.1
18:1(n-7)	3.5	0.6	62.9	38.2	28.0	16.0	14.1	10.8	34.4	24.1	23.5	10.3	32.1	19.7	0.4	0.1	8.8	7.5	8.5	6.8	10.9	2.4
18:2(n-6) cis	0 6	0.2	27.1	11.8	14.5	5.5	8.3	5.6	5.4	3.9	8.2	3.7	10.0	6.2	0.5	0.2	1.0	0.8	1.1	0.5	2.6	1.8
18:3(n-6) & 19:0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	2.0	4.4	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.1	0.4	0.7
18:3(n-3)	0.9	0.3	42.6	23.2	66.5	25.6	29.8	11.4	8.6	5.6	35.3	16.0	43.5	26.3	2.7	1.3	2.7	2.8	5.1	3.6	19.2	7.4
18:4(n-3)	0.1	0.1	1.6	1.2	1.7	2.2	1.6	3.0	2.8	4.6	0.0	0.0	0.1	0.2	0.0	0.0	0.3	0.4	0.0	0.1	2.5	4.4
20:0	0.0	0.1	1.5	1.1	0.3	0.1	1.1	2.1	1.2	0.8	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.2	0.0	0.1	0.6	0.7
20:4(n-6)	0.0	0.1	1.2	0.6	1.3	0.5	0.6	0.3	1.5	1.0	0.5	0.2	0.6	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.7
20:3(n-3)	0.0	0.0	0.6	0.6	1.1	0.4	0.8	0.7	1.6	3.1	0.6	0.3	0.6	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.6
20:4(n-3)	0.0	0.0	0.0	0.0	1.9	2.6	0.0	0.0	5.4	10.1	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.8	1.5
20:5(n-3)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.8	3.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.8
22:5(n-3)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.9	1.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	1.2	2.4
24:0	0.2	0.2	0.0	0.0	1.0	1.8	0.0	0.0	0.2	0.4	0.0	0.0	0.0	0.0	0.4	0.1	1.2	0.8	0.0	0.0	0.1	0.3
22:6(n-3)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.2	1.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.7
Total Σ fatty acids	14.7	3.6	427.0	182.2	259.8	122.2	134.7	82.1	173.8	57.1	158.9	66.3	247.2	178.4	9.1	1.7	44.7	30.9	50.3	24.5	90	16.5
SAFA	7.2	2.2	234.7	115.5	116.3	58.8	63.5	45.9	79.1	22.6	72.7	32.7	156.3	85.6	4.8	2.2	26.5	14.4	29.7	16.5	38.2	13.6
MUFA	5.6	0.9	118.8	67.7	55.8	30.8	29.0	24.0	57.2	30.6	41.1	17.6	79.0	23.6	0.9	0.2	13.5	12.3	13.5	9.1	23.2	11.3
PUFA	1.9	0.7	73.4	27.7	87.8	33.0	42.2	15.2	37.4	29.3	45.1	19.0	72.1	14.4	3.5	1.6	4.7	4.6	7.1	4.3	31.9	19.6
MUFA/PUFA	2.9		1.6		0.6		0.7		1.5		0.9		1.1		0.3		2.9		1.9		0.7	
SAFA/MUFA	1.3		2.0		2.1		2.2		1.4		1.8		2.0		5.3		2.0		2.2		1.6	

stored at −80 °C until extraction. For extraction, filters were homogenized by ultrasonication in dichloromethane:methanol (2:1, v/v) following the method described by Folch et al. [25]. An internal standard was added (23:0 FAME) prior to extraction. For gas–liquid chromatography of FA, methyl esters were prepared from aliquots of the extracted cyanobacteria by transesterification with 3% sulfuric acid in absolute methanol for 4 h at 80 °C. After extraction with hexane, fatty acid methylesters (FAMEs) were analyzed with a gas–liquid chromatograph (HP 6890, Hewlett-Packard GmbH, Waldbronn, Germany) on a capillary column (30 m × 0.25 mm I.D.; film thickness: 0.25 μm; liquid phase: DB-FFAP, J&W, Cologne, Germany) using temperature programming [26]. FAMEs were identified by comparison with known standard mixtures. If necessary, identification of FAMEs was confirmed by gas chromatography–mass spectrometry (GC–MS) measurements. Total lipid concentration refers to the sum of total FAME.

2.2. Pigment analysis

For each treatment, 20 mL from each of the five replicates was filtered on GF/F filters (Whatman, Maidstone, UK), frozen in liquid nitrogen and stored for two months at −80 °C before extraction and analysis. Pigments on filters, except phycocyanin samples, were extracted according to Wright and Jeffrey [27] and Wulff et al. [28] in 1.5 mL 100% methanol by ultrasonication (Vibra-cell) equipped with a 3 mm diameter probe operating at 80% in 5 s pulses. Vials with filtered extracts (0.45 μm) were transferred to a cooled autosampler and analyzed via HPLC [27] using an absorbance diode-array detector (Spectraphysics UV6000LP, Santa Clara, USA). The column used was a Kinetex 2.6 μm C18, 150 × 3.00 mm (Phenomenex, Torrance, USA) equipped with a guard column (SecurityGuard, Phenomenex C18, 4 mm × 3.0 mm, Torrance, USA). To identify peaks, the HPLC system was calibrated with pigment standards (DHI Water and Environment, Hørsholm, Denmark). Identification of peaks was confirmed by online recording of absorbance spectra (400–700 nm) as described in Jeffrey and Wright [27] and are presented as concentrations (mg L^{-1}) or ratios (w/w) to chlorophyll a (Chl a). For phycocyanin (PC) analysis, PC was extracted by the thaw–freeze method according to Sarada et al. [29] and Siegelmann and Kycia [30] and measured spectrophotometrically (UV-2401PC, Shimadzu, Kyoto, Japan) in a quartz cuvette. The PC content was calculated using the formula $PC = (OD_{615} - 0.474 * OD_{652}) / 5.34$ [31] where OD_{615} is the optical density at 615 nm and OD_{652} the optical density at 652 nm, giving mg mL^{-1}. The result was subsequently converted to mg PC per biovolume of cyanobacteria (mg PC mm^{-3}).

2.3. Filament length and growth

For each treatment, 4 mL from each of the five replicates was preserved with acidified Lugol's solution, kept in the dark and analyzed within six months. Each Lugol sample was gently mixed before being analyzed in 40× magnification (Axiovert 40CFL, micrometerocular 44 42 32 E-Pl 10×/20, Zeiss, Oberkochen, Germany) in a gridded Sedgewick rafter (1801-G20 Wildlife Supply Company, Yulee, USA). The length and width for each filament in 100 randomly selected squares (100 μL) were measured and the total biovolume (mm^3 L^{-1}) per species was calculated by considering each filament a cylinder. The growth for each species was measured by specific growth rate (μ day^{-1}) and calculated according to $(\ln D_B - \ln D_A) / (t_B - t_A)$ where D_A is the biovolume at the first day of the experiments and D_B the biovolume at the end, t_A as day A and t_B as day B. In addition, the

A. flos-aquae						*N. spumigena*													
14						0		7						14					
−P		−N		f/2		Initial		−P		−N		f/2		−P		−N		f/2	
Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
4.7	1.5	8.2	5.2	3.8	2.0	0.9	0.5	0.1	0.1	0.4	0.5	0.4	0.1	0.1	0.0	0.0	0.0	0.4	0.1
2.4	0.8	4.7	4.7	3.5	2.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.1
0.8	0.3	1.6	1.4	2.6	2.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1
1.1	0.6	1.1	1.2	0.6	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
18.9	6.4	36.3	26.6	20.0	10.7	30.5	12.3	4.1	4.0	25.5	14.5	21.2	9.1	2.2	0.4	1.5	0.1	27.2	8.2
4.6	2.1	5.6	6.6	4.5	3.2	5.8	2.6	1.1	1.1	2.1	1.3	2.8	0.7	0.4	0.1	0.3	0.0	3.9	0.7
0.0	0.0	0.0	0.0	0.1	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.1	0.3	0.2	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.2	0.0
0.0	0.0	0.0	0.0	0.3	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1
0.0	0.0	0.0	0.0	0.1	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
8.2	3.7	14.1	13.9	3.2	1.6	5.2	2.3	0.7	0.6	4.3	1.5	3.4	1.8	0.3	0.0	0.2	0.0	4.0	1.3
3.8	2.6	8.4	7.1	3.4	1.9	8.0	3.5	0.8	0.7	2.5	1.0	2.7	1.2	0.3	0.1	0.2	0.1	4.0	0.6
13.6	7.3	17.0	21.2	12.9	10.0	1.5	0.6	0.4	0.4	2.5	1.3	4.6	2.3	0.4	0.1	0.3	0.1	5.9	0.8
5.2	1.6	4.0	3.5	2.3	1.0	2.8	1.4	0.5	0.5	0.8	0.6	0.9	0.3	0.2	0.0	0.1	0.0	1.5	0.5
0.0	0.0	0.0	0.0	0.2	0.5	0.9	0.6	0.1	0.1	0.3	0.3	0.1	0.2	0.1	0.0	0.0	0.0	0.5	0.3
9.0	6.0	17.1	14.2	14.0	6.6	7.0	3.7	1.3	1.2	3.6	2.3	4.2	1.4	0.6	0.1	0.5	0.1	6.3	2.0
0.6	0.3	0.4	0.8	0.0	0.0	9.2	5.0	1.2	1.1	4.1	2.7	5.0	2.0	0.8	0.2	0.6	0.1	7.9	2.9
0.3	0.1	0.2	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.3	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.2	0.1
0.0	0.0	0.0	0.0	0.1	0.2	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.2	0.1
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.4	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.2	0.0	0.0	0.0	0.0	0.0	0.1
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
73.7	30.5	119.4	104.0	71.9	36.4	71.9	30.6	10.6	9.8	46.3	21.0	45	15.7	5	0.9	9	0.4	62	11.7
36.5	12.8	66.8	53.1	34.0	19.1	36.7	14.8	5.0	4.8	30.3	16.1	25.2	11.0	2.6	0.4	1.8	0.1	32.1	9.3
22.0	11.9	31.0	34.5	20.8	14.7	15.3	6.6	2.3	2.2	7.0	3.4	10.1	3.9	1.2	0.2	0.8	0.1	13.8	0.4
15.1	7.6	21.6	18.3	17.2	6.8	19.9	10.7	3.3	2.9	8.9	5.7	10.4	4.0	1.7	0.4	1.3	0.2	16.7	5.8
1.5		1.4		1.2		0.8		0.7		0.8		1.0		0.7		0.6		0.8	
1.7		2.2		1.6		2.4		2.2		4.3		2.5		2.2		2.2		2.3	

number of cells and heterocysts were counted in 30 random filaments from each sample.

2.4. Photosynthetic activity

To measure the maximum photosynthetic activity of PSII in the cyanobacteria, the F_v/F_m yield was measured [32] with a Pulse Amplitude Modulation (PAM) fluorometer (WATER-PAM, Walz GmbH, Effeltrich, Germany) in all treatments at each sampling day. F_v/F_m is calculated according to $(F_m - F_0) / F_m = F_v/F_m$, where F_m is the maximum fluorescent yield and F_0 the fluorescent yield before the light pulse in a dark-adapted state. The measurements were obtained in the emitter–detector unit of the CUVETTE version, with red LED light (650–730 nm) optimized for cyanobacteria (WATER-ED 8, 487, Walz GmbH, Effeltrich, Germany) and equipped with a stirring device (WATER-S, Walz GmbH, Effeltrich, Germany) to homogenize the sample prior to measurement [33]. For effective quantum yield measurement, 3 mL of each sample was transferred to the quartz cuvette, kept dark for 3 min and stirred 10 s before a light pulse of 600 ms was applied.

2.5. POC, PON & POP analyses

For each treatment, 20 mL from each of the five replicates was filtered onto precombusted (400 °C for 4 h) 25 mm GF/C filters (Whatman, Maidstone, UK) for POC/PON and additional 20 mL for POP analysis. Filters for POP were washed prior to filtering with 0.1 M HCl and rinsed with Milli-Q. All filters were then frozen at −20 °C and freeze-dried for 36 h (Heto Power Dry PL3000, Thermo Scientific, Waltham, USA). POP samples were analyzed within six months [34] at Tvärminne Zoological Station, University of Helsinki, Finland. For POC/PON analysis, filters were ground into fine powder (MM301, Retsch, Haan, Germany) and analyzed in an elemental analyzer (EA 1108 CHNS-O, Fisons Instruments, Ipswich, UK) applying 2,5-bis-[5-tert-butyl-benzoxazol-2-yl]-thiophen as a standard. Dry weight calculations were derived from the POC, PON and POP measurements in mol L^{-1} and the molar mass for C, N and P.

2.6. Nutrient analysis

For each treatment, 10 mL from each of the five replicates was 0.2 μm filtered (Filtropur, Sarstedt, Numbrecht, Germany) and stored at −80 °C until analysis of inorganic nitrite, nitrate, phosphate and silicate. The nutrient analysis, based on colorimetric methods [35], was performed by the Swedish Meteorological and Hydrological Institute (SMHI, Göteborg, Sweden).

2.7. Statistics

Data was analyzed by one-way ANOVA and Tukey's Post-Hoc test, using SPSS software (PASW Statistics ver. 20, IBM, Armonk, USA) for each sampling day, with either species or nutrient treatment as factor. Homogeneity was tested with Cochran's test and, where needed, data was transformed according to Underwood [36]. Significant differences were set as $p < 0.0005$ after Bonferroni correction [37].

Table 3
Pigment concentration [μg mm^{-3}] within *D. lemmermannii*, *A. flos-aquae* and *N. spumigena* for phosphorus depleted f/2 medium (-P), nitrogen depleted f/2 medium (−N) and full nutrient treatment (f/2 medium) after 14 days in comparison to the initial concentration. SD refers to standard deviation.

Treatment	*D. lemmermannii*								*A. flos-aquae*								*N. spumigena*							
	Initial		−P		−N		f/2		Initial		−P		−N		f/2		Initial		−P		−N		f/2	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Pigments																								
Aphanizophyll	0.69	0.05	0.95	0.17	0.67	0.04	0.57	0.04	0.45	0.04	0.23	0.03	0.48	0.03	0.96	0.21	–				–		–	
β-Carotene	0.19	0.03	0.11	0.02	0.12	0.01	0.09	0.01	0.09	0.00	0.02	0.00	0.07	0.01	0.13	0.03	0.16	0.01	0.05	0.00	0.03	0.00	0.05	0.01
β-Cryptoxanthin	0.005	0.00	0.01	0.00	0.007	0.00	0.006	0.00	0.02	0.00	0.02	0.00	0.03	0.00	0.08	0.02	–				–		–	
Canthaxanthin	0.03	0.01	0.33	0.05	0.12	0.04	0.10	0.03	0.11	0.01	0.05	0.00	0.08	0.01	0.19	0.05	0.07	0.00	0.12	0.01	0.07	0.00	0.08	0.01
Chl a	2.08	0.27	2.13	0.48	1.39	0.13	1.12	0.12	1.06	0.07	0.26	0.04	0.68	0.11	1.39	0.26	1.83	0.11	0.56	0.05	0.38	0.03	0.45	0.07
Echinenone	0.14	0.03	0.33	0.06	0.22	0.01	0.18	0.01	0.16	0.01	0.04	0.00	0.12	0.01	0.28	0.07	0.21	0.00	0.14	0.01	0.09	0.00	0.11	0.02
4-Keto-myxoxanthophyll	–		–		–		–		–		–		–		–		0.44	0.02	0.32	0.02	0.26	0.01	0.29	0.04
Myxoxanthophyll	0.39	0.03	0.89	0.16	0.60	0.02	0.57	0.04	0.09	0.00	0.20	0.03	0.43	0.03	0.76	0.17	0.05	0.00	0.03	0.00	0.04	0.00	0.04	0.00
Oscillaxanthin	0.04	0.00	0.07	0.03	0.04	0.01	0.05	0.00	0.01	0.00	0.02	0.00	0.04	0.00	0.07	0.01	–				–		–	
Zeaxanthin	0.03	0.00	1.42	0.29	0.07	0.01	0.07	0.00	0.02	0.00	0.01	0.00	0.04	0.01	0.06	0.01	–				–		–	

3. Results and discussion

For screening reasons, we have obtained multiple parameters during the experiment. Based on the direction of the present publication, we decided to show information only relevant to potential future applications. More detailed information can be accessed in the supplementary material provided.

3.1. Total and single fatty acids

Total lipid content rather than profile is often the main important factor for industrial applications such as biofuel production (biomass to fuel) [38,39]. At Day 0 of our study, average total fatty acid (TFA) content per biovolume was lowest, but not statistically different (Table A.1) in *A. flos-aquae* (9.1 μg mm^{-3}), almost double in *D. lemmermannii* (14.7 μg mm^{-3}) and largest in *N. spumigena* (71.9 μg mm^{-3}) (Table 2). Due to optimum lipid composition and content being seldom related to optimal industrial biomass production [40], we modeled both natural as well as assumingly optimal nutrient conditions (f/2) to get indications about industrial harvest and production under natural seasonal conditions. According to our obtained FA profiles (Table 2) and [41], all three species investigated can be classified as type 4, based on the assumption that cyanobacteria can be classified into four groups in terms of their FA composition [42]. Group 4 is characterized by the presence of the FA 18:1, 18:2, 18:3a (α-linolenic acid), 18:3γ (γ-linolenic acid) and 18:4 which relative proportions can be affected by growth conditions. The FA 16:1 is present in low levels [41]. The most promising of the three species investigated for biofuel production according to the TFA content was *D. lemmermannii*, reaching average maximum TFA values (Table 2) after 7 days in the P depleted treatment (427.0 μg mm^{-3}). Maximum TFA of *A. flos-aquae* was obtained after 14 days within the N depleted treatment (119.4 μg mm^{-3}). *N. spumigena* had highest TFA initially. However, the TFA was statistically significantly higher after 14 days in the f/2 treatment and lowest under N depletion for *N. spumigena* (Table A.1). The enhanced FA production under nutrient depletion could be explained by the need for carbon storage under suboptimal conditions, as observed also by Siron et al. [43] and Malzahn et al. [44]. This may prove advantageous for industrial FA production.

In contrast to biofuel production, single FA are used in the food and pharmaceutical industry due to their inter alia antioxidant, anti-inflammatory and anti-microbial activities [45]. The FA 14:0, 15:0, 16:0, 17:0, 18:0, 19:0, 20:0 and 24:0 are indicated in the following as saturated FA (SAFA),16:1(n-7), 18:1(n-7), 18:1(n-9) as monounsaturated FA (MUFA) and the FA 16:2(n-4), 16:3(n-4), 16:4(n-1), 18:2(n-6), 18:3(n-3), 18:3(n-6), 18:4(n-3), 20:3(n-3), 20:4(n-3), 20:4(n-6), 20:5(n-3), 22:5(n-3) and 22:6(n-3) as polyunsaturated FA (PUFA). Of particular interest in commercial production [45,46] and for use in several anti-cancer and anti-heart disease drugs of the pharmaceutical industry are the monounsaturated hexadecanoic acid (16:1(n-7)), octadecanoic acid (18:1(n-9)), polyunsaturated octadecatrienoic acid (18:3(n-3)), eicosapentaenoic acid (EPA; 20:5(n-3)) and docosahexaenoic acid (DHA; 22:6(n-3)), which are present in the three investigated species (Table 2). Nevertheless, the amounts of essential FA are known to be dependent on species and growing conditions [44,47].

3.1.1. Species differences in FA

Initial values indicated that *N. spumigena* contained high amounts of SAFA (51.3%), MUFA (21.6%) and PUFA (27.1%), while the proportion of MUFA was highest in *D. lemmermannii* (27.8%) and lowest in *A. flos-aquae* (9.9%). Results are related to the overall TFA contents (Table 2) and indicate how the ratios of SAFA, MUFA and PUFA may develop under certain nutrient conditions. Galhano et al. [48] observed SAFA of 61.7%, MUFA of 24.8% and PUFA of 13.5% in *Aphanizomenon gracile* and SAFA of 46.3%, MUFA of 17.7% and PUFA of 36.0% in *Anabaena cylindrica*. The results for both species are, in terms of SAFA, similar to our species

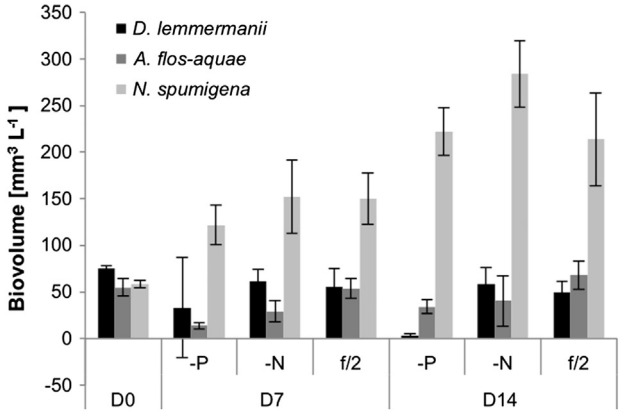

Fig. 1. Biovolumes [$mm^3 L^{-1}$] for *D. lemmermannii*, *A. flos-aquae* and *N. spumigena* at Day 0, Day 7 and Day 14 for phosphorus depleted f/2 medium (−P), nitrogen depleted f/2 medium (−N) and full nutrient treatment (f/2 medium). Error bars show standard deviation, n = 5.

before the experiment started, but vary up to four fold within MUFA and PUFA. Additionally, Li et al. [49] observed 3-hydroxyl FA 12:0 and 15:1 in *A. flos-aquae* and 15:1 in *Anabaena affinis*; these FA could not be identified in our analysis but seem to be of less taxonomic value [50]. As earlier studies indicate, e.g., [51], the morphological distinction of *Anabaena* (*Dolichospermum*) and *Aphanizomenon* is difficult. FA results from the present study (Table 2) and those obtained in Li et al. [52] suggest that FA seem to be more sensitive to environmental parameters than anticipated, leading to consequences in the FA use as characteristic markers. Consequently, ratios between MUFA, PUFA and SAFA seem to be highly variable for different cyanobacterial species [48], but appear rather constant within certain species under comparable environmental conditions [53].

The amount of 18:1(n-7) in *D. lemmermannii* and *A. flos-aquae* throughout the treatments is far higher compared to *N. spumigena*. The latter shows, in contrast, higher values of 18:1(n-9). Due to 18:1(n-7) being more related to bacterial metabolism [54,55], one could suspect that *D. lemmermannii* and *A. flos-aquae* are more bacterial related species, while *N. spumigena* is a more autotrophic species, exhibiting an algal related biosynthesis. Although the FA-signal from heterotrophic bacteria, commonly associated with the cyanobacteria, might not be strong enough, interference thereof cannot be fully excluded.

3.1.2. Treatment effect on FA

Nutrient starvation and high radiation regimes for a limited period are known to increase the lipid yield in outdoor algal cultures [56]. In our study, ratios of SAFA/MUFA + PUFA (Table 2) in *D. lemmermannii* under f/2 and N depletion and in *A. flos-aquae* under N depletion are comparable to ratios obtained by Galhano et al. [48].

As previously mentioned, there is a strong negative relationship between lipid content, growth and nutrient availability [57] leading to important conclusions for future culturing conditions and biomass production of the desired species. The results of the present study and of De Figueiredo et al. [58] show decreasing growth rates in *Aphanizomenon* strains under P depletion and varying responses to N depletion, which point to the carbon storage hypothesis of Siron et al. [43] and Malzahn et al. [44]. Recent results [59] highlight the physiological response cascade of cyanobacteria to N starvation occurring at different time scales, ranging from an immediate response to a long term scaled reaction. This might indicate a connection between results obtained in the present study of *A. flos-aquae* after 7 and 14 days and transcriptome regulation of cyanobacteria.

3.2. Pigments

It is known that increased lipid content reduces other valuable compounds in the biomass, suggesting that, "the high lipid containing algae may not necessarily be the most favorable candidate organisms" [9]. Cyanobacterial pigments are characterized by high diversity and richness, which could revolutionize the industrial use of color in the near future [60].

For total carotenoids, at Day 0 *D. lemmermannii* already had statistically significantly higher total pigment content than both *A. flos-aquae* and *N. spumigena* (Table 3, Table A.1). This observation continued after Day 7 and Day 14 in both N and P depleted treatments.

Phycobiliproteins in particular are used as fluorescent tracers and natural dyes in the food and cosmetic industries [61,62]. Regarding phycobiliprotein content, *D. lemmermannii* would be an excellent candidate with contents up to 19% of dry weight [63]. Within the carotenoid subgroup of xanthophyll, the present cyanobacteria (Table 3) comprise canthaxanthin, β-cryptoxanthin (except *N. spumigena*), echinenone,

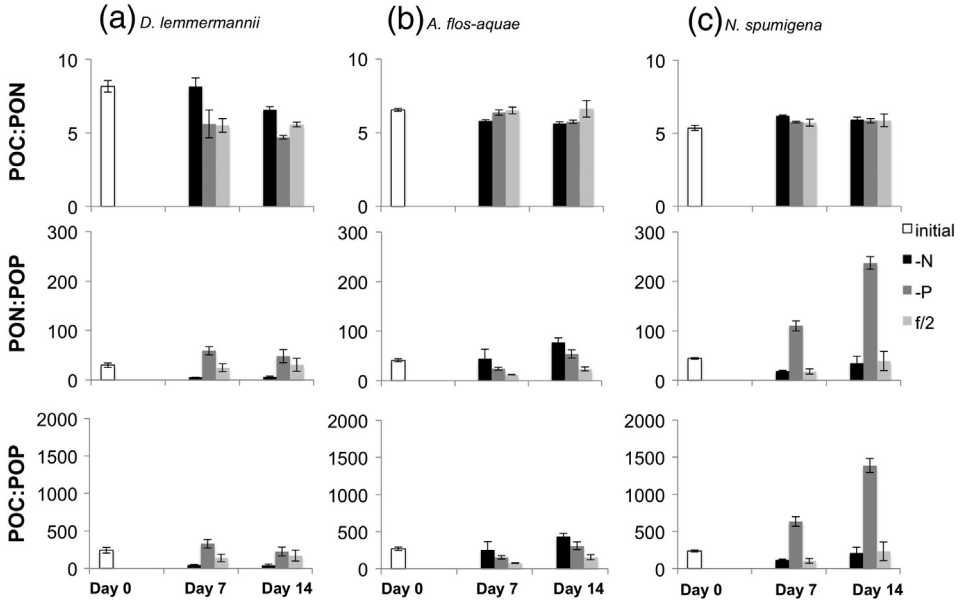

Fig. 2. POC:PON, PON:POP and POC:POP ratios for (a) *D. lemmermannii*, (b) *A. flos-aquae* and (c) *N. spumigena* at Day 0, Day 7 and Day 14 for phosphorus depleted f/2 medium (−P), nitrogen depleted f/2 medium (−N) and full nutrient treatment (f/2 medium). Error bars show standard deviation, n = 5.

myxoxanthophyll and oscillaxanthin (except *N. spumigena*). In addition, *N. spumigena* contains the species-specific xanthophyll 4-keto-myxoxanthophyll [64]. It is known that carotenoids are especially affected by radiation intensity and quality [28,64,65], nitrogen source and concentration [28], species and strain type [64] as well as growth stage [28,66]. In this study, carotenoids in *D. lemmermannii* were positively affected by P depletion, *A. flos-aquae* by N depletion and under full nutrients, while *N. spumigena* showed no response to the applied treatments (Table 3). In particular, the zeaxanthin concentration in *D. lemmermannii* increased in P depletion at Day 14. Zeaxanthin is a radiation protective pigment and in the low biovolume concentration (P depletion) this pigment could protect the cells from excess radiation. In addition, *N. spumigena* and *D. lemmermannii* (former *Anabaena*) are reported to contain UV absorbing pigments porphyra 334 and shinorine [67–70].

Phycocyanin levels in mg mm^{-3} were not significantly different between any of the species or treatments, nor between phycocyanin levels, biovolume or cell concentration (data not shown). We hypothesize that the method used [29] was not optimal for extraction of phycocyanins in these species. A new extraction method [71] has, in a later pilot study (Karlberg et al., unpublished), been proven to better extract phycobilins in *N. spumigena* (21.3 SD8.5 μg mm^{-3} compared to 7.6 SD2.5). The extraction efficiency of this new method in *D. lemmermannii* and *A. flos-aquae* is yet to be performed.

3.3. Biovolume and growth

At Day 7 *N. spumigena* had statistically significantly higher biovolume than both *A. flos-aquae* and *D. lemmermannii* in full f/2 medium and N depletion, but not under P depletion. This trend continued at Day 14 and then also under P depletion (Fig. 1, Table A.1). The higher biovolume for *N. spumigena*, compared to *A. flos-aquae* and *D. lemmermannii* in all treatments on both Day 7 and Day 14, may also be due to *N. spumigena* high irradiance tolerance [64,72,73]. During the summer-blooms in the Baltic Sea, *N. spumigena* is distributed in the top 5 m of the water column, *Anabaena* sp. (*Dolichospermum* sp.) down to 10 m depth and *Aphanizomenon* sp. equally distributed between 0 and 20 m throughout the water column [74]. The comparably high radiation intensities in this study may therefore have favored *N. spumigena* over the other two species. However, the F_v/F_m showed only a possible photoinhibition in *A. flos-aquae* and *D. lemmermannii* after 14 days under P depletion, with yields of 0.08 (SD0.01) and 0.08 (SD0.01) respectively, compared to 0.27 (SD0.02) for *N. spumigena* (Table A.2). Potential photoinhibition can occur when the biovolume is low and all cells are exposed to high irradiances with no chance of self-shading. The lower F_v/F_m in P depleted treatments could have been a result of this effect. However, since all three species showed low F_v/F_m under P depletion, regardless of biovolume, it is more likely that P depletion has a strong negative effect on F_v/F_m for these nitrogen fixating species, as phosphorus is also the limiting nutrient during bloom conditions in the Baltic Sea [75,76]. After 14 days there was no statistical difference in biovolume between the nutrient treatments for either *A. flos-aquae* or *N. spumigena* (Fig. 1, Table A.1), but the negative effect of the P depleted treatment, seen by the low F_v/F_m, was significant for the biovolume of *D. lemmermannii*.

N. spumigena had continuously positive specific growth rate in all treatments throughout the experiment (data not shown). *N. spumigena* generally has higher specific growth rate than *A. flos-aquae* ([73], Wulff et al., unpublished). This may be a competitive advantage, allowing *N. spumigena* to reach and maintain high biovolume and cell concentration during the bloom. *A. flos-aquae* exists as vegetative cells in filaments in the water column throughout the year [77], meaning it has an advantage when light and temperature reaches optimal levels in early summer and need not only germinate from akinetes, as *D. lemmermannii* and *N. spumigena* do. Although the specific growth rate for *A. flos-aquae* was negative between Day 0 and Day 7 in all treatments, it is positive between Day 7 and Day 14. This indicates a longer acclimatization time for *A. flos-aquae* and it would have been interesting to continue the experiment (compare e.g., [59,78], Wulff et al., unpublished). *D. lemmermannii* had negative specific growth rate in all treatments throughout the experiment. Since the cyanobacteria strains were reared at similar radiation conditions and in full nutrient medium, the negative growth rate results cannot be linked to non-adaptation towards the light regime, temperature or medium. In contrast, Moreno et al. [63] observed production rates for *D. lemmermannii* (former *Anabaena*) of up to 24 g DW m^{-2} per day under N depleted outdoor conditions; the highest reported growth rate under manipulated experimental outdoor conditions. Our study obtained up to 93.7 mg DW L^{-1} in *N. spumigena*, 61.0 mg DW L^{-1} in *A. flos-aquae* and 54.3 mg DW L^{-1} in *D. lemmermannii* (Tables A.1 and A.3), which is comparable to the studies of Reichert et al. [79] with *Spirulina* cultures.

Overall, in all treatments and on both Day 7 and Day 14, *N. spumigena* had statistically significantly higher biovolume and specific growth rate than both *A. flos-aquae* and *D. lemmermannii*, but not TFA per biovolume (Table A.1). Highest values of TFA per biovolume were observed in *D. lemmermannii* after 7 days in the P depleted treatment (Table 2). Increased concentration of FA under nutrient stress is common for many microalgal genera and species, e.g., [80,81] and references therein. For *D. lemmermannii*, the biovolume L^{-1} after 7 days under P depletion was very low. Therefore the TFA L^{-1} was highest after 7 days in the N depleted treatment (~16 mg L^{-1}) compared to *A. flos-aquae* and *N. spumigena* and the other treatments. *A. flos-aquae* had highest TFA L^{-1} after 7 days in f/2 (~6 mg L^{-1}) while *N. spumigena* reached highest TFA L^{-1} in f/2 after 14 days (14 mg L^{-1}). Naturally, these values cannot be compared to genetically modified *Synechocystis* sp. with a maximum TFA of 197 mg L^{-1} [82]. Consequently, one has to distinguish between the use of biomass for fuels and the use of lipids, derived from biological organisms, to obtain the maximum output with a certain species.

3.4. POC, PON & POP to FA ratios with applications for energy yield

We can support the hypothesis that nutrient deficient cyanobacteria and microalgae are favorable food for higher trophic levels regarding their FA profiles. This finding is of special interest to applications such as the recently introduced and seminal multi-trophic aquacultures, a co-culturing and interaction of species with benefits for both the environment and economy.

Earlier studies have shown (summarized in [83]) that phytoplankton stoichiometry is most variable at low growth rates, with PON:POP ratios ranging from 5 to 1000 and POC:POP from 60 to 1200. In Fig. 2,

Table 4
Conclusion summary of the present study addressing the questions: which species appear to be the most suitable for harvesting of different compounds, which harvesting period seems the most promising and which culturing conditions appear to be the most efficient.

Desired parameter/compound	Species with highest values in desired parameter/compound	Potentially best "harvesting" period for desired parameter/compound	Nutrient conditions with highest results of desired parameter/compound
Biovolume	*N. spumigena*	8–14 days	−N (−P, f/2)
FA/biovolume	*D. lemmermannii*	1–7 days	−P
FA/L	*D. lemmermannii*	1–7 days	−N
Pigments/biovolume	*A. flos-aquae*	1–7 days	f/2
Pigments/L	*A. flos-aquae*	1–7 days	f/2

ratios of POC:PON, ranging from 5.2 to 8.5, PON:POP (5.7 to 237.4) and POC:POP (30.4 to 1454.4) are shown for all three cyanobacteria species and all treatments over the experimental period. According to Goldman et al. [84], culturing phytoplankton under N depletion results in PON:POP ratios of less than 10:1, while under P depletion, ratios of more than 30:1 occur. In our study, POC, PON and POP differed slightly initially due to species-specific compositions, with *D. lemmermannii* having statistically lowest POC and PON values and *A. flos-aquae* highest (Table A.1, Table A.4). The wide range of POC, PON and POP might be related to low growth rates with respect to observations in phytoplankton, matching nutrient input ratios at low growth rates [83].

Consumers in higher trophic levels are often constrained with respect to their body C:N:P ratios while the actual primary production reflects the nutrient ratios of the surrounding environment [85]. The body C:N:P is therefore influenced by the food quality constraints on growth/reproduction, resource competition, trophic efficiency and nutrient recycling [86]. Effects of nutrient depletion and/or full nutrient treatments on stoichiometry (Table 2) and FA profiles were observed in cyanobacteria (present study) and in the cryptophyte *Rhodomonas salina* [87]. For industrial applications culturing and/or harvesting cyanobacteria, it is of high relevance to consider these stoichiometry effects. For example, *D. lemmermannii* contained the most FA under P depletion, while the most TFAs were produced under N depletion in *A. flos-aquae*. Likewise, *R. salina* [77] has shown significant differences between nutrient treatments, with generally higher TFA and higher unsaturated FA contents (e.g., Ω-3 and Ω-6 FA) in nutrient depleted treatments.

3.5. Inorganic nutrients

In Table 1, the initial nutrient treatments for the different experimental scenarios with the three different species are presented. Nutrient conditions remained stable due to the addition of treatment-specific nutrients, on Day 7, to all experimental bottles. Under natural bloom conditions, the elemental content of phytoplankton can reflect the ratio of N:P supply, while the chemico-physical context or the presence or absence of N-fixating organisms can modify this expectation [88]. Since nutrient depleted treatments mimic reasonably natural conditions before, during and after a summer bloom of the three dominant N-fixing cyanobacteria species, with low values of both N and P, the results obtained can be used to optimize nutrient conditions within laboratory or seasonal in-situ harvest, dependent on desired parameter or compound.

3.6. Costs, feasibility and other issues

Although the presented results sound promising, we have to keep in mind that mass cyanobacteria production is not simply extrapolating controlled laboratory experiments to large scale outdoor production systems [89]. There is an urgent need to develop a detailed and feasible procedure for the production of biochemically active compounds and secondary metabolites of cyanobacteria [15] in cooperation with the industry. As scientists, we can only give advice and point out knowledge gaps; the technical challenges are a different kettle of fish. The obtained results might be of interest to an endless group of buyers, such as the aquaculture industry, animal farms, biomass production and incineration. In addition, regulatory and commercial factors might inhibit the large-scale deployment of algae farms for production of biofuel [90], food additives and pharmaceuticals. Consequently, it may have to be explored in more detail in the future. By-products of algae [91], the algae meal, and cyanobacteria seem to be promising as feed for animals, but toxic substances such as nodularin in *N. spumigena* have to be considered. Two possible solutions were suggested by Vuori et al. [92]; removing nodularin effectively by reverse osmosis or vacuum distillation [93] and destroying nodularin by ultraviolet and high PAR. Overall biomass production costs of aquatic microorganisms could additionally be optimized by usage of the remaining biomass cake as fertilizer, or to obtain biogas via aerobic fermentation [94,95].

Our experiment suggests laboratory rearing, as well as harvest of algal biomass under natural conditions, every few days or on a daily basis, depending on their growth rates within favorable nutrient and temperature conditions (compare [96,97]). Since the nutrient depleted treatment worked best, it would lower the costs of the culture medium in bioreactors.

4. Concluding remarks

From the initial results of this pilot experiment, we can draw the following conclusions.

The choice of species strongly depends on the desired compounds. Each species originally has a species dependent chemical fingerprint that may be modified by the culture conditions and harvesting period to meet the needs of the consumer. The conclusions presented in Table 4 only indicate that the investigated cyanobacteria could be of interest for biofuel and secondary metabolites, in addition to already existing genetically modified cyanobacteria as well as other biofuels. Further research needs to be carried out in terms of technological feasibility on large scales, outdoor bioreactors, natural occurrences, impact on ecosystems and toxicity issues.

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