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CULTIVATION AND PHOTOPHYSIOLOGICAL CHARACTERISTICS OF DESMIDS IN MODERATELY SALINE AQUACULTURE WASTEWATER[1]

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Although desmids typically inhabit freshwater environments characterized by low amounts of nutrients and low salinity, several desmid species have been recorded in eutrophic waters, indicating their adaptation to elevated pollution and conductivity. This study aimed to determine whether desmids could be used for remediation of moderately saline aquaculture wastewater (AWW) from a fish farm situated in the southeast of Sweden. Fourteen desmid strains isolated from different climates (tropical to polar) and trophic conditions (oligotrophic to eutrophic) were cultivated in diluted AWW and we estimated their growth rates, biomass, nutrient removal efficiency, chlorophyll fluorescence parameters and cellular C, N and P quotas. Despite being grown at moderate salinity, unfavourable N:P ratio, and relatively low light/temperature regime the eutrophic strains, *Cosmarium humile*, *Cosmarium laeve* and a meso-oligotrophic species *Cosmarium impressulum*, completely absorbed nitrate and phosphate from AWW media after 7 d, indicating their potential for remediation of fish effluents in colder climates. These species, along with the typical eutrophic species, *Cosmarium meneghinii* and *Staurastrum chaetoceras*, had biomass in the range 0.45–1.19 $g \cdot L^{-1}$ while maximum growth rates ranged from 0.36 to 0.51 $\cdot d^{-1}$, similar to published rates for several fast-growing green microalgae cultivated in various AWW types. Tropical desmids had distinctly high values of saturating irradiance (I_k > 1,000 µmol photons $\cdot m^{-2} \cdot s^{-1}$), and, along with eutrophic desmids, had high potential electron transport ($rETR_{max}$ > 155 rel. units). Hence, the desmids studied demonstrated inherent photophysiological responses corresponding to their climate and trophic origin under the suboptimal growth conditions.

Key index words: **aquaculture wastewater; biomass; green algae; growth rate; maximum quantum yield; photosynthetic capacity and efficiency; remediation; saturating irradiance; Zygnematophyceae**

***Abbreviations*: AWW, aquaculture wastewater; F_V/F_M, maximum quantum yield of PSII; I_k, saturating irradiance; $rETR_{max}$, maximum relative electron transport rate; α, slope of rETR curve-photosynthetic efficiency**

The conjugating algae group (Zygnematophyceae, Streptophyta) that involves two orders, Zygnematales (families Zygnemataceae and Mesotaeniaceae – saccoderm desmids) and Desmidiales (placoderm desmids), is a cosmopolitan group of algae widely distributed in all main types of freshwater ecosystems (Guiry 2013, Stamenković and Hanelt 2017). A vast amount of floristic and ecological studies on conjugating algae show that the placoderm ('true') desmids are generally regarded as typical inhabitants of oligotrophic and unpolluted habitats characterized by low amounts of nutrients and dissolved salts (Coesel 1983, Gerrath 1993). In addition, acidic, highly colored, dystrophic lakes may also contain large desmid populations (e.g., Willén 1980, 1992, Howell and South 1981, Kouwets 1988, 1991, 1997). Fewer desmids are found in nutrient-rich water bodies, and some of them are recognized as reliable indicators of eutrophic conditions, such as *Closterium acutum*, *Closterium acutum* var. *variabile*, *Closterium aciculare*, *Closterium acerosum*, *Cosmarium botrytis*, *Staurastrum paradoxum* var. *parvum*, *Staurastrum chaetoceras*, *Staurastrum planctonicum*, *Staurastrum tetracerum* and *Staurastrum pingue* (Padisák 1980, Rosén, 1981, Coesel 1983, ten Cate et al. 1991, Coesel and Meesters 2007). In addition, more recent floristic investigations demonstrate that many desmids known as indicators of oligotrophic conditions have been commonly found in meso-eutrophic to eutrophic waters, while eutrophic taxa have been

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frequently recorded in effluents from agricultural complexes (Fehér 2003, Stamenković and Cvijan 2008, Ferragut and Bicudo 2009, 2012, de Silva et al. 2018). In general, this indicated that desmids shifted their optima to high concentrations of nutrients and various pollutants, thus, some of them could possibly be used as absorbents of excess nutrients in wastewaters.

Even in some unpolluted habitats such as peat bogs, peat pits, ditches and marshes that do not possess freshwater influents, the amounts of dissolved solids and salts in water may occasionally be very high (Wetzel 2001). In these closed environments, microalgae may face rapidly changing osmolarities due to evaporation during high temperature periods and by dilution during rain. Additionally, in their habitats, desmids may be exposed to increases in salinity due to fertilization from agriculture or road salt application (Affenzeller et al. 2009). While salt-tolerant green algae such as *Dunaliella*, *Chlamydomonas* or *Chlorella* have developed metabolic strategies to cope with elevated salinity (Pelah et al. 2004, Yoshida et al. 2004, Goyal 2007), there are fewer data on the influences of increased salinity on desmid growth and physiological characteristics. Experimental studies of *Micrasterias denticulata* grown under salt stress showed that increased osmolality of the nutrient solution (from the usual level < 2 mosm $\cdot$ kg^{-1} up to 26 mosm $\cdot$ kg^{-1}) gradually inhibited cell division although the cells retained the ability to divide if subsequently placed in distilled water (Meindl et al. 1989). The addition of high quantities of KCl or NaCl (200 mmol $\cdot$ L^{-1}: 11.7 g $\cdot$ L^{-1} and 14.9 g $\cdot$ L^{-1}, respectively) to the culture medium led to severe ultrastructural and physiological changes indicating programmed cell death (PCD) in *M. denticulata* and these alterations were clearly distinguished from changes induced by osmotic stress using iso-osmotic sorbitol (Affenzeller et al. 2009, Lütz-Meindl 2016). Several ecological studies revealed that some desmids thrived in polluted water bodies which, beside the high amount of nutrients, also had relatively high quantities of dissolved salts (Fehér 2003, 2007, Krasznai et al. 2008, Stamenković and Cvijan 2008a,b,c, 2009), but these concentrations were lower than those which induced PCD. Hammer et al. (1983) found that *Staurastrum gracile* was abundant in Canadian lakes containing 3–5 g $\cdot$ L^{-1} of total dissolved solids and common in lakes with around 10 g $\cdot$ L^{-1} of total dissolved solids. These facts provoked the need to investigate whether desmids can be cultivated in moderately saline wastewaters which also possess relatively high amounts of nutrients.

Compared to physical and chemical treatment processes, algae-based wastewater treatment can potentially achieve nutrient removal in a cheaper and more environment-friendly way with the added benefits of resource recovery and recycling (Renuka et al. 2015). In Northern Europe, there have been many projects investigating the growth and performance of microalgae in waste resources in laboratory or pilot-scale outdoor studies performed by researchers, often in cooperation with industry (Cheregi et al. 2019). Investigations have shown that both fresh and dried biomass of Desmidiaceae and Zygnemataceae appear to be an efficient substrate for the biosorption of heavy metals, nitrogen, and phosphorous compounds (Elgavish et al. 1980, 1982, Kumar et al. 2016, Lütz-Meindl 2016, Ge et al. 2018), indicating their potential for purification of various wastewater types. Therefore, the main aim of our study was to examine whether desmids could be used for the remediation of the moderately saline aquaculture wastewater (AWW) from a fish farm situated in the southeast of Sweden. We also aim to reveal the desmid ecophysiological features that may contribute to explaining their tolerance of, and existence in moderately saline AWW. Hence, we cultured a number of small to large-celled desmid strains collected from various climate and trophic conditions using batch mode in media containing AWW. Parameters usually considered in the selection of microalgae for wastewater bioremediation are growth rates, biomass amount and nutrient absorption (Renuka et al. 2015, Gonçalves et al. 2017). However, measuring chlorophyll *a* fluorescence characteristics as well as cellular C, N, and P quotas are needed to provide important data on the physiological performance of selected strains during wastewater treatments (e.g., Whitton et al. 2016, Ansari et al. 2017, Liu et al. 2019).

MATERIALS AND METHODS

Fish (aquaculture) wastewater sampling and chemistry. The fish farm is situated near the coast of the Baltic Sea south of Stockholm. The fish species, zander (*Sander lucioperca*), is grown in brackish water in indoor tanks at 18°C. The effluent was collected from the final stage of juvenile-fish production, settled to remove large particles, and filtered through 47 mm Whatman GF/F filters immediately before the cultivation of desmids. We measured the pH and conductivity of the wastewater using a pH meter (827 pH lab Metrohm, Herisau, Switzerland) and a conductivity meter (EC-215, Hanna Instruments, Kungsbacka, Sweden). The nutrients and elements in the AWW were analyzed in the supernatant of cultures, obtained by filtering 10 mL culture medium with a 0.2 μm filter (Filtropur, Sarstedt, Numbrecht, Germany) and stored at −80°C until analysis. Detailed chemical analyses were performed by the Lennart Månsson AB company, Helsingborg, Sweden (from three randomly taken samples), while analyses of nutrients were done at the start and after 7 and 14 d of the cultivation in the Kristineberg Marine Research Station according to methods from Grasshoff et al. (1999). Chemical characteristics of AWW are shown in Table 1.

Algal strains. A total of 14 desmid strains (10 *Cosmarium* and 4 *Staurastrum* taxa) of different cell size, trophic preference, climate origin, and time of isolation were selected for the investigation of cultivation in moderately saline AWW (Table 2). Desmids were purchased from the Microalgae and Zygnematophyceae Collection Hamburg (MZCH, Germany;

TABLE 1. Characteristics of the undiluted filtered aquaculture effluent.

Characteristic	Measure in AWW
pH	7.2
Conductivity	8.7 mS · cm^{-1}
Ca	63.4 mg · L^{-1}
Cu	0.026 mg · L^{-1}
Fe	0.0037 mg · L^{-1}
K	21 mg · L^{-1}
Mg	69 mg · L^{-1}
Mn	0.094 mg · L^{-1}
Mo	0.0026 mg · L^{-1}
Na	540 mg · L^{-1}
Zn	0.036 mg · L^{-1}
S	48 mg · L^{-1}
Cl	655 mg · L^{-1}
B	0.22 mg · L^{-1}
SiO_2	6.3 mg · L^{-1}
PO_4^{3-}	1.98 mg · L^{-1} (20.9 μmol · L^{-1})
NO_3^-	96.60 mg · L^{-1} (1557.9 μmol · L^{-1})
NO_2^-	0.0060 mg · L^{-1} (0.13 μmol · L^{-1})
NH_4^+	0.0103 mg · L^{-1} (0.57 μmol · L^{-1})

SDs typically < 10% of mean, $n = 3$.

von Schwartzenberg et al. 2013) and the Coimbra Collection of Algae (ACOI, Portugal; Santos and Santos 2004). The cell morphology as well as the length and width of algal cells were estimated using a light microscope (Zeiss, Axiovert 40, Jena, Germany) from measurements of >50 cells of each strain. In this study, the desmid taxa with length < 22 μm were regarded as small-celled while desmids of 22–40 μm were considered medium-celled (Fig. 1). The thickness of the mucilaginous cell sheath (in μm) was measured microscopically at the cellular apex in 50 randomly chosen cells in cell suspension stained by Indian ink (Stamenković and Hanelt 2011).

Culture conditions and experimental set-up. Prior to experiments, all the desmids were transferred into new medium every 2 weeks for 4 months and acclimated at 18°C and 90 – 100 μmol photons · m^{-2} · s^{-1} (light:dark regime 16:8 h) in the modified Woods Hole medium prepared with deionized water (WH or WC; Nichols 1973). The chemical composition of the WH medium was: $CaCl_2$ * $2H_2O$ (36 mg · L^{-1}), $MgSO_4$ * 7 H_2O (37 mg · L^{-1}), K_2HPO_4 * $3H_2O$ (11.4 mg · L^{-1}), $NaHCO_3$ (12.6 mg · L^{-1}), $NaNO_3$ (85 mg · L^{-1}), $CoCl_2$ * 6 H_2O (0.01 mg · L^{-1}), $CuSO_4$ * $5H_2O$ (0.01 mg · L^{-1}), $FeCl_3$ * $6H_2O$ (3.15 mg · L^{-1}), H_3BO_3 (1 mg · L^{-1}), $MnCl_2$ * $4H_2O$ (0.18 mg · L^{-1}), Na_2EDTA (4.36 mg · L^{-1}), Na_2MoO_4 * $2H_2O$ (0.006 mg · L^{-1}), $ZnSO_4$ * $7H_2O$ (0.022 mg · L^{-1}), thiamine (0.1 mg · L^{-1}), biotin (0.0005 mg · L^{-1}), cyanocobalamin (0.0005 mg · L^{-1}), Hepes buffer (4.77 mg · L^{-1}). The pH of the WH medium was adjusted to 6 by adding 0.1 M HCl.

To test the influence of AWW on growth and photophysiological behaviour of desmids, we grew the strains in 200 mL Nunc flasks filled with 100 mL WH and 60 mL filtered AWW (three replicates), thus, large salinity stress to oligotrophic and polar desmids was avoided. Flasks with medium were inoculated from the desmid samples in the logarithmic growth phase to starting biomass (cell dry weight, CDW) of approximately 0.03 g · L^{-1}. The desmid cultures were grown 14 d in a climate chamber at 18°C (16:8 h light:dark); the light was provided by white florescent tubes (Philips Master, TL-D 58W/840, Reflex Eco, Amsterdam, the Netherlands) to 100 μmol photons · m^{-2} · s^{-1}. The climate chamber was also equipped with red LED lamps (Plant Climatics GroLEDs). The light intensity was adjusted using a cosine quantum sensor (LI-COR, LI-1400, Lincoln, NE, USA). The "control" desmid cultures were grown in 200 mL Nunc flasks, filled with 160 mL WH under the same cultivation conditions.

The five desmid strains that exhibited the highest growth rates and chlorophyll fluorescence parameters were selected to examine nutrient uptake and biomass production in AWW diluted with WH or water. Desmids were grown in modified 1 L borosilicate glass flasks filled with: 700 mL WH (control), 460 mL WH with 240 mL AWW (WH + A), and 460 mL deionized water with 240 mL AWW (DI + A), three replicates for each desmid strain. Flasks with medium were inoculated with the sample in the logarithmic growth phase, to the starting biomass of 0.03 g · L^{-1} (approximately 2.5 * 10^7 cells · L^{-1}). The cultures were continuously bubbled with humidified air at a rate of about 10 L · h^{-1} with no additional CO_2 added, and mixed using a magnetic stirrer to prevent self-shading. The desmid cultures were grown 14 d at the same conditions as for the pre-test cultures.

Determination of growth rate and biomass. The cell number was routinely estimated using a gridded Sedgewick Rafter counting chamber under a light microscope (Zeiss, Axiovert 40, Jena, Germany), samples were taken every second day. Specific growth rate per day (μ) was calculated by the formula:

$$\mu = \frac{\ln\left(\frac{N_1}{N_0}\right)}{t_1 - t_0}$$

where N_1 and N_0 are the cell concentrations at the end and beginning of a period of time t days. The doubling time was estimated using the formula: $d = \ln(2) \cdot \mu^{-1}$ (Guillard 1973).

The samples for CDW were collected at the start of experiments using 1 L bottles (0 d) and at the end (14 d). For the CDW determination, a sample of 10–20 mL from each culture flask was filtered onto a precombusted (at 400°C for 5 h, stored in a desiccator) and pre-weighted 47 mm Whatman GF/F filter. The filters were placed overnight in an oven at 105°C and weighted again to obtain dry weight for each desmid strain (g · L^{-1}).

Chlorophyll fluorescence measurements. Photosynthetic efficiency was measured as a the fluorescence of PSII, determined by using a pulse amplitude modulation fluorometer (Water PAM, Walz GmbH, Effeltrich, Germany) connected to a computer with WinControl software (Walz GmbH). Prior to measurement, the number of cells was equalized by adding a quantity of the corresponding medium to achieve approximately 6,000 cells · mL^{-1} for medium- and large-celled strains, or 9,000 cells · mL^{-1} for small-celled taxa. Immediately after sampling, the algal suspension was acclimated in darkness for 5 min at 18°C and put in 5 mL Quartz cuvettes (Hellma, Müllheim, Germany). The suspensions were gently stirred using a small magnetic bean during the fluorescence measurements. The maximum quantum yield (F_V/F_M; the ratio of variable to maximum chlorophyll fluorescence from photosystem II) was measured at time zero ($n = 6$) as described by Hanelt (1998). After dark incubation, a pulse of weak, far-red light was applied to empty the electron pool from Q_A. The initial fluorescence (F_0) was measured with red measuring light (~0.3 μmol photons · m^{-2} · s^{-1}, 650 nm) and the maximum fluorescence (F_M) was determined using a 600 ms completely saturating white light pulse (~3,500 μmol photons · m^{-2} · s^{-1}).

Photosynthesis (in terms of the relative electron transport rate, rETR = PFR * $\Delta F/F_M'$) versus irradiance curves were also measured (rETR curves, $n = 3$, chosen at random from the six replicates) as described by Bischof et al. (1998). Here, PFR refers to photon fluence rate; F_M' is the maximum fluorescence from a light adapted sample; ΔF (or F_q') refers to

TABLE 2. List of desmid strains used in this study and sorted according to the average cell length (±SDs, $n = 50$), trophic preferences, climate at the site of collection, and the year of isolation.

Strain	Cell length (μm)	Approx. cell size	Trophic preference	Climate of origin	Year of isolation	Mucilage abundance	Maximum growth rate (d^{-1})	Minimum doubling time (d)
Cosmarium obtusatum MZCH 509	60.5 (9.1)	Large	Eutrophic	Tropical	2002	**	0.29 (0.08)	2.4
Cosmarium formosulum MZCH 536	43.5 (5.3)	Large	Eutrophic	Moderate	1993	**	0.27 (0.06)	2.6
Cosmarium crenatum var. *boldtianum* MZCH 561	35.4 (6.3)	Medium	Oligotrophic	Polar	1995	***	0.15 (0.02)	4.6
Cosmarium laeve MZCH 508	22.0 (4.2)	Medium	Eutrophic	Tropical	2001	*	0.31 (0.11)	2.2
Cosmarium impressulum MZCH 506	20.8 (3.5)	Small	Meso-oligotrophic	Tropical	2003	***	0.34 (0.10)	2.0
Cosmarium meneghinii MZCH 59	18.7 (0.5)	Small	Eutrophic	Moderate	1927	–	0.42 (0.01)	1.6
Cosmarium humile ACOI 1879	16.1 (2.3)	Small	Eutrophic	Subtropical	2006	*	0.38 (0.04)	1.8
Cosmarium regnellii var. *pseudoregnellii* ACOI 370	15.3 (3.7)	Small	Meso-eutrophic	Subtropical	1989	*	0.40 (0.09)	1.6
Cosmarium regnesii var. *polonicum* MZCH 465	12.6 (2.8)	Small	Meso-oligotrophic	Moderate	1998	***	0.18 (0.02)	3.8
Cosmarium dilatatum MZCH 463	10.2 (1.9)	Small	Oligotrophic	Moderate	1998	***	0.26 (0.06)	2.5
Staurastrum punctulatum MZCH 501	27.3 (3.1)	Medium	Meso-eutrophic	Tropical	2003	**	0.24 (0.07)	2.9
Staurastrum boreale MZCH 631	24.9 (2.9)	Medium	Meso-eutrophic	Subtropical	2014	**	0.21 (0.08)	3.3
Staurastrum polymorphum MZCH 628	24.6 (3.0)	Medium	Meso-eutrophic	Subtropical	2014	**	0.23 (0.03)	3.0
Staurastrum chaetoceras MZCH 547	17.2 (1.1)	Small	Eutrophic	Moderate	1984	–	0.38 (0.06)	1.8

Trophic preference is established according to Stamenković et al. (2019). Maximum measured growth rates and minimum doubling times (±SDs, $n = 3$) are given for the strains grown in Nunc flasks with WH and AWW. The abundance of the mucilaginous envelope was determined after 3 d of cultivation using Indian ink staining and it was categorized as: *** – high abundance (>20 μm mean thickness), ** – moderate abundance (5–20 μm mean thickness), * – low abundance of mucilage (<5 μm mean thickness). MZCH – Microalgae and Zygnematophyceae Collection Hamburg, Germany; ACOI – Coimbra Collection of Algae, Portugal. The five strains selected for the tests on nutrient absorption are underlined.

the difference in fluorescence between F_M' and F'; F' is the fluorescence emission from an irradiated sample (Baker 2008). Thirteen levels of light intensity from white light LED of the Water PAM, ranging from 5 to 2,076 μmol photons $\cdot m^{-2} \cdot s^{-1}$, were used to create the rETR curves, the duration of each intensity being 30 s. The hyperbolic tangent model of Jassby and Platt (1976) was used to estimate rETR curve parameters described as:

$$ETR = rETR_{max} * \tan h\,(\alpha * PFR * rETR_{max}^{-1}).$$

where $rETR_{max}$ is the maximum relative electron transport rate, tan h is the hyperbolic tangent function and α is the electron transport efficiency. The saturation irradiance for electron transport (I_k) was calculated as the light intensity at which the initial slope of the curve (α) intercepts the horizontal asymptote ($rETR_{max}$ – the maximum relative electron transport rate). The curve fit was calculated with the Solver Module of MS-Excel using the least squares method and comparing differences between measured and calculated data. The parameters of rETR curves: $rETR_{max}$ which determines the photosynthetic capacity, the slope of rETR curve (α) referring to the photosynthetic efficiency, and the saturating irradiance (I_k) appeared as essential in the assessment of abiotic-stress influences on the physiological state of PSII (White and Critchley 1999, Hanelt et al. 2003, Serôdio et al. 2006, Cruz and Serôdio 2008).

POC, PON and POP analyses. For the five selected desmid strains, the content of particulate organic carbon (POC), particulate organic nitrogen (PON) as well as particulate organic phosphorous (POP) were determined 24 h after the start of cultivation and then after 14 d for all the cultivation media. For each treatment, 20 mL was filtered onto precombusted (400°C for 4 h) 25 mm GF/F filters (Whatman, Maidstone, UK) for POC/PON, and additional 20 mL for POP analysis. After the filtration of desmid samples, filters for POC/PON were frozen at −20°C and then freeze-dried for 36 h (Heto Power Dry PL3000, Thermo Scientific, Waltham, MA, USA). Filters for POP analyses were washed with 0.1 M HCl followed by a rinse with deionized water prior to combustion. Filter blanks were prepared by filtering the corresponding volume of deionized water. The filter blanks were used to subtract background concentrations. The filters were left to dry at room temperature before being analysed for POP by Tvärminne Zoological Station, Finland, according to the method described in Solórzano and Sharp (1980). For POC and PON analysis, filters were ground into fine powder (MM301, Retsch, Haan, Germany) and analyzed in an

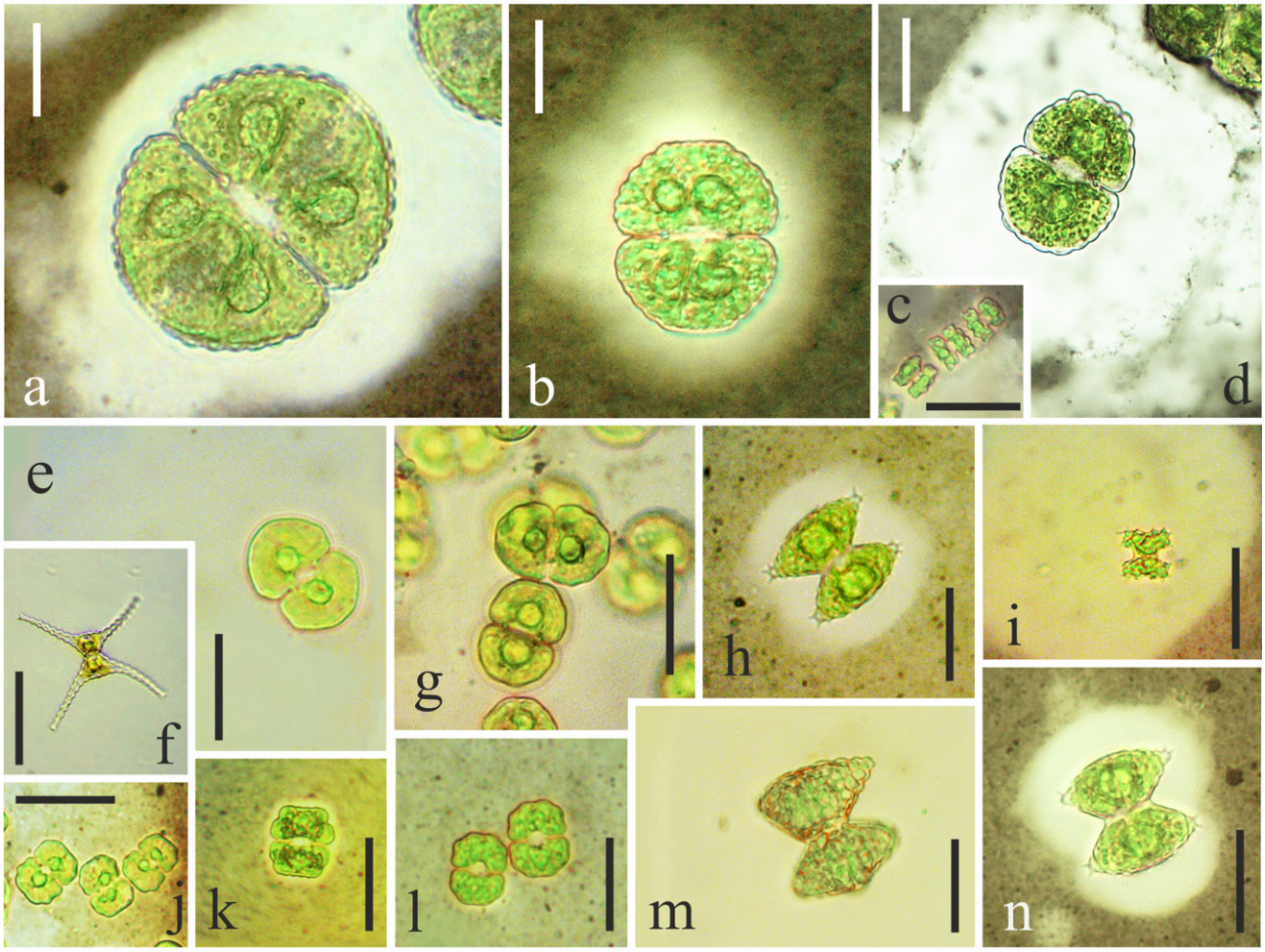


FIG. 1. Light micrographs of the desmid strains grown in aquaculture wastewater, sampled at the start of cultivation and stained in Indian ink: (a) *Cosmarium obtusatum*, (b) *Cosmarium formosulum*, (c) *Cosmarium dilatatum*, (d) *Cosmarium crenatum* var. *boldtianum*, (e) *Cosmarium laeve*, (f) *Staurastrum chaetoceras*, g) *Cosmarium impressulum*, (h) *Staurastrum polymorphum*, (i) *Cosmarium regnesii* var. *polonicum*, (j) *Cosmarium regnellii* var. *pseudoregnellii*, (k) *Cosmarium humile*, (l) *Cosmarium meneghinii*, (m) *Staurastrum punctulatum*, (n) *Staurastrum boreale*. Scale bars 20 μm. [Colour figure can be viewed at wileyonlinelibrary.com]

elemental analyzer (EA 1108 CHNS-O, Fisons Instruments, Ipswich, UK) applying 2,5-bis-[5-tertbutyl-benzoxazol-2-yl]-thiophen as a standard. The cellular C, N, and P quotas were converted into mol · L^{-1} to estimate molar POC:PON, PON:POP and POC:POP ratios.

Statistical analysis. Data were tested for normality (the Kolmogorov-Smirnov test) and homogeneity of variance (Levene statistics). Independent-samples *t*-tests were done to determine whether chlorophyll fluorescence parameters differed between the start of cultivation in WH medium (control) and after 24 h of cultivation in WH with AWW in Nunc flasks. We used a series of one-way ANOVA tests with Tukey HSD post-hoc tests to determine whether differences for chlorophyll fluorescence parameters at the start of cultivation in WH medium (control) and after 3 d of cultivation in WH + A or DI + A in aerated 1 L flasks were significant. Furthermore, a series of one-way ANOVAs (including the Tukey HSD post-hoc tests) were performed to determine whether nutrient concentrations differed after 0, 7, and 14 d. The same tests were used to estimate differences in POC, PON, POP quotas and their ratios of desmids grown in AWW media compared to those of the desmids grown in WH (at days 1 and 14). Statistical analyses were performed using IBM SPSS 20.0 software (SPSS, Chicago, IL, USA).

RESULTS

Pre-test with 14 desmid strains in WH medium + aquaculture wastewater. In WH with 60 mL AWW the average conductivity was 2.9 mS · cm^{-1}, while the pH of desmid cultures increased from 6.5 to 8.5 within 5 d. The small-celled taxa typical of meso- to eutrophic habitats, such as *Cosmarium meneghinii*, *C. regnellii*, *Cosmarium humile*, and *Staurastrum chaetoceras* had higher growth rates compared to the other strains (up to 0.42 · d^{-1} in *C. meneghinii*; Table 2). The small-celled desmids from oligotrophic environment, *Cosmarium regnesii* and *Cosmarium dilatatum*, had noticeably lower growth rates (0.18 and 0.26 · d^{-1} respectively). Interestingly, a eutrophic large-celled desmid, *Cosmarium obtusatum*, had higher growth rates (0.29 · d^{-1}) compared to that of the small-celled oligotrophic desmids. Although we noted a tendency of tropical and subtropical desmids to have relatively high growth

rates, (e.g., the tropical strains *C. laeve* and *Cosmarium impressulum* showed growth rates of up $0.34 \cdot d^{-1}$), the medium-celled subtropical desmid *Staurastrum boreale* had a rather low maximum growth rate ($0.21 \cdot d^{-1}$). This species, along with *C. regnellii*, showed somewhat shrunken chloroplasts after 3 d of cultivation, while cells of *C. obtusatum*, *Cosmarium crenatum*, *Staurastrum punctulatum,* and *Staurastrum polymorphum* displayed slight shrinking of the protoplasts after 6 d. All the meso-oligotrophic and oligotrophic desmids had copious mucilaginous envelopes around cells during the entire cultivation period, while the eutrophic desmids had typically a low amount of mucilage.

Chlorophyll fluorescence parameters of desmids in the pre-test experiment. After a decrease in F_V/F_M after 3 h to around 90% of the control in the eutrophic species *Cosmarium humile*, *C. meneghinii*, and *C. formosulum*, their maximum quantum yield recovered to 100% after 24 h (Fig. 2). Moreover, the eutrophic species *C. obtusatum*, *Staurastrum chaetoceras* as well as *S. punctulatum* displayed an increase in F_V/F_M after 3 h (up to 111.5% in *S. chaetoceras*). The species that had a constant decrease in F_V/F_M were the oligotrophic taxa *C. crenatum*, *C. dilatatum* and *C. regnesii*, along with the meso-eutrophic species *S. boreale* and *S. polymorphum*. The meso-oligotrophic species *C. impressulum*, fully recovered the yield after 6 d of cultivation after the initial decrease in F_V/F_M (72.2%).

In general, the strains collected from tropical and subtropical regions such as *Cosmarium laeve*, *C. impressulum*, *Staurastrum boreale* and *Staurastrum polymorphum* had higher F_V/F_M values than the desmids from other climates, with values up to 0.72 in *C. laeve* (Table 3). Desmids from these climates were also characterized by a high photosynthetic capacity as concluded from the high $rETR_{max}$ values (from 126.8 to 180.3 rel. units), except for *S. boreale*. Yet, *C. meneghinii*, the eutrophic desmid from the moderate climate had by far the highest photosynthetic capacity (220.2 rel. units), which only slightly decreased during the cultivation in AWW. Furthermore, the tropical strains (*C. obtusatum*, *C. laeve*, *C. impressulum,* and *S. punctulatum*) displayed consistently high values of I_k, indicating that high light intensity is needed to saturate rETR curves (>900 μmol photons $\cdot m^{-2} \cdot s^{-1}$).

The desmids confined to oligotrophic habitats (*Cosmarium crenatum*, *C. regnesii*, and *C. dilatatum*) exhibited lower values of all chlorophyll fluorescence parameters at the start of cultivation. After 24 h cultivation in the moderately saline medium, $rETR_{max}$ and I_k values appeared to be higher in the small-celled eutrophic desmids (*C. laeve*, *C. meneghinii,* and *Staurastrum chaetoceras*) compared to the other taxa. Interestingly, the oligotrophic desmids, *C. crenatum* and *C. dilatatum* as well as *S. punctulatum*, did not have a significant decrease in $rETR_{max}$ after 24 h while I_k decreased, which caused steeper rETR curve slopes – consequently increasing photosynthetic efficiency (α).

Experiments with selected desmids in 1 L flasks. Nutrient absorption: Five strains that exhibited fair growth and chlorophyll fluorescence parameters (high F_V/F_M and $rETR_{max}$ values) as well as no morphological changes during the pre-test, were selected for the test of absorption of nutrients in moderately saline AWW: *Cosmarium humile*, *C. laeve*, *C. meneghinii*, *C. impressulum*, and *Staurastrum chaetoceras*. Changes of the nutrient characteristics of the Woods Hole cultivation media (WH, control), WH with AWW (WH + A), and deionized water with AWW (DI + A) during the cultivation of desmids are shown in Figure 3. The average conductivity of WH medium was 0.28 mS $\cdot$ cm^{-1}, while the average conductivity of WH + A and DI + A was 2.9 mS $\cdot$ cm^{-1}. pH increased slowly from 6.5 to over 8 in most desmid cultures after 7 d and remained high during the experimental period (Table S1 in the Supporting Information). On average, 1,180 μmol $\cdot$ L^{-1} NO_3^- was measured in WH medium, and *C. humile*, *C. leave,* and *C. impressulum* absorbed 100% nitrate after 7 d of cultivation, while *C. meneghinii* and *S. chaetoceras* had around 70% absorption. Although NO_3^- concentration in WH +

A was comparable to that in WH, *C. humile*, *C. leave*, and *C. impressulum* showed somewhat lower percentages of absorption (59, 89, and 70%, respectfully), higher than in *C. meneghinii* and *S. chaetoceras* (30% of control). All desmids efficiently absorbed nitrate after 14 d in WH + A, apart from *S. chaetoceras* (90% of the starting value). This species also had slower absorption of nitrate in DI + A (76%) after 7 d cultivation. All the other desmids fully absorbed both nutrients after 7 d in DI + A. Concentrations of nitrite and ammonium ion in WH + A and DI + A at the start of the experiment were very low: <0.2 μmol $\cdot$ L^{-1} (NO_2^-) and 1–5.3 μmol $\cdot$ L^{-1} (NH_4^+).

Growth parameters. The highest maximum growth rates (μ_{max}) of desmids grown in WH were observed for *Cosmarium impressulum* ($0.52 \cdot d^{-1}$) and *C. laeve* ($0.44 \cdot d^{-1}$; Table 4). Except for *C. impressulum*, all the desmids had higher μ_{max} in WH + A compared to WH. During the cultivation in DI + A, μ_{max} of the selected desmids were slightly lower compared to the control (WH), while *C. meneghinii* showed the highest μ_{max} – $0.42 \cdot d^{-1}$. Biomass of the desmids cultivated in WH after 14 d varied from 0.73 g $\cdot$ L^{-1} in *S. chaetoceras* to 1.01 g $\cdot$ L^{-1} in *C. meneghinii*. *Cosmarium laeve* and *C. impressulum* showed an increase in CDW when cultivated in media containing AWW.

Chlorophyll fluorescence parameters. The selected desmid strains preserved a constant maximum quantum yield (100% of the beginning value) during 10 d of cultivation in WH medium, later the F_V/F_M values slightly decreased (not shown). The desmid strains showed only a small decrease in F_V/F_M (down to 90%) after 3 h treatment in WH + A and

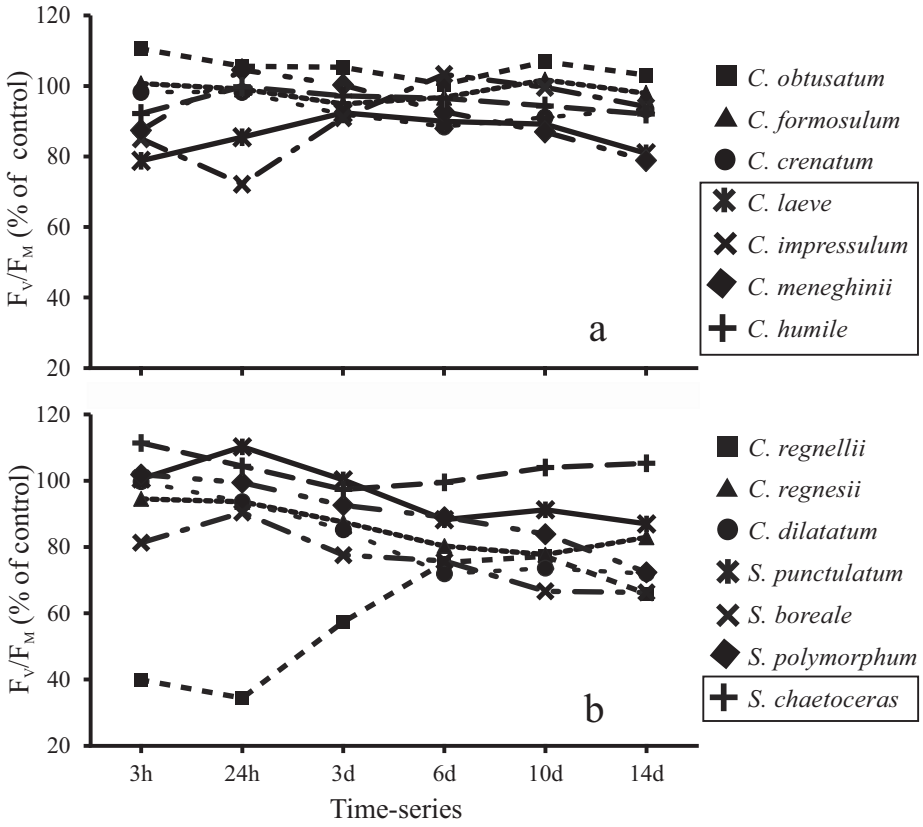


FIG. 2. Changes of the mean of maximum quantum yield (F_V/F_M) of the desmid strains grown in Nunc flasks containing WH medium with AWW, expressed as percentages of controls. Controls were F_V/F_M of desmids cultured in WH, at the start of cultivation. SDs typically < 10% of mean ($n = 6$); not shown for clarity. (a) *Cosmarium obtusatum*, *Cosmarium formosulum*, *Cosmarium crenatum* var. *boldtianum*, *Cosmarium laeve*, *Cosmarium impressulum*, *Cosmarium meneghinii*, *Cosmarium humile*, (b) *Cosmarium regnellii* var. *pseudoregnellii*, *Cosmarium regnesii* var. *polonicum*, *Cosmarium dilatatum*, *Staurastrum punctulatum*, *Staurastrum boreale*, *Staurastrum polymorphum*, *Staurastrum chaetoceras*. The strains selected for the investigation on nutrient absorption are outlined in black.

TABLE 3. Chlorophyll fluorescence parameters of desmids at the start of growth in WH (control), and after 24 h cultivation in WH with AWW.

Strain	F_V/F_M control	F_V/F_M 24 h	$rETR_{max}$ control	$rETR_{max}$ 24 h	I_k control (µmol photons · m^{-2} · s^{-1})	I_k 24 h (µmol photons · m^{-2} · s^{-1})	alpha control	alpha 24 h
Cosmarium obtusatum	0.57	0.61*	155.1	96.7**	1,099	442**	0.204	0.213*
Cosmarium formosulum	0.65	0.65^{ns}	141.3	98.1**	815	482**	0.202	0.209*
Cosmarium crenatum var. *boldtianum*	0.66	0.64^{ns}	119.1	120.8^{ns}	692	680*	0.189	0.188^{ns}
Cosmarium laeve	0.72	0.68^{ns}	170.2	167.5^{ns}	1,035	$1,082^{ns}$	0.212	0.155**
Cosmarium impressulum	0.69	0.49**	180.3	159.2*	1,011	$1,064^{ns}$	0.211	0.150**
Cosmarium meneghinii	0.63	0.59^{ns}	220.2	217.3^{ns}	658	666^{ns}	0.210	0.179**
Cosmarium humile	0.64	0.62^{ns}	135.6	95.9**	851	691*	0.180	0.145*
Cosmarium regnellii var. *pseudoregnellii*	0.68	0.23**	112.1	34.0**	888	800**	0.167	0.063**
Cosmarium regnesii var. *polonicum*	0.58	0.54*	104.4	78.3**	693	589**	0.157	0.133*
Cosmarium dilatatum	0.62	0.58*	97.9	98.3^{ns}	599	600^{ns}	0.160	0.165*
Staurastrum punctulatum	0.59	0.65*	135.3	135.6^{ns}	909	531**	0.192	0.199*
Staurastrum boreale	0.71	0.64*	99.6	96.4*	825	712**	0.140	0.135*
Staurastrum polymorphum	0.69	0.68^{ns}	126.8	87.9**	656	309**	0.153	0.147*
Staurastrum chaetoceras	0.60	0.58^{ns}	132.7	101.2**	671	697*	0.208	0.189*

F_V/F_M – maximum recorded quantum yield, $rETR_{max}$ – maximum relative electron transport rate, I_k – saturating irradiance, the light intensity at which the initial slope of curve (α) intercepts the horizontal asymptote ($rETR_{max}$), determined using the hyperbolic tangent equation from Jassby and Platt (1976). Significant differences from control values are marked with asterisks: $P < 0.05$*, $P < 0.001$**, ns – not significant (*t*-tests, $n = 6$; SDs typically < 10% of mean). The five strains selected for the tests on nutrient absorption are underlined.

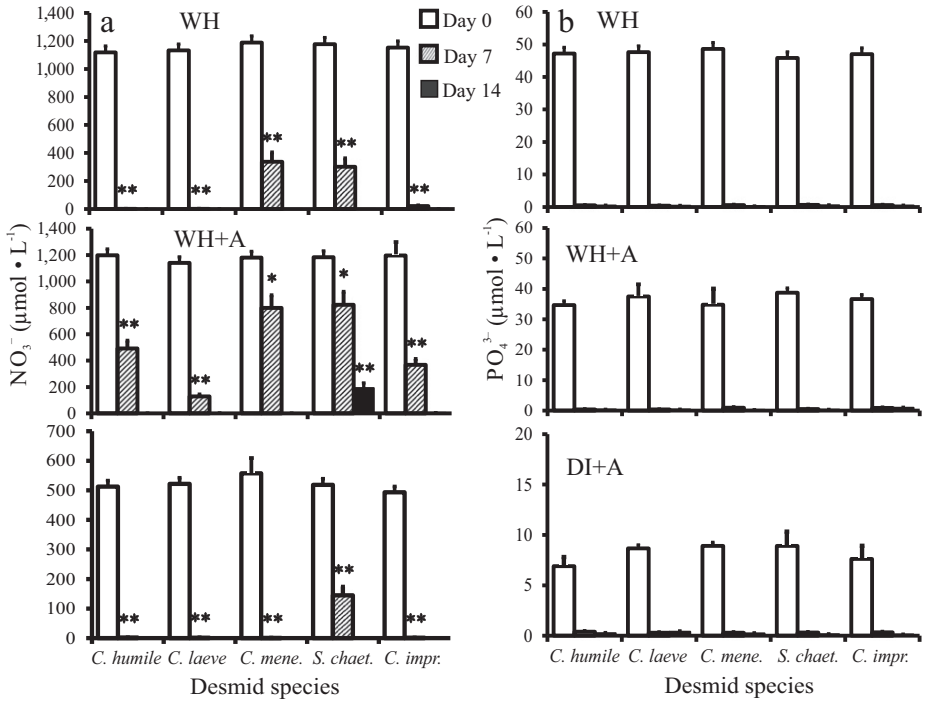


FIG. 3. Changes of nitrate (a) and phosphate (b) concentrations during the cultivation of five selected desmids in Woods Hole (WH), WH with AWW (WH + A) and deionized water with AWW (DI + A). Error bars are SDs, $n = 3$. For nitrate concentrations, significant differences compared to the samples taken at the start of cultivation (day 0) are marked with asterisks: $P < 0.05$ *; $P < 0.001$ ** (Tukey HSD post-hoc tests). At day 14, all the nitrate concentrations were significantly different from day 0 at $P < 0.001$ (Tukey HSD post-hoc tests, not shown). The phosphate concentrations at days 7 and 14 were significantly different from day 0, $P < 0.001$ (Tukey HSD post-hoc tests, not shown).

TABLE 4. Maximum measured growth rates, minimum doubling times, and cell dry weight (CDW) achieved at the end of experiment (14 d) of the desmids cultivated in Woods Hole (WH), WH with AWW (WH + A) and deionized water with AWW (DI + A)

Growth condition	Growth parameter	*Cosmarium humile*	*Cosmarium laeve*	*Cosmarium meneghinii*	*Cosmarium impressulum*	*Staurastrum chaetoceras*
WH	Max. growth rate (d^{-1})	0.40 (0.09)	0.44 (0.03)	0.39 (0.08)	0.52 (0.10)	0.38 (0.07)
	Min. doubling time (d)	1.73	1.57	1.78	1.32	1.82
	CDW ($g \cdot L^{-1}$)	0.94 (0.12)	0.82 (0.14)	1.01 (0.18)	0.80 (0.09)	0.73 (0.11)
WH + A	Max. growth rate (d^{-1})	0.51 (0.06)	0.45 (0.04)	0.43 (0.05)	0.42 (0.10)	0.39 (0.08)
	Min. doubling time (d)	1.36	1.54	1.61	1.65	1.78
	CDW ($g \cdot L^{-1}$)	0.84 (0.09)	0.98 (0.13)	0.83 (0.12)	1.19 (0.14)	0.45 (0.05)
DI + A	Max. growth rate (d^{-1})	0.39 (0.07)	0.36 (0.09)	0.42 (0.10)	0.50 (0.14)	0.37 (0.09)
	Min. doubling time (d)	1.77	1.93	1.65	1.39	1.87
	CDW ($g \cdot L^{-1}$)	0.89 (0.14)	1.02 (0.20)	0.78 (0.13)	0.86 (0.11)	0.63 (0.07)

SDs are given in parentheses, $n = 3$. At the start of cultivation CDW was 0.03 $g \cdot L^{-1}$ for all the desmid strains.

DI + A; afterwards, the desmids fully recovered maximum quantum yield after 3 d (Fig. 4). Interestingly, after 7 d of cultivation in WH + A, *C. humile*, *C. meneghinii*, and *C. impressulum* increased F_V/F_M to over 110% and it remained high till the tenth day of cultivation. The ameliorating effect of AWW was also observed in *C. meneghinii*, *C. impressulum*, and *S. chaetoceras* when grown in DI + A (increase 108, 111 and 110% of control values, respectively).

The photosynthetic capacity increased in all the desmids in both AWW treatments compared to the control, being the highest in *Cosmarium meneghinii* in WH + A medium (211.5 rel. units; Table 5). A significant decrease in I_k was observed for *C. laeve*, *C. meneghinii*, and *C. impressulum* in WH + A, while I_k values remained constant in *C. humile* and *Staurastrum chaetoceras*. The steeper rETR curves caused an increase in photosynthetic efficiency (α) in all the desmid species cultivated in WH + A (up to 0.23 in *C. meneghinii*). The α values were higher than that when desmids were grown in Nunc flasks, both in control and WH + A medium.

Changes of cellular carbon, nitrogen, and phosphorus quotas (POC, PON, and POP) in the desmid

strains. Except for *Cosmarium meneghinii* and *Staurastrum chaetoceras*, all desmids cultivated 24 h in WH + A and DI + A had higher cellular carbon and nitrogen quantities than when they were cultivated in WH (Fig. 5, Table S2 in the Supporting Information). Desmids which possess a mucilaginous envelope (*C. humile*, *C. laeve*, and *C. impressulum*) had high POC values both after 24 h and 14 d cultivation in all the media. As expected, larger values for cellular P were observed for all desmids grown in WH and WH + A, compared to that in DI-A due to the higher phosphate content in these media. *Cosmarium meneghinii* and *S. chaetoceras* had lowest POP values in all the cultivation conditions after 14 d, while *C. impressulum* had highest POP values among all the desmids in all the cultivation conditions.

Molar POC:PON ratios increased in all the desmid strains after 14 d (except in *Cosmarium meneghinii* and *Staurastrum chaetoceras* cultivated in WH + A). Cultivation in DI + A increased molar POC:PON between 27.4 and 36.3 (Fig. 6). *Cosmarium impressulum* had lowest molar PON:POP after 24 h cultivation in WH (26.8) compared to that of the other desmids, followed by *C. humile* and *C. laeve* (36.1 and 39.2, respectively). In WH + A medium, all desmids except *S. chaetoceras* had higher PON:POP ratios after 24 h cultivation compared to that when grown in WH. *Cosmarium meneghinii* and *S. chaetoceras* showed significant increases in PON:POP and POC:POP ratios after 14 d of cultivation in all the media. In contrast, *C. impressulum* showed smaller ratios at the end of growth in all the media compared to the other desmids.

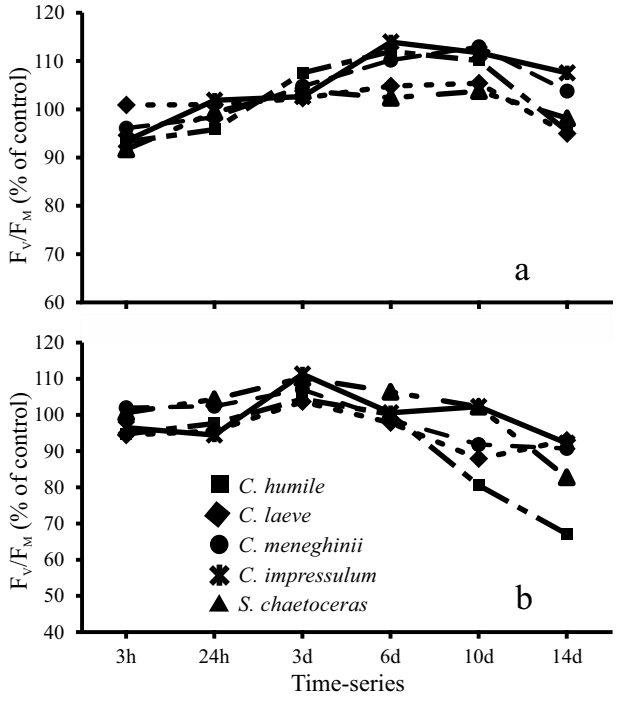


FIG. 4. Changes of the mean of maximum quantum yield (F_V/F_M) of the desmid strains cultivated in WH with AWW (WH + A) (a), deionized water with AWW (DI + A) (b). Values are expressed as percentages of controls (F_V/F_M of strains at the start of cultivation in WH). SDs typically < 10% of mean ($n = 6$); not shown for clarity.

DISCUSSION

Our study revealed that the small-celled eutrophic desmids have potential for AWW bioremediation based on chlorophyll fluorescence parameters, growth rates and biomass when cultivated in media containing moderately saline effluent from a fish farm. In general, microalgae effectively reclaim municipal, industrial, agricultural wastewaters (Gonçalves et al. 2017). Yet, this method has not been used as much in the treatment of aquaculture effluents (Lananan et al. 2014, Gao et al. 2016, Ansari et al. 2017). Recent floristic-ecological investigations showed that several desmid taxa could thrive in waters containing high quantities of dissolved salts and organic biodegradable compounds (see Introduction). Hence, it appeared justified to test if desmids could be used for the remediation of moderately saline AWW. Both AWW media used in our study were characterized by 10 times higher conductivity compared to WH (~2,900 $\mu S \cdot cm^{-1}$) as well as high pH values after 7 d (>8.5) which, along with the cultivation temperature (18°C), are not usual conditions for abundant desmid growth (Brook 1981, Coesel and Wardenaar 1990). The nutrient composition of AWW used in this study basically corresponded to modified WH, a common and widely used medium for long-term cultivation of conjugatophycean algae (Coesel 1991, Spijkerman and Coesel 1996a, Stamenković et al. 2019). If the dominant nutrients in media (71.5 $mg \cdot L^{-1}$ NO_3^- and 4.4 $mg \cdot L^{-1}$ PO_4^{3-} in WH; 74 $mg \cdot L^{-1}$ NO_3^- and 3.5 $mg \cdot L^{-1}$ PO_4^{3-} in WH + A) were taken into account for the calculation of the molar N:P ratio, WH and WHA would have N:P ratios 11.3 and 14.6, belonging to the N:P range of the optimal nutrient-replete growth conditions (5–19; Geider and La Roche 2002). On the other hand, DI + A (with 33 $mg \cdot L^{-1}$ NO_3^- and 0.8 $mg \cdot L^{-1}$ PO_4^{3-}) had the N:P ratio 28.6, which indicated P deficiency. Considering the composition of micronutrients in AWW (Table 1), the quantities of Fe, Mn, and Mo appeared manifold lower in WH + A and DI + A than that in WH and the dominant ions were Na^+ (~180 $mg \cdot L^{-1}$) and Cl^- (~220 $mg \cdot L^{-1}$). This is in contrast with inland waters that usually have Mg^{2+}/Na^+ and HCO_3^- as predominant ions (Reynolds 1984, Wetzel 2001).

Although the desmids were grown in the AWW media that were characterized by moderate salinity, unsuitable micronutrient composition, and at lower temperature than their recommended temperature optimum (21–25°C; Stamenković and Hanelt 2013a), high fluorescence parameters ($rETR_{max}$ and F_V/F_M) indicated that all of the strains tolerated

TABLE 5. Chlorophyll fluorescence parameters of the selected desmids at the start of cultivation in WH (control), and after 3 d cultivation in WH, WH with AWW (WH + A), deionized water with AWW (DI + A)

Strain	Condition	F_V/F_M control	F_V/F_M 3 d treat.	$rETR_{max}$ control	$rETR_{max}$ 3 d treat.	I_k control (μmol photons · m^{-2} · s^{-1})	I_k 3 d treat. (μmol photons · m^{-2} · s^{-1})	alpha control	Alpha 3 d treat.
Cosmarium humile	WH	0.66	0.68^{ns}	127.4	133.2^{ns}	624	670*	0.197	0.199^{ns}
	WH + A		0.71*		157.0**		622^{ns}		0.226**
	DI + A		0.71*		180.6**		709**		0.207*
Cosmarium leave	WH	0.68	0.70*	151.3	159.2^{ns}	872	898*	0.199	0.189^{ns}
	WH + A		0.69^{ns}		150.2^{ns}		803*		0.214**
	DI + A		0.72*		183.8**		902*		0.221**
Cosmarium meneghinii	WH	0.63	0.66^{ns}	194.4	210.6*	679	711*	0.201	0.198^{ns}
	WH + A		0.65*		211.5*		614*		0.230**
	DI + A		0.68*		210.4*		751**		0.206^{ns}
Cosmarium impressulum	WH	0.67	0.65^{ns}	177.7	171.1^{ns}	1,087	802**	0.190	0.199^{ns}
	WH + A		0.67^{ns}		186.9*		857**		0.220**
	DI + A		0.71*		191.2**		862**		0.222**
Staurastrum chaetoceras	WH	0.65	0.66^{ns}	143.3	163.2*	825	843*	0.191	0.194^{ns}
	WH + A		0.70*		149.5^{ns}		826^{ns}		0.203*
	DI + A		0.72*		184.0**		914**		0.205*

F_V/F_M – maximum quantum yield, $rETR_{max}$ – maximum relative electron transport rate, I_k – saturating irradiance, the light intensity at which the initial slope of curve (α) intercepts the horizontal asymptote ($rETR_{max}$), α – slope of rETR curve, determined using the hyperbolic tangent equation from Jassby and Platt (1976). Significant differences from control are marked with asterisks: $P < 0.05$*; $P < 0.001$**; ns – not significant (Tukey HSD post-hoc tests, $n = 6$, SDs typically < 10% of mean).

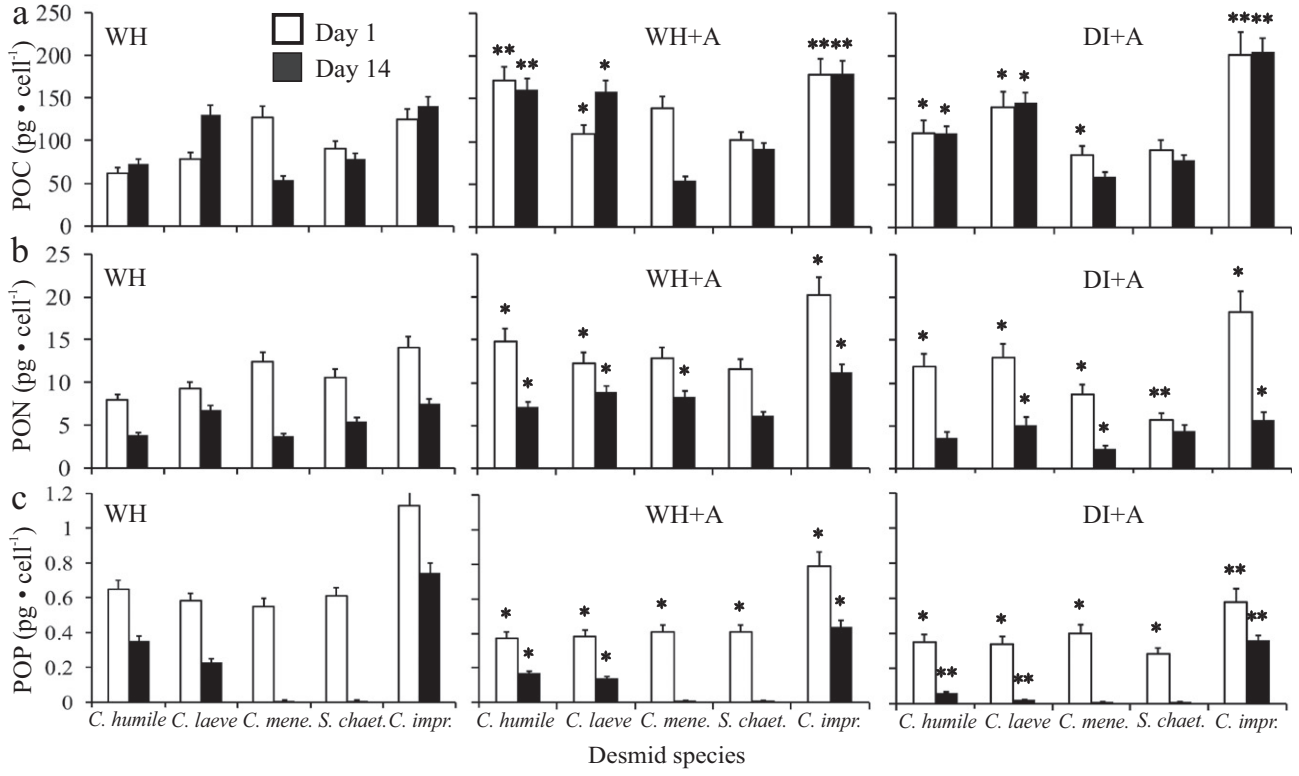


FIG. 5. Cellular carbon, nitrogen, and phosphorus quotas (a POC, b PON, c POP) for the selected desmid strains after 1 and 14 d of cultivation in WH, WH with AWW (WH + A), deionized water with AWW (DI + A). Error bars are SDs, $n = 3$. Significant differences in comparison to the WH samples at the respective days are marked with asterisks: $P < 0.05$ *; $P < 0.001$ ** (Tukey HSD post-hoc tests).

these conditions. When grown in Nunc flasks, all the desmids from eutrophic habitats as well as a meso-oligotrophic strain, *C. impressulum*, were characterized by higher growth rates compared to the oligotrophic strains. With the addition of ambient CO_2 to WH, the selected desmids (*C. humile*, *C. laeve*, *C. meneghinii*, *C. impressulum*, and *S. chaetoceras*) achieved higher growth rates than when grown in

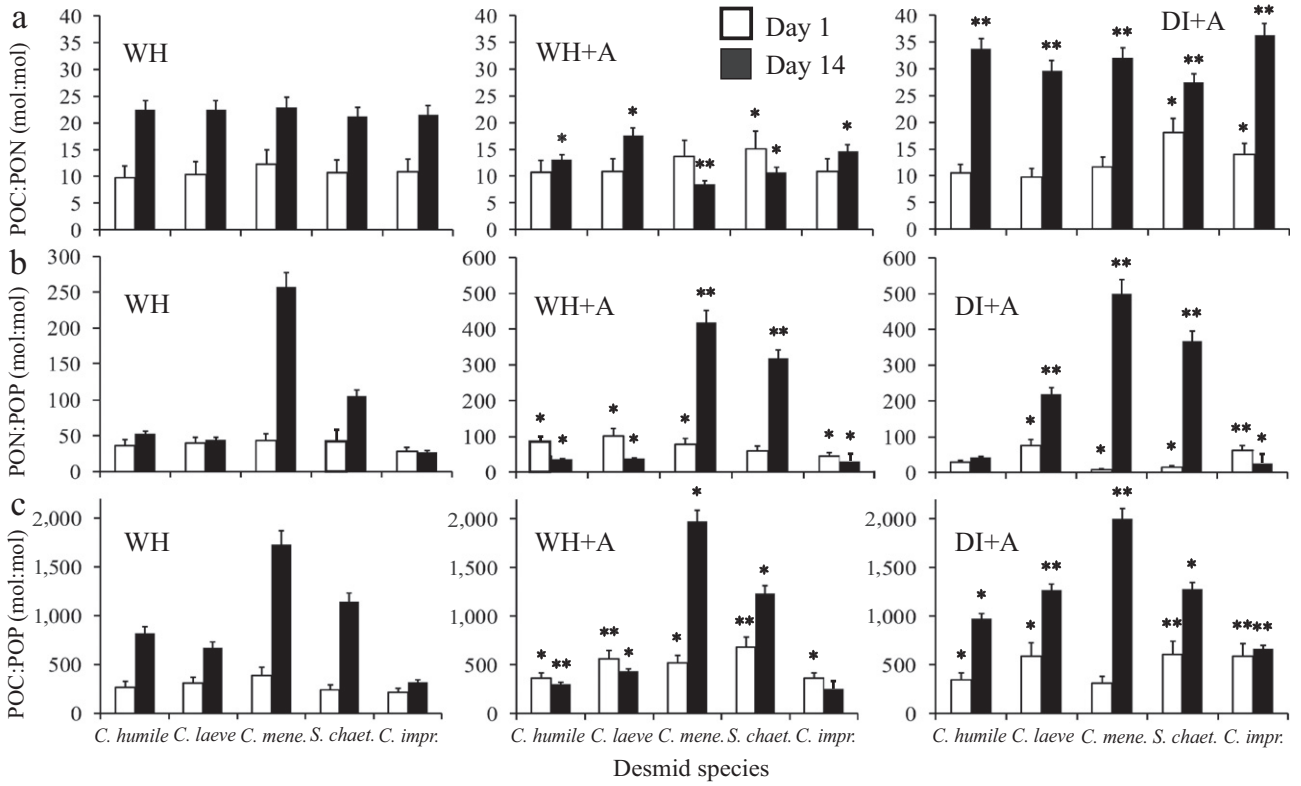


FIG. 6. Molar POC:PON (a), PON:POP (b) and POC:POP (c) ratios for the desmid strains grown in WH, WH with AWW (WH + A), deionized water with AWW (DI + A) after 1 and 14 d. Error bars are SDs, $n = 3$. Significant differences in comparison to the WH samples at the respective days are marked with asterisks: $P < 0.05$ *; $P < 0.001$ ** (Tukey HSD post-hoc tests).

Nunc flasks, and the μ_{max} values were even higher with the addition of AWW (up to $0.51 \cdot d^{-1}$ in *C. humile*). Our results are in line with the fact that eutrophic desmid taxa are characterized by high photosynthetic capacities, as estimated both by chlorophyll fluorescence and oxygen production measurements, and they may achieve rather high growth rates (around 50% higher than those found in the typical oligotrophic taxa), consequently predominating over microalgae and cyanobacteria in nutrient-rich habitats (Coesel and Wardenaar 1990, Spijkerman and Coesel 1998a, Spijkerman et al. 2004, Stamenković and Hanelt 2011). The high performance of PSII in the large-celled eutrophic species, *C. obtusatum* explained its higher growth rates compared to that of the small-celled oligotrophic desmids. Although we know that small-celled microalgae exhibit higher intrinsic growth rates compared to medium-celled taxa (Fogg 1975, Reynolds 1984), the influence of the trophic origin obviously had a large impact on the ecophysiological characteristics of eutrophic desmids, which displayed consistently high F_V/F_M values and growth rates in AWW.

The growth rates of the desmids grown in air-bubbled 1 L flasks fell within the range of growth rates in commercially grown green microalgae cultivated in various types of AWW. Their μ_{max} were higher than in the fast-growing *Parachlorella kessleri* that had the specific growth rates decreasing from 0.12 to $0.037 \cdot d^{-1}$ with increasing inoculum concentrations, cultivated in AWW with lower N amounts than in our study (Liu et al. 2019). The selected desmids had higher μ_{max} than the fast-growing species *Chlorella* sp., *Scenedesmus* sp., and *Monoraphidium* sp. ($0.006–0.018 \cdot d^{-1}$) grown in the tilapia effluent medium which contained $24 \text{ mg} \cdot L^{-1}$ NH_4^+ and $10 \text{ mg} \cdot L^{-1}$ PO_4^{3-} (Guerrero-Cabrera et al. 2014), and *Platymonas* (*Tetraselmis*) *subcordiformis* which had growth rates from $0.12 \cdot d^{-1}$ at low NO_3^- ($1.7 \text{ mg} \cdot L^{-1}$) to $0.26 \cdot d^{-1}$ at higher NO_3^- ($47.8 \text{ mg} \cdot L^{-1}$; Guo et al. 2013). The desmid μ_{max} values were also in the range of growth rates of green algal strains grown in various synthetic wastewaters, which varied between $0.32 \cdot d^{-1}$ for *Selenastrum capricornutum*, $0.38 \cdot d^{-1}$ for *Tetradesmus (Scenedesmus) obliquus*, $0.52 \cdot d^{-1}$ for *Chlorella vulgaris* (Zhao et al. 2016), and $0.34–0.68 \cdot d^{-1}$ for *Selenastrum* sp. (Tossavainen et al. 2017, 2019). The selected desmids, with the exception of *S. chaetoceras*, demonstrated over 25-fold increase in biomass during the 14 d cultivation in AWW media, from $0.03 \text{ g} \cdot L^{-1}$ to over $0.8 \text{ g} \cdot L^{-1}$ (the highest CDW values were $1.02 \text{ g} \cdot L^{-1}$ in *C. laeve* in DI + A, and $1.19 \text{ g} \cdot L^{-1}$ in *C. impressulum* in WH + A). These CDW values fall within the range of the

same strains cultivated at more favourable conditions, at 23°C and 150 μmol photons · m^{-2} · s^{-1} in WH (Stamenković et al. 2019). The CDW values of desmids were higher than in some commercially grown microalgae characterized by high robustness and rapid growth in wastewaters. For example, *C. vulgaris*, *Scenedesmus quadricauda*, *P. kessleri*, and *Chlorococcum* sp. achieved only 0.23, 0.24, 0.26 and 0.25 g · L^{-1} when cultivated in freshwater fish effluent which had low nitrate (0.35 mg · L^{-1}) and high nitrite amounts (24.5 mg · L^{-1}), while ammonium was 6.5 mg · L^{-1}, and total P 1.8 mg · L^{-1} (Liu et al. 2019). Furthermore, the desmids had somewhat lower biomass compared to that of *Chlorella sorokiniana*, *Tetradesmus obliquus* and *Ankistrodesmus falcatus* grown 14 d in the Nile tilapia effluent, containing 5.3 mg · L^{-1} NH_4^+, 40.7 mg · L^{-1} NO_3^-, 8.8 mg · L^{-1} PO_4^{3-}, and the algae achieved 1.25–2.25 g · L^{-1} CDW when the average inoculum was 0.2 g · L^{-1} (Ansari et al. 2017).

Although NH_4^+ is regarded as preferable for microalgae compared to NO_3^- (Crofcheck et al. 2012), *Cosmarium humile*, *C. laeve*, and *C. impressulum* completely absorbed relatively high amounts of nitrate in WH and DI + A within 7 d. Most desmids utilize nitrate as a nitrogen source, however, some desmids inhabiting eutrophic water bodies such as *Staurastrum tetracerum* and *C. aciculare* may utilize NH_4^+ instead (Venkateshwarlu 1983, Coesel 1991). The inability of *C. aciculare* to grow in media with nitrate was explained by the complete lack of nitrate reductase activity in this desmid (Coesel 1991). The lower NO_3^- absorption in *C. meneghinii* and *S. chaetoceras* could be explained by their requirement of NH_4^+ as nitrogen source. These species are considered typical eutrophic desmids and they have been commonly found in heavily polluted habitats containing high ammonium and phosphorus loads (Lenzenweger 1999, 2003, Coesel and Meesters 2007). Yet, both species decreased nitrate quantities in all the media after 14 d indicating that they possessed nitrate reductase and that it might have taken time for enzyme synthesis/activation. The absence of ammonium ions in AWW obviously increased the high nitrate uptake in desmids, since ammonium may have a negative effect on nitrate assimilation at both transcriptional and posttranscriptional levels (Sanz-Luque et al. 2015, Taziki et al. 2015). A Dutch strain of *S. chaetoceras* and the eutrophic species *Closterium limneticum* displayed a marked nitrate reductase activity when grown in ammonium-deficient medium (Coesel 1991). Furthermore, desmids are regarded as an algal group adapted to high light intensities – having the onset of saturation at >800 μmol photons · m^{-2} · s^{-1}, and they prefer warm temperatures (25°C) compared to other microalgae from moderate climate (Stamenković and Hanelt 2013a,b, 2017). As photosynthetic rates are enhanced at higher light and warmer temperature regimes, nutrient assimilation and other energy- and reductant-requiring processes, including nitrate uptake, also increase (Taziki et al. 2015). Therefore, if the light/temperature conditions are improved (e.g., using the natural sunlight that ranges about 1,000–2,000 μmol photons · m^{-2} · s^{-1} in summer) the nutrient absorption might be even faster in the eutrophic desmids.

The AWW media and cultivation conditions did not exert stress to the photosynthetic machinery in most desmids, as concluded from the small inhibitions and relatively high percentages of F_V/F_M compared to control during the growth in Nunc flasks. The ameliorating effect of AWW to F_V/F_M in the eutrophic desmids *Chlorococcum obtusatum*, *Staurastrum chaetoceras* and *S. punctulatum* in Nunc flasks, and in *C. meneghinii* and *C. impressulum* in WH + A and DI + A (Figs. 2 and 4) indicated that no non-photochemical quenching driven by the xanthophyll cycle pigments occurred. Comparably, *Micrasterias denticulata* demonstrated no large changes in F_V/F_M and pigment composition when the cells were treated with 200 mmol · L^{-1} NaCl while the chloroplasts had minor alterations (Affenzeller et al. 2009). The species exhibiting some protoplast shrinkage (*C. obtusatum*, *C. crenatum*, *S. punctulatum*, and *S. polymorphum*) had F_V/F_M values in the range of the other desmids studied. In general, the high stability of the PSII machinery in conditions that cause the morphological changes may partly explain the resistance of desmids to salt stress and nutrient changes in their habitats, similarly as noted after applications of temperature/irradiation stress (Stamenković and Hanelt 2017). The increase in the photosynthetic efficiency (α) in the eutrophic desmids in 1 L flasks demonstrated the low-light acclimation (i.e., behaviour corresponding to "shade-adapted" plants) as a result of the cultivation at low light levels (Raven and Geider 2003, Ralph and Gademann 2005, Stamenković and Hanelt 2017). This attribute was also observed in oligotrophic desmids grown in Nunc flasks, while the eutrophic desmids consumed CO_2 rapidly in the closed flasks due to the high growth, causing the decrease in chlorophyll fluorescence parameters.

The desmid strains originating from the tropical climate (*Cosmarium obtusatum*, *C. laeve* and *C. impressulum*) were characterized by higher $rETR_{max}$ (>155 rel. units) and I_k values (>1,000 μmol photons · m^{-2} · s^{-1}) compared to the desmids collected from the other climates. This revealed their adaptation to high light intensities at low latitudes, comparable to what was described for tropical macro- and microalgae (Hanelt et al. 2003, Stamenković and Hanelt 2017). The polar taxon, *C. crenatum*, as well as the meso-oligotrophic desmids from moderate climate (*C. regnesii* and *C. dilatatum*) had lowest photosynthetic capacity and saturating irradiance (up to 119.1 rel. units for $rETR_{max}$, and 693.3 μmol photons · m^{-2} · s^{-1} for I_k), while the subtropical desmid strains (*C. humile*, *C. regnellii*, *Staurastrum boreale*, and *S. polymorphum*) were in between. It has been revealed that algae show the decrease in ETR_{max} and

I_k from medium to high latitudes corresponding to the decrease of solar irradiance from the equator to the polar regions (Lüning 1990, Wiencke et al. 1993, Weykam et al. 1996, Roleda et al. 2005, 2006). Hence, both the climate origin and the trophic preference of the desmids studied had substantial impacts on chlorophyll fluorescence parameters and, consequently, on the growth rates and biomass. It appeared that the time of isolation (i.e., the age of cultures) did not have a large influence on the physiological state of PSII and growth rates of desmids in this study. As noted earlier, desmids might have stable genomes and consistent species- and strain-specific photophysiological responses under PAR/UV radiation and temperature stress conditions (Stamenković and Hanelt 2017). Thus, our study additionally pointed that the desmid strains could preserve inherent physiological responses with regard to their climate and trophic origin, even when grown in the moderately saline AWW and under the suboptimal light/temperature regime.

The values of the cellular C, N, and P quotas of desmids cultivated 24 h in WH generally corresponded to the values known for microalgae and cyanobacteria (Spijkerman and Coesel 1996a,b, Giovagnetti et al. 2012, Perrin et al. 2016, Whitton et al. 2016). Since the mucilage of Zygnematophyceae contains predominantly carbohydrates (Kiemle et al. 2007), the increase in POC in *Cosmarium humile*, *C. laeve*, and *C. impressulum* in WH + A and DI + A after 14 d cultivation can be caused by the increasing of mucilaginous sheaths in these species in nutrient-deficient conditions. The thickness of the desmid extracellular matrix may increase in the nutrient-poor stationary phase since it has an important role in the trapping and concentration of nutrients (Stamenković and Hanelt 2011). Furthermore, the increase in fatty acid content in the nutrient-deficient phase in desmids (Stamenković et al. 2019, 2020) certainly resulted in an increase in POC:PON ratios in all the media.

Interestingly, cellular P quotas in the eutrophic species, *Cosmarium meneghinii* and *Staurastrum chaetoceras*, decreased from the range 0.28–0.61 pg · cell^{-1} to the barely detected quantities after 14 d in all the media, which pointed to the strong P starvation in these species. On the other hand, a meso-oligotrophic species, *C. impressulum*, had highest POP values both after 24 h in WH (1.13 pg · cell^{-1}) and after 14 d in all the cultivation media (up to 0.74 pg · cell^{-1} in WH). Spijkerman and Coesel (1996a,b, 1998a,b) demonstrated that the eutrophic desmids, *S. pingue* and *S. chaetoceras*, had higher maximum P uptake rates and higher initial growth rates with a short lag phase than in an oligotrophic species *Cosmarium abbreviatum*, and hence they are well adapted to a P pulse of short duration occurring in eutrophic water bodies. Accordingly, *C. meneghinii* and *S. chaetoceras* likely had a rapid P uptake during the first days of cultivation and they were not capable of long-term storage of intracellular P in contrast to *C. impressulum*. The eutrophic desmids are adapted both to high nutrient amounts and to high variations in nutrient concentrations, which may occur due to the resuspension from sediments in shallow eutrophic lakes (Spijkerman and Coesel 1998a,b). On the contrary, having the higher storage capacity, *C. impressulum* may appear competitively superior when exposed to an infrequent but lasting P pulse in meso-oligotrophic habitats. Tilman and Kilham (1976), Kromkamp et al. (1989) and Elgavish et al. (1980, 1982) found a large difference in storage ability for P for microalgae with comparable growth rates, which also supported our study.

Cultivation of microalgae under N depletion resulted in molar PON:POP ratios of less than 10:1, while under P depletion, ratios of more than 30:1 occurred (Goldman 1979, Larsdotter 2006, Gonçalves et al. 2017). Although the desmid PON:POP ratio was around 35 after 24 h cultivation in WH, it reached over 50 in WH + A after only 24 h pointing to the P deficiency in the selected desmids, which was significantly high in *Cosmarium humile*, *C. laeve*, and *C. meneghinii*. *Cosmarium impressulum* displayed a slight decrease in PON:POP at the end of cultivation in WH + A and DI + A, thus showing the highest tolerance to the limited P source. Therefore, desmids revealed the species-specific ability to adjust the N and P concentration in their biomass in relation to the surrounding concentration in the water, in accordance to what is known for the other freshwater microalgae (Beuckels et al. 2015, Choi and Lee 2015).

Using a small start inoculum (0.03 g · L^{-1}) and at relatively low light/temperature regime *Cosmarium humile*, *C. laeve*, *C. meneghinii*, and *C. impressulum* absorbed high amounts of nitrate and achieved relatively high growth rates, and this all indicated their potential for the remediation of fish effluents in colder climates. Considering that the south of Sweden has long summer days (over 14 h of light) this could favour high biomass production as losses of biomass due to respiration would decrease. As desmids synthesize high amount of valuable metabolites such as specific fatty acids and carbohydrates (Ekelhof and Melkonian 2017a,b, Stamenković et al. 2019, 2020), the production of these metabolites may be sustainable if the cultivation of desmids is coupled with wastewater treatments. Several members of this primarily oligotrophic group of algae showed high plasticity and robustness at moderate salinity, unfavourable nutrient and light/temperature regimes and, thus, they appear to be interesting for wastewater bioremediation.

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AUTHOR CONTRIBUTION

M. Stamenković: Conceptualization (lead); formal analysis (lead); investigation (lead); methodology (equal); visualization (equal); writing – original draft (lead); writing – review & editing (equal). **E. Steinwall:** Conceptualization (equal); investigation (equal); writing – original draft (equal). **A. Wulff:** Conceptualization (equal); methodology (equal); project administration (equal); supervision (equal); validation (equal); visualization (equal); writing – original draft (equal); writing – review & editing (equal).

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web site:

Table S1. Nutrient characteristics of media during the cultivation of five selected desmid strains: Woods Hole (WH), WH with AWW (WH + A) and deionized water with AWW (DI + A). SDs typically < 10% of mean, $n = 3$.

Table S2. Cellular carbon, nitrogen and phosphorus quotas for the desmid strains after 1 and 14 d of cultivation in WH, WH with AWW (WH + A), deionized water with AWW (DI + A). SDs typically < 10% of mean, $n = 3$.