KU900225 Isochrysis galbana CCAP 927-1 AJ549230 Zygogonium tunetanum UTCC 136 100 95 KM020185 Zygnema circumcarinatum SAG 698-1b AJ428078 Mesotaenium endlicherianum SAG 12.97 99 MG652622 Streptosarcina arenaria SAG 2560 100 MG652624 Streptosarcina costaricana SAG 36.98 FN562443 Pyramimonas parkeae CCMP 726 100 AB017123 Pyramimonas propulsa NIES 251 82 FN562441 Pyramimonas tetrarhynchus SCCAP K-0002 100 KF615764 Polyblepharides amylifera CCMP720 FN562434 Nephroselmis rotunda CCAP 1960-1 93 FR865651 Nephroselmis rotunda CCAP 1960-3 100 FN562435 Nephroselmis rotunda M0932 93 MT833289 Nephroselmis sp. N3C46 X74754 Nephroselmis olivacea SAG 40.89 100 - FN562433 Nephroselmis astigmatica NIES-252 -AB158376 Nephroselmis pyriformis UTEX LB2001 AY425306 Nephroselmis pyriformis RCC949 100 97 1 X75565 Pseudoscourfieldia marina CCMP 717 AY762602 Lobosphaera incisa SAG 2007 100 KM020112 Trochisciopsis tetraspora SAG 19.95 MN267185 Xerochlorella sp. SAG 2582 KM020066 Pseudostichococcus monallantoides SAG 380-1 100 KJ756841 Stichococcus bacillaris CCAP 379-5 95 MT078183 Tetratostichococcus jenerensis SAG 2138 100 LT560364 Pseudochlorella signiensis CCAP 211-57 HE610126 Koliella longiseta SAG 470-1 83 LT560358 Pseudochlorella pyrenoidosa SAG 18.95 89

Figure S1. Phylogenetic tree of Nephroselmis sp. N3C46 based on 18S rRNA sequences

AB006050 Pseudochlorella subsphaerica CCAP 264-3

LT560356 Pseudochlorella pringsheimii SAG 211-1a

0.02



**Figure S2.** Residual phosphate concentration  $(\mu M)$  in the medium over time of *Nephroselmis* sp. cultures in PBRs in batch and continuous mode. Black dots represent sample collection for antioxidant activity measure and carotenoids analysis. Data are expressed as mean ± standard error (SE, n = 2).



**Figure S3.** HPLC chromatogram at 450 nm of ethanol extract of *Nephroselmis* sp.. Siph, siphonaxanthin; Neo, neoxanthin; Viola, violaxanthin; Anthe, antheraxanthin; Zea, zeaxanthin; Lut, lutein; Chl, chlorophyll; Lyco, lycopene;  $\beta$ -car,  $\beta$ -carotene; Car 54, unidentified carotenoid (see Serive et al. [103] for UV-vis spectrum of Car 54 in HPLC system)



Figure S4. Microphotography of *Nephroselmis* sp.



**Figure S5**. Absorbance at 680 nm and residual nitrate concentration ( $\mu$ M) over time of *Nephroselmis* sp. cultures in PBRs in batch and continuous mode. Black dots represent sample collection for antioxidant activity and carotenoids analysis. Data are expressed as mean ± standard error (SE, n = 2).



**Figure S6**. Pearson's correlation analysis between cell concentration in cell mL<sup>-1</sup> and light absorbance at 680 nm

**Table S1.** Pigment composition of *Nephroselmis* sp. (mg g<sup>-1</sup> DW) at different time of the culture. Siph, siphonaxanthin; Neo, neoxanthin (*trans* and *cis*); XCP, Xanthophyll Cycle Pigments (violaxanthin + antheraxanthin + zeaxanthin); Lut, lutein; Lyco, lycopene;  $\beta$ -Car,  $\beta$ -carotene; TC, total carotenoids; Chl *a*, chlorophyll *a*; Chl *b*, chlorophyll *b*. Data are expressed as mean ± standard error (SE, n = 2). Different letters indicate statistically significant differences (p<0.05).

			Siph	Neo	ХСР	Lut	Lyco	β-car	TC	Chl a	Chl b
		Day 2	$5.00 \pm 0.80^{b}$	$7.35 \pm 1.47^{b}$	$9.50 \pm 0.44^{b}$	$5.93 \pm 0.10^{\rm bc}$	$2.93 \pm 0.12^{abc}$	$11.84 \pm 0.73^{ab}$	42.55 ± 2.99 <sup>b</sup>	73.75 ± 11.24 <sup>b</sup>	$79.48 \pm 6.40^{b}$
Batch		Day 3	$6.36 \pm 0.33^{a}$	$9.58\pm0.75^{\rm a}$	$12.14 \pm 0.35^{a}$	$7.16\pm0.06^{ab}$	$3.32 \pm 0.37^{a}$	$12.14 \pm 0.55^{ab}$	$50.70 \pm 1.34^{a}$	$110.15 \pm 3.79^{a}$	$102.97 \pm 0.51^{a}$
		Day 4	$4.07 \pm 0.32^{b}$	$6.08 \pm 0.61^{b}$	$9.57 \pm 0.36^{b}$	$7.13 \pm 0.21^{ab}$	$2.99 \pm 0.34^{ab}$	$11.71 \pm 0.65^{ab}$	$41.55 \pm 1.64^{b}$	$65.57 \pm 2.81^{bc}$	$64.21 \pm 3.30^{\circ}$
		Day 6	$2.35 \pm 0.22^{cd}$	$4.04 \pm 0.34^{\circ}$	$7.43 \pm 0.19^{cd}$	$6.82 \pm 0.24^{abc}$	$2.48 \pm 0.02^{bcd}$	$10.63 \pm 0.40^{bc}$	$33.74 \pm 1.30^{\circ}$	$41.67 \pm 2.18^{de}$	$44.20 \pm 2.02^{d}$
Continuous N-lim	N-resupply	Day 7	$4.01 \pm 0.53^{b}$	$6.05 \pm 1.12^{b}$	$8.60 \pm 0.64^{bc}$	$7.97 \pm 0.84^{a}$	$2.89 \pm 0.17^{abc}$	$12.89 \pm 1.43^{a}$	$42.40 \pm 4.06^{b}$	$66.10 \pm 5.93^{b}$	$63.94 \pm 6.83^{\circ}$
	Steady state	Day 23-24	$2.66 \pm 0.24^{\circ}$	$3.37 \pm 0.47^{cd}$	$8.08\pm0.75^{\rm bc}$	$5.64 \pm 0.54^{\rm bc}$	$2.58\pm0.28^{\rm bc}$	$8.86 \pm 0.74^{\circ}$	$31.20 \pm 2.63^{cd}$	$51.82 \pm 5.43^{cd}$	$45.92 \pm 3.87^{d}$
Batch N-starvation		Day 28	$2.33 \pm 0.07^{cd}$	$2.94 \pm 0.00^{cde}$	$7.63 \pm 0.29^{cd}$	$5.92 \pm 0.36^{bc}$	$2.70 \pm 0.09^{abc}$	$9.24 \pm 0.59^{\circ}$	$30.76 \pm 0.05^{cd}$	$45.66 \pm 2.36^{d}$	$41.58 \pm 2.79^{de}$
		Day 29	$1.32 \pm 0.12^{de}$	$2.09\pm0.06^{\rm de}$	$6.08 \pm 0.52^{de}$	$5.33 \pm 0.47^{\circ}$	$2.26 \pm 0.01^{cd}$	$9.42 \pm 0.54^{\circ}$	$26.51 \pm 0.59^{d}$	$29.61 \pm 2.08^{\text{ef}}$	$30.05 \pm 2.20^{\text{ef}}$
		Day 30	$1.05 \pm 0.16^{\rm e}$	$1.94 \pm 0.15^{e}$	$5.84 \pm 0.77^{de}$	$5.56 \pm 0.85^{bc}$	$1.85 \pm 0.23^{d}$	$10.61 \pm 0.14^{bc}$	26.84 ± 2.26 <sup>cd</sup>	$24.25 \pm 2.70^{\rm f}$	$28.07 \pm 2.92^{\rm f}$
		Day 31	$0.74 \pm 0.12^{\rm e}$	$1.72 \pm 0.25^{e}$	$5.24 \pm 0.88^{e}$	$5.22 \pm 1.00^{\circ}$	$0.95 \pm 0.16^{\rm e}$	$10.85 \pm 0.44^{abc}$	$24.72 \pm 2.78^{d}$	$20.69 \pm 2.93^{\rm f}$	$26.03 \pm 2.87^{\rm f}$