

1 **Lipid Productivity and Cell Wall Ultrastructure of Six Strains of *Nannochloropsis*:**
2 **Implications for Biofuel Production and Downstream Processing.**

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11 Running Head: Cell Wall Ultra-Structure of *Nannochloropsis*

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20 **Abstract**

21 Microalgae are generating considerable interest for third generation biodiesel
22 production. However, appropriate strain selection is proving challenging due to the
23 significant variation in cellular physiology, metabolic potential and genetics observed
24 even amongst strains deemed morphologically similar. Six strains of *Nannochloropsis*
25 from the CCAP culture collection were assessed for their lipid productivity and cellular
26 structure, as proxies for oil production and harvesting ease, to assess their suitability as
27 biodiesel production platforms. Differences in growth rate and lipid accumulation across
28 the strains were observed. *N. oculata* strain 849/7 showed significantly reduced
29 doubling time compared to *N. salina* strain 849/3, whilst *N. oceanica* 849/10 produced
30 the highest lipid content. In addition the six strains could be differentiated in to 3 distinct
31 classes based on their cell wall thickness, which varied across the strains from 63-119
32 nm and which is independent of both species and geographical isolation location. The
33 importance of these variations in ultrastructure and physiology for biodiesel production
34 is discussed.

35

36 **Key words**

37 *Nannochloropsis*, Microalgae; fatty acid; lipid; ultrastructure; biofuels

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39 **1.1 Introduction**

40 There has been considerable interest in microalgae as a potential alternative, low
41 carbon, renewable source of bioenergy and chemical feedstock [1, 2]. In particular, the
42 need for alternative sources of liquid transportation fuels to replace the over-reliance on
43 diminishing sources of mineral oil is crucial to economic stability and development. This
44 is compounded by the need to reduce CO₂ emissions to mitigate global climate change
45 resulting from increasing levels of atmospheric CO₂. In 2011 global energy demand
46 rose 2.5% with 33% of all consumption derived from oil [3]. Research aimed at the
47 development of carbon neutral liquid, gaseous and solid sources of renewable energy
48 has in recent years started to move away from biofuels primarily derived from food
49 crops and oily seeds which impact negatively on both food and water security and
50 contribute to the destruction of the world's forests [4,5,6]. Biofuels from waste cooking
51 oil and animal fat have grown in production but cannot satisfy the current demand for
52 transport fuel [4] and have been shown to make a limited contribution to climate change
53 mitigation [7].

54 Microalgae show promise as a high impact source of biomass for biofuel production.
55 These photosynthetic microorganisms have low input requirements for light and nutrition
56 whilst producing large amounts of biomass over short periods of time, including
57 processing lipids for biofuels and valuable co-products [6].

58 When compared to current approaches with terrestrial crops, algae have much higher
59 predicted energy yields per area [5, 8] and whilst algae production is known to consume

60 more water than other biofuel sources produced from terrestrial growth [8], use of
61 marine species would not impact on decreasing fresh water supplies.

62 The economic and technical barriers involved in micro algal derived biofuel production
63 have begun to be addressed in recent years with heavy investment from both
64 government and industrial sponsors [5, 9]. These research programs have seen
65 technology improvements enabling higher biomass / hectare output combined with
66 reduced cost of dewatering, extraction and refining. Despite these technical advances
67 algal derived biofuels remain uncompetitive with present day fossil fuels [5]. It is well-
68 known that many algae accumulate triacylglycerol in large quantities during the
69 stationary phase of growth [4, 10], however, to be commercially viable production
70 requires a semi-continuous culture maintained at mid-logarithmic phase suitable for
71 non-stop culture.

72 Traditional cross breeding in plants is an effective method for improving the yield of oil
73 seed crops, yet such an approach with algae is not feasible. Whilst bioengineering
74 offers the possibilities for improving lipid accumulation and a raft of other important traits
75 such as thermotolerance and photosynthetic efficiency, many species of algae remain
76 resistant to the standard molecular techniques available. For these reasons optimal
77 microalga strain selection is critical to maximise biofuel production.

78 An initial analysis of several microalgal species suggested that *Nannochloropsis* sp.
79 showed the most promise as a potential feedstock for biofuel production. The
80 *Nannochloropsis* genus is a diverse collection of microalgae comprising 6 species
81 containing several sub strains within this. In fact, a recent genomic analysis of 6

82 species of *Nannochloropsis* has revealed considerable genetic diversity amongst
83 species despite strong conservation of 18S rRNA genes [11]. To assess the potential of
84 *Nannochloropsis* as a biofuel producing strain and to develop a better understanding of
85 the physiological differences which may exist between the different *Nannochloropsis*
86 species a total of four *Nannochloropsis* species representing six different strains were
87 analysed for fatty acid composition and lipid productivity. Since these species are also
88 known to be very robust and refractory to cellular disruption [12, 13, 14] a detail that
89 could impact on the ease and efficiency of oil extraction, an ultra-structural assessment
90 of the cell wall of each species using electron microscopy was performed. Considerable
91 variations in both lipid accumulation and cell wall thickness were observed and the
92 implication of these observations for biofuel production and downstream processing of
93 algal biomass is discussed.

94

95 **2. Methods**

96 **2.1 Strains.**

97 Algae strains were obtained from the Culture Collection of Algae and Protozoa (Scottish
98 Association for Marine Science, Oban, Scotland, U.K.). Cultures assessed in this
99 investigation: *Nannochloropsis salina* (CCAP 849/3 isolated from Skate Point, Isle of
100 Cumbrae, Scotland and CCAP 849/6 isolated from Great South Bay, Long Island, New
101 York, USA), *N. gaditana* (CCAP 849/5 isolated from Cadiz Bay, Cadiz, Spain),
102 *N. oculata* (CCAP 849/1 from Skate Point, Isle of Cumbrae, Scotland and CCAP 849/7

103 isolated from Lake of Tunis, Tunisia), *N. oceanica* (CCAP 849/10; no geographical
104 data).

105 **2.2 Culture conditions.**

106 Stock cultures were maintained under batch culture conditions (1L) in F/2 medium [15]
107 and sub-cultured on a weekly basis. Experimental cultures (250 ml in 1 L flasks) were
108 maintained under 100 $\mu\text{mol photons m}^2 \text{ sec}^{-1}$ irradiance on a 16 h: 8 h light: dark cycle
109 at 25 °C ($\pm 1^\circ\text{C}$) and agitated daily but provided no additional aeration. Cultures for
110 comparison of 90% salinity (~30.6ppt) and 10% salinity (~3.4ppt) acclimated cells were
111 grown in 2 L growth vessels and bubbled with air under the same light conditions. All
112 experimental cultures were inoculated at a density of 1×10^5 cells ml^{-1} and maintained
113 without sub-culturing into stationary growth phase. Samples were removed and
114 analysed for cell growth, cell wall phenotype, and cellular lipids analysed. Culture
115 growth rates and cell densities were monitored as described below.

116 **2.3 Growth rate determination.**

117 Culture density was determined via light microscope cell enumeration in a
118 haemocytometer following staining with Lugols iodine solution (2 %). Specific growth
119 rates (K) were calculated according to the following equation:

$$120 \quad K = \ln(N_2/N_1) / (t_2 - t_1)$$

121 where N_2 and N_1 are the total cells mL^{-1} at time point (t_2) and time point (t_1)
122 respectively, and where $t_2 > t_1$.

123 **2.4 Lipid analyses.**

124 For each strain, at each sampling point 5 x 50ml samples were subject to lipid analysis.
125 Fatty acid concentrations and profiles in microalgal cells were determined post
126 conversion to fatty acid methyl esters (FAMES) using GC-MS (Agilent 7890A GC and
127 5975C inert MSD, Agilent Technologies Ltd., Edinburgh, UK). Culture samples were
128 centrifuged (10,000 × g), washed in distilled water and resulting pellets lyophilised.
129 Nonadecanoic acid (C19:0) was added as an internal standard and cellular fatty acids
130 were converted directly to FAMES by adding 1mL of transesterification mix (95:5 v/v 3 N
131 methanolic HCl; 2,2-dimethoxypropane) followed by incubation at 90 °C for 1h. After
132 cooling, FAMES were recovered by addition of 1 % w/v NaCl solution (1 mL) and *n*-
133 hexane (1 mL) followed by vortexing. The upper hexane layer was injected directly onto
134 the GC-MS system as previously described in White *et al.* [16].

135 **2.5 Electron microscopy.**

136 Exponentially growing cells harvested 5 days after culture initiation were washed in
137 fresh F/2 medium and re-suspended in fixative (2.5 % glutaraldehyde in PBS) at an
138 approximate cell density of 1×10^9 cells ml⁻¹ for 4 hours at 4 °C. Fixed cells were
139 washed twice in PBS and then immobilised in 2 % low melting point agarose. The
140 agarose pellet (just the area with cells) was cut into small blocks (1x1x1 mm) which
141 were post-fixed in 1 % osmium tetroxide in PBS, dehydrated in an alcohol series and
142 embedded in Agar Low Viscosity Resin. Ultra-thin sections were cut with a Leica EM
143 UC7 ultra microtome, stained with uranyl acetate and lead citrate, and examined in a
144 JEOL 1200EX transmission electron microscope. Analysis of the cell wall thickness was
145 performed using ImageJ [17] with individual cells measured in 5 separate places and a
146 total of 50 individual cells per strain assessed.

147 **2.6 Statistical analysis**

148 Data was assessed for normality and then subject to ANOVA and 2-sample T testing. P
149 values of less than 0.05 were considered to be significant.

150

151 **3. Results and Discussion**

152 The growth rate has a major impact on the maximum productivity that a strain can
153 achieve, and also impacts on any lag time in culture recovery post-harvest, especially
154 where a culture has been allowed to enter stationary phase. The growth properties of
155 the six strains grown in batch culture over 27 days is summarised in Figure 1. All
156 strains demonstrated a substantial biomass production before entering stationary phase
157 with an average maximum cell density across all strains of 32 million cell mL⁻¹ (Figure
158 1).

159 Both *N. oculata* strains (849/1 and 849/7) achieved significantly higher cell densities
160 than the other species assessed ($p < 0.05$) with strain 849/1 achieving a 38% higher cell
161 density than the least prolific strain *N. salina* (849/6) (Table 1). The average doubling
162 time during the initial growth phase was fairly consistent across the *N. salina*, *N.*
163 *gaditana* and *N. oceanica* strains (40-46 hours). *N. oculata* strain 849/7, however,
164 showed significantly reduced doubling time of 35.3 hours compared to *N. salina* strain
165 849/3 with a doubling time of 45.9 hours ($p = 0.042$).

166 Lipid content steadily increases during the growth phase [4, 10] and it can vary
167 significantly between the early exponential and stationary phases of growth. To be

168 commercially viable however, algae will need to be maintained under continuous culture
169 conditions without any lag post-harvest, and as such lipid analysis to measure the
170 FAME content and composition was performed during the exponential growth phase
171 (Day 5). Total FAME content (Figure 2) varied widely both across the species from 3.5%
172 in *N. oculata* strain 849/1 to 14.7% in *N. oceanica* strain 849/10, and within sub-strains
173 of the same species with the second *N. oculata* strain (849/7) achieving 8.86% total
174 FAME - more than double that of 849/1 ($P \leq 0.05$).

175 Diversity of fatty acid saturation was relatively low and with the exception of 849/1, all
176 strains showed a preference for saturated and monounsaturated fatty acid production
177 over the long chain polyunsaturated fatty acids. Strain 849/1 showed an equal FAME
178 content of SFAs, MUFAs and PUFAs (Fig. 2). The lower level of oil accumulation
179 combined with the high polyunsaturated nature of the FAME components make this
180 strain particularly unsuited to biofuel production.

181 *N. oceanica* strain 849/10 had both the highest total FAME accumulation and the lowest
182 overall level of unsaturation (Fig. 2). *N. salina* strain 849/3 and *N. oculata* strain 849/7
183 both had high levels of FAME and though the level of unsaturation was elevated
184 ($p < 0.05$) in comparison to 849/10, the PUFA content of these two strains was not
185 considered to be incompatible as biofuel feedstock. In addition, analysis of the individual
186 fatty acid profiles (Table 2) showed 849/3 and 849/7 produced a significantly higher
187 ratio of palmitic acid (16:1) to oleic acid (18:1) compared to 849/10 and 849/6. With
188 winter and summer blends of biodiesel having differing requirements in terms of
189 viscosity and flash point the availability of strains with different FAME profiles raises the

190 possibility of biofuel production where the algal strain is alternated depending on the
191 fuel requirements.

192 Cell wall thickness is an important trait to be considered, since it affects not only the
193 carbon budget of the organism, ease of downstream extraction of the oil and processing
194 of residual biomass [18] but is also a major barrier to bioengineering. The
195 *Nannochloropsis* strains were subjected to TEM analysis. Figure 3 shows a
196 representative image from each strain and the average cell size and cell wall thickness.
197 Inter and intraspecies cell size (Figure 3A) varied significantly from 2.31 – 2.77 μm with
198 *Nannochloropsis gaditana* 849/5 attaining a size significantly larger than all the other
199 species ($p < 0.05$). No significant difference in cell size was observed between the *N.*
200 *salina* sub strains, but the *N. oculata* strains varied from 2.36 μm (849/1) to 2.59 μm
201 (849/7) $p < 0.05$.

202 Cell wall thickness varied widely both between the 4 different species and surprisingly
203 between the sub strains of the same species. The strains fell into three statistically
204 distinct groupings as summarised in Figure 3b, with very thick (849/1, 849/6, 849/10) in
205 the range 107-119nm, thick (849/5, 849/7) in the range 82-90nm or thin cell walls
206 (849/3) in the range 63-69nm. *N. salina* strains were most divergent with 849/3 having
207 the thinnest cell wall of all the strains, measuring on average 66nm, and 849/6 having
208 one of the thickest cell walls, measuring on average 108 nm.

209 There appears to be no geographical correlation between cell wall thickness and
210 original isolation sites and it is interesting to note that 849/3 (thin cell walls) and 849/1
211 (very thick cell walls) were both isolated from the same location at Skate Point, Isle of

212 Cumbrae, Scotland. In addition, since all the strains have been maintained for several
213 years under the same laboratory conditions it would suggest that the observed
214 differences in cell wall thickness are not phenotypic fluctuations due to some transient
215 environmental condition but are a distinct genetic trait of each strain. For an alga to be
216 integrated into a commercially viable industrial scale process it needs to demonstrate a
217 significant level of robustness in its tolerance to changes in growth conditions such as
218 salinity and temperature. This is especially so where waste-water or growth medium
219 recycling is likely to feature which may cause significant variations in the chemical
220 composition and salinity of the medium.

221 The effect of low salt conditions on *Nannochloropsis* growth and the effect of salinity on
222 cell ultrastructure were determined. *N. salina* strain 849/3 was acclimated from standard
223 90% seawater F/2 medium (approximately 3.1% total salt) to a 10% seawater F/2
224 medium (approximately 0.35% total salt) stepwise in 4 increments (70%, 50%, 30%,
225 10% seawater) over a period of two months. Greater dilution of the seawater at each
226 step caused the culture to die.

227 Changes in lipid productivity, growth and ultra-structure were assessed as before.
228 Reduced saline growth conditions caused a thickening of the cell wall of around 20% in
229 response to the elevated osmotic potential (Table 3). A representative TEM image of
230 cells grown under each condition is given in Figure 4 and the ultra-structure change is
231 clearly visible. Growth analysis showed no difference in doubling time or maximum cell
232 density.

233

234 Comparative analysis of lipid productivity and quality (Table 3 and Figure 4A) indicated
235 little difference in total FAME content between the 10% and 90% salinity cultures with
236 only a slight though significant increase in the 90% salinity cells during mid-exponential
237 growth phase ($p < 0.05$). No significant changes in the fatty acid profile were observed
238 between the cultures in the exponential phase of growth, however, during the stationary
239 phase the level of PUFAs was reduced when cells were grown at 90% salinity. Long
240 (16-18C) and very long chain (>18C) PUFAs are primarily used by micro algae in cell
241 wall structure, and are involved in membrane fluidity maintenance, plastid structure and
242 function and are often substrates for lipoxygenase. The oxylipins produced have roles in
243 signalling both wound-healing and stress and many have roles in innate immunity with
244 antimicrobial and anti-fungal properties [19, 20, 21, 22]. We consider that the difference
245 the level of saturation and the elevation in the long and very long chain fatty acid
246 between the 10 and 90% salinity cells (Table 3) and elevated requirement for these
247 structurally important fatty acids during stationary phase is due to both the increased
248 cell wall thickness and the presence of altered consortia of symbiotic and competitive
249 microorganisms in the reduced saline growth environment.

250 These results indicate that moderate salinity changes (up to 20%) in culture are unlikely
251 to hinder the culture growth. The shift from 90% salinity to 10% salinity and
252 corresponding increase in cell wall thickness did however have small negative impact
253 on lipid productivity during exponential growth (the optimal growth phase for lipid
254 production) and supports other work indicating that diverting carbon from cell wall
255 production towards lipid production may be beneficial for lipid-based biofuel production.
256 Increased cell wall thickness due to growth under decreased salinity may provide

257 increased residual biomass for either anaerobic digestion [23] or conversion to another
258 bulk chemical feedstock through, for example, pyrolysis. Such an approach would result
259 in the need for a more intense method for cell lysis and an increased use of freshwater,
260 negating a major reason for developing marine strains for feedstock production.

261 Choice of the species to be used in microalgal derived biofuel is therefore vital to ensure
262 maximum productivity. Modern photobioreactor (PBR) technologies lend themselves to
263 continuous or semi continuous biomass production maintaining the algal culture in the
264 growth phase. This type of culturing with an algal species such as *Nannochloropsis*
265 *salina* 849/3, which is capable of accumulating relatively high amounts of lipid during
266 mid-to-late growth phase, could improve the productivity over traditional stationary
267 phase harvesting. Based on the data shown in Figure 4A and Table 3 for cells grown
268 under 90% salinity conditions, assuming 45% lipid content (10pg/cell) and a maximum
269 cell density of 1.17×10^8 cells ml^{-1} , yields of around 468g oil would be obtained from a
270 400L scale culture after 25 days (harvesting cells during stationary phase). This
271 compares to a semi-continuous harvesting of 20% volume daily of an actively growing
272 culture at 17.5% lipid content (3.88pg/cell) and a cell density of 9.8×10^7 million cells ml^{-1} ,
273 which would yield around 30g oil per day or 762g oil over a 25 day period. This equates
274 to a productivity increase of approximately 39%.

275 Quin *et al.* [24] have developed a 174,000 L outdoor photobioreactor where the major
276 variable is light. Of particular interest is their use of *Nannochloropsis oculata* CCMP
277 525 which is the same as CCAP 849/1 used in this study, so allowing a direct
278 comparison of productivity. Quin *et al.* report an average annual productivity of $0.15 \text{ g L}^{-1} \text{ d}^{-1}$
279 with the highest productivity recorded in the summer months reaching 0.37 g L^{-1}

280 d^{-1} which is comparable to this study ($0.432 \text{ g}^{-1}/\text{L}^{-1}/d^{-1}$ when grown in a small scale
281 indoor PBR as described above). This suggests that similar productivity is observed
282 under laboratory conditions as in outdoor systems and any shortfall in productivity would
283 be due to seasonal variations rather than issues of large scale cultivation. When
284 considering that agricultural growth is dominated by seasonal cultivation, seasonal
285 variation in both light and temperature should not be seen as a negative factor in the
286 development of large-scale outdoor cultivation and continued production in the winter
287 months considered an advantage. However, not only can the total lipid content in
288 outdoor facilities vary by up to 50% over the year but this variation can affect the fatty
289 acid profile so altering the quality of the final product [25]. Reducing the environmental
290 variability in outdoor cultivation is thus desirable but the added energy (and carbon)
291 costs must be carefully considered.

292 The present study demonstrates the wide physiological variation across
293 *Nannochloropsis* species and strains with differences observed both in lipid
294 biosynthesis and cell ultrastructure. Several of the *Nannochloropsis* strains assessed
295 demonstrated high lipid accumulation during the growth phase combined with fast
296 growth rates and an ability to grow to high cell densities. For example, *N. oceanica*
297 strain 849/10 combines both high oil production and low levels of polyunsaturation with
298 substantial levels of cell proliferation accumulating at high cell densities. However this
299 strain also has one of the thickest cell walls of all the strains tested which could affect
300 the efficiency of downstream processing. In contrast, the good growth and metabolic
301 characteristics coupled with a much thinner cell wall found for *Nannochloropsis salina*
302 strain 849/3 may make it a better prospect for biofuel production overall if downstream

303 processing is found to be better for this strain. The increased cell wall thickness this
304 strain exhibited when exposed to decreased salinity also suggests that the composition
305 of the growth medium (and possibly CO₂ concentration) could play an important role in
306 algal growth beyond influencing the quantity and quality of lipids produced by a given
307 strain with unexpected implications for downstream harvesting and processing of the
308 resultant biomass.

309 Thus, a better understanding of the cell biology and physiology of microalgae beyond
310 simply lipid content is necessary if microalgae-derived biofuels are to become an
311 economic reality. This study further supports the diversity of *Nannochloropsis* species
312 as demonstrated by the recent genomic sequencing [11, 26, 27] on 6 *Nannochloropsis*
313 species and suggests that rDNA sequencing is an inadequate marker in isolation for
314 algal species classification.

315

316 **4. Conclusion.**

317 Investigation of 6 individual *Nannochloropsis* species including phenotypic assessment
318 of growth and cell wall thickness as well as GC-MS analysis of fatty acid content and
319 composition showed that *Nannochloropsis salina* strain 849/3 has the best combination
320 of the desired characteristics essential to ensure the high productivity of biomass
321 required for biofuel feedstock. Further, we have demonstrated that the robustness of *N.*
322 *salina* (849/3) to changes in salinity will allow for flexibility in growth media compatible
323 with waste water recycling and also makes this strain particularly suitable for

324 bioengineering. The relatively narrow cell wall thickness of this strain should also aid in
325 the ease of cell rupture for oil extraction, thus reducing downstream processing costs.

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398

399 **Table 1. Average initial growth rate (doubling time) and the maximum cell density**
400 **achieved \pm SEM for 6 *Nannochloropsis* strains.**

Strain	Average doubling time (hours)	Maximum cell density (cell/ml)
849/1	39.8 \pm 1.1	42 $\times 10^6 \pm 2.4 \times 10^6$
849/3	45.9 \pm 2.4	28 $\times 10^6 \pm 0.4 \times 10^6$
849/5	43.3 \pm 2.8	34 $\times 10^6 \pm 1.1 \times 10^6$
849/6	40.0 \pm 2.4	26 $\times 10^6 \pm 1.7 \times 10^6$
849/7	35.3 \pm 2.3	36 $\times 10^6 \pm 1.2 \times 10^6$
849/10	40.6 \pm 0.5	30 $\times 10^6 \pm 1.7 \times 10^6$

401

402

403 **Table 2. Fatty acid profile of six *Nannochloropsis* strains during early**
 404 **exponential phase of growth. Data presented as percentage of total FAME**
 405 **content \pm S.D.**

406

Strain	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:3	C20:4	C20:5	Other
849/1	4.7 \pm 0.1	28.1 \pm 0.6	28.5 \pm 0.2	0.5 \pm 0.0	4.5 \pm 0.8	2.4 \pm 0.1	1.0 \pm 0.0	6.1 \pm 0.1	6.0 \pm 0.1	16.0 \pm 0.2	2.3 \pm 1.0
849/3	3.2 \pm 0.0	38.5 \pm 0.5	34.2 \pm 0.1	1.4 \pm 0.0	5.5 \pm 0.2	1.3 \pm 0.0	0.8 \pm 0.0	3.5 \pm 0.7	3.3 \pm 0.1	6.6 \pm 0.0	1.7 \pm 0.3
849/5	3.4 \pm 0.2	36.8 \pm 2.5	33.4 \pm 1.4	2.1 \pm 0.1	4.3 \pm 0.6	1.7 \pm 0.0	0.8 \pm 0.1	1.4 \pm 1.6	4.3 \pm 0.2	9.4 \pm 0.5	2.3 \pm 0.6
849/6	2.5 \pm 0.5	36.3 \pm 7.0	33.0 \pm 5.6	1.7 \pm 0.6	13.3 \pm 15	1.5 \pm 0.3	0.8 \pm 0.2	1.5 \pm 0.2	3.0 \pm 0.5	5.3 \pm 0.9	1.2 \pm 0.5
849/7	5.2 \pm 0.2	37.0 \pm 0.7	36.4 \pm 0.5	1.2 \pm 0.1	4.8 \pm 0.9	2.2 \pm 0.1	0.3 \pm 0.2	0.9 \pm 0.0	3.3 \pm 0.1	7.7 \pm 0.3	1.1 \pm 0.6
849/10	6.8 \pm 0.1	37.1 \pm 0.5	31.6 \pm 0.6	1.9 \pm 0.1	11.3 \pm 0.2	0.8 \pm 0.0	0.3 \pm 0.0	0.7 \pm 0.0	2.2 \pm 0.0	5.7 \pm 0.1	1.6 \pm 0.2

407

408

409 **Table 3. Comparative analysis of the effect of salinity on important physiological**
 410 **parameters**

Salinity	Mid exponential					Stationary			
	Cell wall thickness (nm)	Cell density (cells/ml)	SFA	MUFA	PUFA	Maximum Cell density (cells/ml)	SFA	MUFA	PUFA
90%	65.5 ± 2.8	9.8.E+07	39.4	35.0	25.6	1.17.E+08	41.1	40.8	18.1
10%	82.7 ± 2.6	9.5.E+07	40.1	34.4	25.5	1.21.E+08	31.1	45.4	23.5

411

412

413 **Figure Legends**

414 **Figure 1. Comparison of population growth over 27 day period for 6**

415 ***Nannochloropsis* strains grown under batch culture conditions.** Density is given as
416 10^6 cells ml^{-1} . Strains: 849/1 and 849/7 *Nannochloropsis oculata*, 849/3 and 849/6
417 *Nannochloropsis salina*, 849/5 *Nannochloropsis gaditana*, 849/10 *Nannochloropsis*
418 *oceanica*.

419 **Figure 2. Comparison of Total FAME content \pm S.E.M and degree of fatty acid**

420 **saturation in six *Nannochloropsis* strains.** PUFA; Polyunsaturated fatty acids,
421 MUFA; Monounsaturated fatty acids, SFA; saturated fatty acids

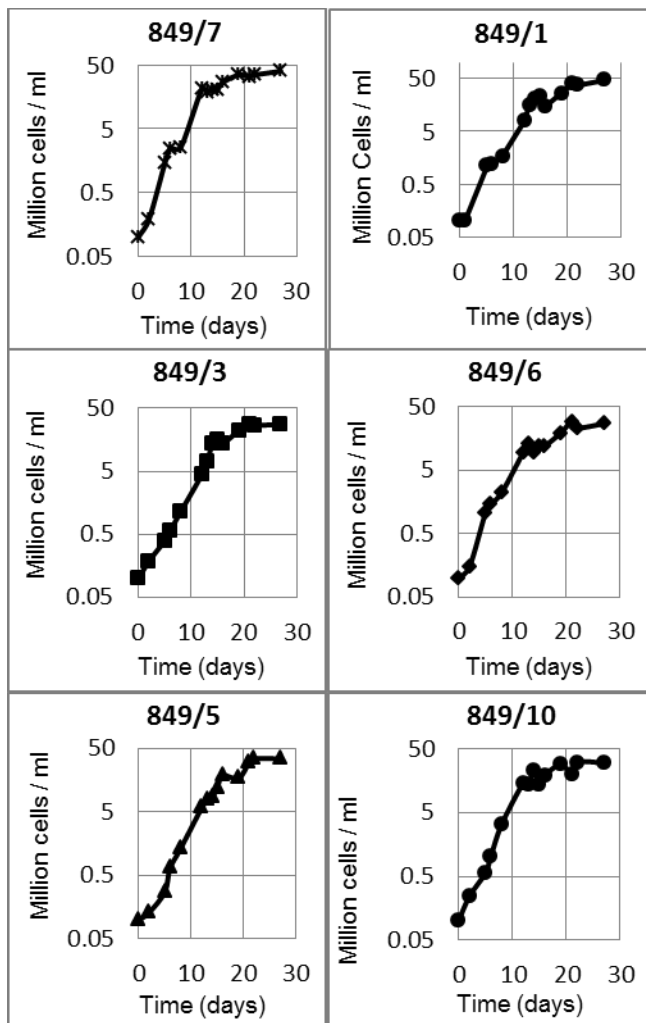
422 **Figure 3. Comparison in cell wall thickness of six *Nannochloropsis* strains. (A)**

423 Cell wall thickness in tabular form \pm SEM. Interval plot (B) provides a graphical
424 representation of the data indicating the three statistically distinct size groupings
425 ($p < 0.05$). (C) Representative sample image of each strain, with the scale bar indicating
426 $1 \mu\text{m}$. Six *Nannochloropsis* strains were fixed and then subject to TEM analysis. The
427 cell wall of individual cells was measured in 5 separate places and a total of 50
428 individual cells per strain were measured. CCMP strains: 849/1 and 849/7
429 (*Nannochloropsis oculata*), 849/3 and 849/6 (*Nannochloropsis salina*), 849/5
430 (*Nannochloropsis gaditana*), 849/10 (*Nannochloropsis oceanica*).

431 **Figure 4. *Nannochloropsis salina* strain 849/3 grown under two salinity**

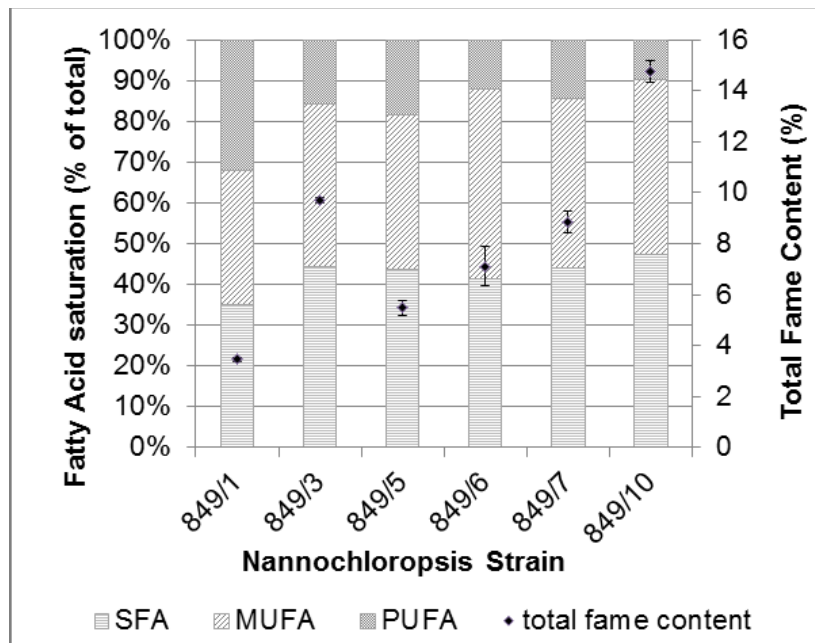
432 **conditions.** Panel A shows total FAME content change during culture growth and
433 decline. exp: exponential, stat: stationary. Panel B Representative sample image of
434 each condition during mid-exponential growth, with the scale bar indicating $1 \mu\text{m}$.

435 **Figure 1**



436

437 **Figure 2**

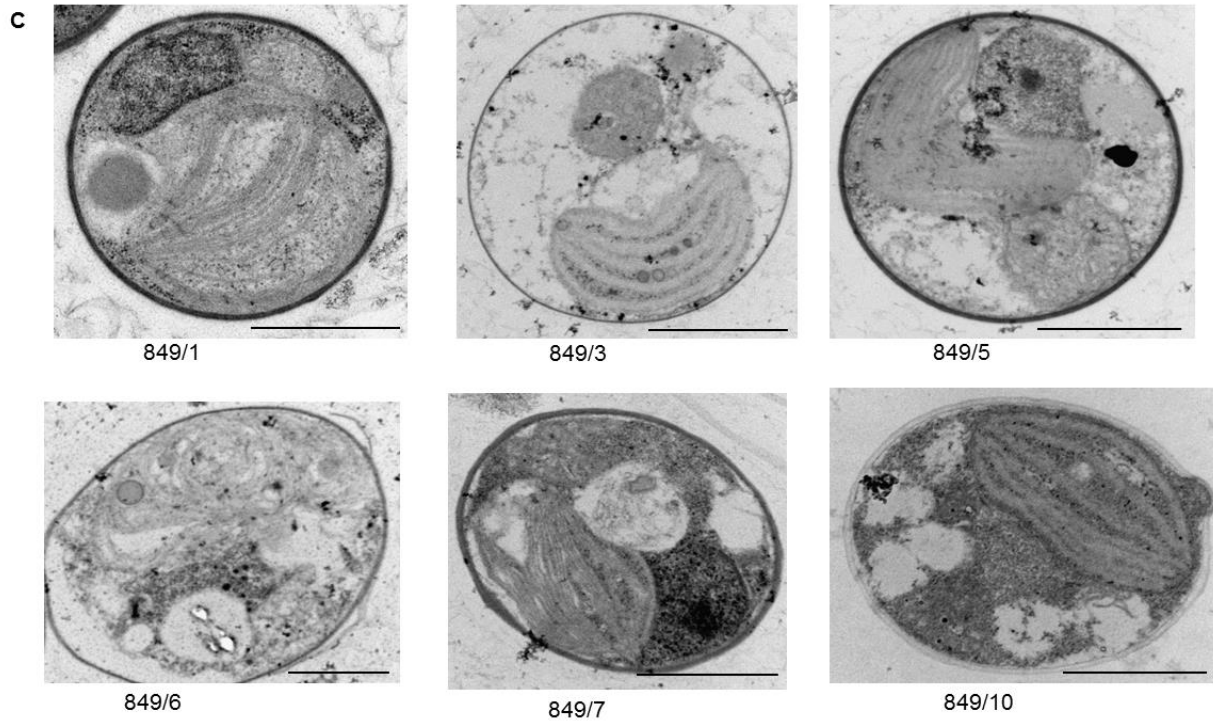
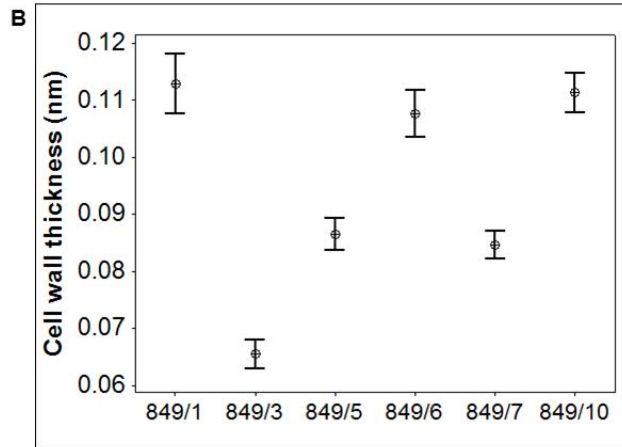


438

439 **Figure 3**

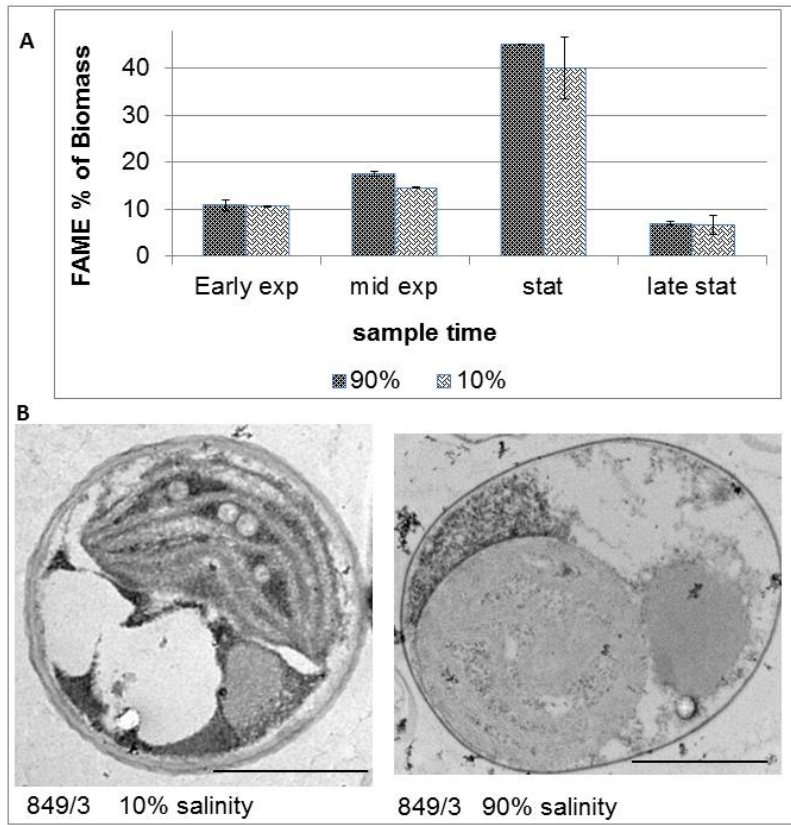
A

Strain	Average cell wall thickness (nm)	Average cell size (μm)
849/1	112.9 \pm 5.9	2.36 \pm 0.04
849/3	65.5 \pm 2.8	2.50 \pm 0.08
849/5	86.5 \pm 3.2	2.77 \pm 0.07
849/6	108.1 \pm 4.7	2.31 \pm 0.09
849/7	84.7 \pm 2.8	2.59 \pm 0.04
849/10	111.3 \pm 3.9	2.47 \pm 0.04



440

441 **Figure 4**



442

443