Light intensity impacts the production of biofuel intermediates in *Heterosigma akashiwo* growing on simulated flue gas containing carbon dioxide and nitric oxide

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Abstract

As a potential biofuel feedstock, the marine microalga, *Heterosigma akashiwo*, accumulates significant lipids, is capable of long-term growth in outdoor photobioreactors, and is an excellent candidate for the bioremediation of industrial emissions. Here, we evaluated resource partitioning in *H. akashiwo* growing on a CO₂ and NO gas mixture under three light intensities: 160, 560, or 1200 $\mu\text{mol quanta m}^{-2} \text{ s}^{-1}$. Light levels had no effect on growth; however, cultures in high light accumulated 2.3-fold more carbohydrates and 17% fewer lipids. Light levels did not affect the percentage of saturated fatty acids, but mono-unsaturates increased by 6% and poly-unsaturates decreased by 12% in high light. The fatty acid profiles reported here suggest that *H. akashiwo* is a good candidate for the production of neutral lipids for biodiesel and also omega-3 fatty acids, and that the quality of biodiesel acquired from feedstocks grown under fluctuating light conditions would be relatively stable.

Keywords: *Heterosigma*, biofuel, irradiance, carbohydrate, bioremediation

1. Introduction

Microalgae are receiving significant attention as a viable feedstock for the production of sustainable biofuels and bioproducts, but algae-derived commodity products are not yet cost competitive with their petroleum-derived counterparts (Davis et al., 2014). Since biomass production accounts for 73% of the final selling price for algal biofuels (Davis et al., 2012), reducing costs associated with farming algae will be essential for overcoming the barriers to economic production of algal bioproducts.

CO₂ supplementation is a costly requirement for commercial biomass production due to carbon limitation at atmospheric CO₂ levels (Benemann 2013), so developing

innovative CO₂ utilization strategies could decrease operating costs significantly. Utilizing waste CO₂ from industrial emissions (i.e. flue gases) would have the dual advantage of simultaneously decreasing biomass production costs while also mitigating the effects of harmful greenhouse gas emissions. Fossil fuel-burning power plants are responsible for over 30% of total greenhouse gas emissions in the United States, and Environmental Protection Agency's Clean Power Plan supports the use of algae-based carbon capture and reuse technologies for power plants to meet new emissions standards (EPA, 2015).

In addition to CO₂, flue gas also contains cytotoxic nitrogen oxides (NO_x, >90% as nitric oxide), and the reaction products of NO_x emissions, ozone (O₃) and nitrous oxide (N₂O), are also potent greenhouse gases (EPA 2014). Previously, we have shown that the marine microalga, *Heterosigma akashiwo* (Raphidophyceae), is an excellent candidate for the bioremediation of industrial emissions, since this alga has the ability to metabolize gaseous nitric oxide (NO) as a nitrogen source (Stewart and Coyne, 2011), and that cultivation on a model flue gas had a positive effect on the growth and productivity of biofuel intermediates in this organism (Stewart et al., 2015). Elsewhere, *H. akashiwo* has been identified as a promising biofuel feedstock (Fuentes-Grünewald et al., 2009) that accumulates significant lipid reserves (Fuentes-Grünewald et al., 2012) and is capable of stable long-term growth in outdoor photobioreactors (Fuentes-Grünewald et al., 2013). Harnessing both CO₂ and NO_x emissions from flue gas as carbon and nitrogen nutrient sources for the growth of microalgae could theoretically reduce operating costs for biomass production by 50% (Douskova et al., 2009; Nagarajan et al., 2013), making *H. akashiwo* an attractive candidate for large scale cultivation.

H. akashiwo is a robust organism that is found in temperate and subtropical regions worldwide (Tobin et al., 2013). Under natural conditions, this alga preferentially migrates to the water surface at mid-day (peak light), and naturally blooms in surface waters where light can exceed 2000 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ (Handy et al., 2005). *H. akashiwo* can grow under a wide range of salinities (5-30 psu), temperatures (4-30 °C), and nutrient regimes (Zhang et al., 2006). The ability of *H. akashiwo* to maintain growth under suboptimal conditions may be due in part to its high photosynthetic efficiency and its ability to rapidly exploit high-light and stratified-light environments (Hennige et al., 2013).

Light intensity has been shown to alter fatty acid composition (e.g. Solovchenko and Khozin-Goldberg, 2013) and starch synthesis (e.g. Li et al., 2010) in microalgae. Under saturating irradiances, many microalgae alter their lipid biosynthetic pathways to accumulate saturated and mono-unsaturated fatty acids (e.g. Hu et al., 2008). Saturated fatty acids are the precursors to quality biodiesel, but supplying maximum light intensity without inducing photodamage presents a difficult challenge. *H. akashiwo* resists photodamage by rapidly acclimating to high-light intensities by utilizing cellular mechanisms to induce photoprotection, such as nonphotochemical quenching and reduced PSII reaction center connectivity (Hennige et al., 2013). These photo-protective mechanisms make *H. akashiwo* a good candidate for the optimization of lipid quality and quantity by light manipulation.

Here, we examined the effect of light intensity on resource partitioning and the production of biofuel intermediates in *H. akashiwo* previously acclimated to growth on a simulated flue gas. High light intensities did not inhibit the growth of *H. akashiwo*, and

did not significantly alter the calculated biodiesel quality of *H. akashiwo*-derived FAMES. After theoretical extraction of high value eicosapentaenoic acid (EPA; C20:5n3), the calculated values for cetane number (CN) met the American Society for Testing Materials (ASTM) minimums for 100% biodiesel stocks.

2. Materials and Methods

2.1. Culture Conditions

Stocks of *Heterosigma akashiwo* CCMP 2393 (NCMA; Boothbay Harbor, ME) were maintained in 20 ppt salinity f/2 (-Si) medium (Guillard, 1975) buffered at pH 7.4 with 20 mM HEPES (Sigma-Aldrich, St. Louis, MO, USA) at 25 °C on a 12:12 hour light: dark cycle with an irradiance of 100 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$, and bubbled with a model flue gas (12% CO₂, 150 ppm NO, balanced in N₂; Praxair Technologies, Danbury, CT, USA) for 3 months before the experiment.

2.2. Experimental Design

Replicate cultures (500 mL; N=4) were acclimated to increasing PAR levels: 160, 560, or 1200 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ (herein defined as low-, medium-, and high-light, respectively) on a 12:12 hour light: dark cycle. After six weeks of growth at the respective light intensity, replicate cultures were maintained semi-continuously in exponential growth. Cultures were diluted to 150,000 cells/mL every other day. Cell counts, *in vivo* chlorophyll *a* (chl *a*), extracted chl *a*, and quantum efficiency of photosystem II (F_v/F_m) were measured every other day before cultures were diluted to monitor the cultures for steady state growth. Steady state growth was reached when cell counts and extracted chl *a* did not vary between dilution days by 5%. Samples were

collected for analysis of total lipid content, fatty acid composition, carbohydrate and protein content, and particulate carbon and nitrogen after 10 days of steady state growth. For cell counts, cells were fixed in a 2% glutaraldehyde solution then counted on a hemocytometer. Growth rates were calculated as:

$$\mu = \ln(N_2/N_1) / (t_2 - t_1),$$

where N is the cell number (cells/mL) and t is the time in days.

In vivo chl *a* fluorescence was measured using an AquaFluor handheld fluorometer (Turner Designs, Sunnyvale, CA, USA). Chl *a* was extracted overnight at -20 °C in 90% acetone and measured using a 10AU field fluorometer (Turner Designs, Sunnyvale, CA, USA).

2.3. Fast Repetition Rate Fluorometry

Maximum quantum yield of PSII fluorescence (F_v/F_m) was measured from a 1 ml sub-sample of culture using a Fast Repetition Rate fluorometer (FRRf, FastOcean FRR plus FastAct Laboratory System, Chelsea Technologies Group Ltd, United Kingdom). Samples were low-light acclimated for 20 min then dark acclimated for 2 min prior to measuring F_v/F_m as previously optimized for photosynthetic measurements of *H. akashiwo* (Hennige et al., 2013). For determination of electron transport rates, a bank of white LEDs within the FastAct was set to deliver an irradiance of 150, 500, or 1200 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ for a 5 min interval prior to the measurement. The electron transport rate (ETR) through photosystem II (PSII) in $\mu\text{mol e}^{-} \text{m}^{-2} \text{s}^{-1}$ was calculated as:

$$\text{ETR}_{(\text{PSII})} = Fq'/Fm' \times E$$

where Fm' is the maximal fluorescence yield in the light acclimated state, Fq' is the difference between Fm' and the steady state fluorescence measured under actinic light (i.e. F), and E is the incident photosynthetically active radiance (PAR).

2.4. Transesterification and Analysis of Fatty Acids

Fatty acid methyl esters (FAMES) were prepared by acid catalyzed direct transesterification and analyzed by gas chromatography as previously described by Stewart et al. (2015).

2.5. Total Lipid, Carbohydrate, and Protein Quantification

Total lipids, carbohydrates, and proteins were quantified by colorimetric methods as previously described by Stewart et al. (2015).

2.6. Particulate Carbon and Particulate Nitrogen

Particulate carbon and particulate nitrogen were quantified using a particulate autoanalyzer (Costech Elemental Analyzer, Costech Analytical Technologies) and standard techniques (Hutchins, 2002). Briefly, the algal cultures were filtered onto precombusted GF/F Whatman glass-fiber filters, stored at -80 °C then dried in an 80 °C oven prior to analysis. Phenylalanine and ethylenediaminetetraacetic acid were used as standards.

2.7. Estimation of Biodiesel Parameters

The saponification number (SN), iodine number (IN), and cetane number (CN) were estimated using empirical formulas from Lei et al. (2012) as previously described by Stewart et al. (2015).

2.8. Statistical Analysis

The experiment was performed in replicate (N=4) for each light level. Data represents means $\pm$ standard deviations. Statistical analysis was performed using JMP Pro v12 software (SAS, Cary, NC, USA). Prior to comparison of means, data were assessed for normality and equality of variance. Raw data that did not meet assumptions of equal variance (by Levene's test) and/or normality (by the Shapiro-Wilk W test) were transformed prior to statistical analysis. A p-value < 0.05 was used as the standard for statistical significance. One-Way Analysis of Variance (ANOVA) was used to determine statistical significance among the light levels followed by Tukey's HSD *post hoc* analysis. For non-parametric analyses, the Kruskal-Wallis Rank Sums test was applied followed by Steel-Dwass *post hoc* analysis.

3. Results and Discussion

3.1. Growth Rates and Photosynthesis

In this study, *H. akashiwo* was cultured with a model flue gas under light intensities ranging from 160 to 1200 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$. Previous studies on algae have shown that irradiance above the level of light saturation will cause a decrease in growth rate due to photoinhibition (Rawat et al., 2013). In contrast, there were no significant differences in growth rates or biomass among the light intensities used in this study (Table 1), showing that *H. akashiwo* (CCMP 2393) is not photoinhibited at intensities equal to or below 1200 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$. For each treatment, the maximum electron transport rate through photosystem II ($\text{ETR}_{(\text{PSII})}$) increased with exposure to increasing PAR settings in *H. akashiwo* (Fig. 1), similar to the response of another closely related raphidophyte, *Chattonella subsalsa* (Warner and Madden, 2007). Notably, $\text{ETR}_{(\text{PSII})}$

values for low-light acclimated cultures were significantly higher than or equal to those for cultures acclimated at medium- and high-light levels when exposed to any of the three PAR settings ($P < 0.02$). For a given PAR intensity (150, 500, or 1200 $\mu\text{moles quanta m}^{-2} \text{s}^{-1}$), there were no significant differences between $\text{ETR}_{(\text{PSII})}$ for the medium- and high-light acclimated cultures. Within treatments, $\text{ETR}_{(\text{PSII})}$ peaked when exposed to 500 $\mu\text{moles quanta m}^{-2} \text{s}^{-1}$ for both the low-light and high-light acclimated cultures ($P < 0.01$), while $\text{ETR}_{(\text{PSII})}$ was highest when exposed to 1200 $\mu\text{moles quanta m}^{-2} \text{s}^{-1}$ for the medium light acclimated culture ($P < 0.001$). These results corroborate previous work, which showed that this strain of *H. akashiwo* rapidly acclimates to shifts in light intensity by utilizing novel photoprotection strategies that enable the alga to exploit high-light regimes in order to rapidly increase growth rates (Hennige et al., 2013). Since light saturation was not observed for the light levels used in the current study, further studies on the photobiology of *H. akashiwo* at very high light intensities ($>1200 \mu\text{moles quanta m}^{-2} \text{s}^{-1}$) would be valuable.

Stable growth rates and rapid ETR responses across light levels are key attributes for successful large-scale cultivation of microalgae in an outdoor commercial setting, where light intensity fluctuates with the weather. Open raceway ponds are commonly used during the mass cultivation of microalgae for biodiesel; however, light limitation due to shading in dense cultures contributes to the low productivities typically seen in open ponds (Rawat et al., 2013). Presumably, the ability of *H. akashiwo* to rapidly exploit fluctuating light intensities contributes to the formation of high-density algal blooms in the natural environment (Hennige et al., 2013). Our study indicates that *H. akashiwo* could be equally productive at the pond surface where irradiance is very high, as well as

below the surface where irradiance is attenuated, and would also be able to exploit the rapidly changing light levels that are characteristic of turbulent mixing conditions.

3.2. Resource Partitioning

The biochemical composition (protein, lipid, and carbohydrate) of *H. akashiwo* varied with light intensity (Fig. 2). Carbohydrate content (pg/cell) was the predominant biochemical constituent for all light treatments, which is not surprising as β -1, 3-glucans are the main storage products of photosynthesis in *H. akashiwo* (Takahashi et al., 2013). Compared to *H. akashiwo* grown in low light, the cultures grown in high light accumulated 2.3-fold more carbohydrates ($P < 0.05$) and 17% fewer lipids ($P < 0.01$; Fig. 2). The decrease in lipids observed here is in contrast to the general trend for microalgae, where lipid content increases with light intensity. Our data reinforces the hypothesis that carbohydrates are the preferred energy storage molecules in *H. akashiwo* when neither light nor CO₂ are limiting. Carbohydrate accumulation is a more efficient energy storage strategy because less ATP and NAD(P)H per carbon are required for carbohydrate synthesis versus TAG synthesis (reviewed in Subramanian et al., 2013). Although TAGs have a higher energy density than storage carbohydrates, it is not enough to compensate for the energy invested in TAG synthesis (Subramanian et al., 2013).

The particulate carbon to particulate nitrogen ratio (C/N) in the high-light treatment was significantly higher than in the low-light treatment (6.33 ± 0.2 and 5.48 ± 0.06 , respectively; $P < 0.01$; Table 1). Neither carbon nor nitrogen content (pg/cell) varied significantly between low- and high-light treatments, but *H. akashiwo* grown in low-light produced 2.8-fold more chl *a* ($P < 0.03$; Fig. 2) and the accumulation of carbohydrates in *H. akashiwo* grown under high light coincided with a trend in

decreasing protein content. These results suggest that *H. akashiwo* may be partitioning nitrogen into nitrogen-containing carbohydrates rather than protein at higher irradiances (e.g. Chizhov et al., 1998). Additionally, Hennige et al. (2013) noted that one of *H. akashiwo*'s strategies for photoprotection in response to high-light might occur through a shift in metabolic demands, where the cell maintains a high carbohydrate to protein ratio and high respiration rate to compensate for low non-photochemical quenching (NPQ). Therefore, the extreme accumulation of carbohydrates in response to increasing light seen here may be a contributing factor to the success of the alga at 1200 $\mu\text{moles quanta m}^{-2} \text{s}^{-1}$ and the apparent lack of light saturation at this level.

3.4. Fatty Acid Analysis and Biodiesel Quality

Fatty acid profiles were determined to assess the effects of light intensity on the biodiesel potential of *H. akashiwo* (Fig. 3). The major fatty acids (>5% total FAMES) in all three treatments were myristic (C14:0), palmitic (C16:0), palmitoleic (C16:1), linolenic (C18:3), stearidonic (C18:4), and eicosapentaenoic (C20:5n3, EPA). Light levels did not affect the percent of SAFAs, but MUFAs increased by 6% ($P < 0.03$) and PUFAs decreased by 12% ($P < 0.05$) from low-light to high-light (Table 2). At the individual fatty acid level, a significant increase in the synthesis of C14:0 (5.1%), C16:0 (5.5%) and C16:1 (4.3%) and a concurrent significant decrease in C18:4 (7.0%) and C20:5n3 (3.9%) was observed with increasing light levels ($P < 0.05$). These results agree with other studies that have shown that PUFAs are reduced under high-light conditions, since they are the major structural lipids of thylakoid membranes, while SAFAs and MUFAs are stored as triacylglycerol in cytosolic lipid bodies (Richmond and Hu, 2013). Additionally, the significant increase in C14:0 with increasing light can be explained by

its requirement for the synthesis of C16:0, since C14:0 is linked to the fatty acid synthase enzyme prior to the two carbon elongation cycle that produces C16:0. Here, it appears that the significant changes in individual fatty acid content are driven by the photosynthetic requirements of cultures growing under different light regimes.

In microalgae, only fatty acids that form triacylglycerides are easily converted into biodiesel, and the fatty acid composition of these neutral lipids determines biodiesel quality. Therefore, FAME profiles and empirical formulas may be used to estimate a cetane number (CN), which is the primary indicator of diesel quality that measures the ignition delay when diesel is injected into a combustion chamber (Lei et al., 2012). A higher CN indicates a shorter ignition time. The American Society for Testing Materials' minimum CN requirement is 40 for blended (6% to 20%) biodiesel and 47 for 100% biodiesel (ASTM, 2014). In the present study, light levels did not significantly affect CN, and the CNs for FAMEs derived from medium- and high-light cultures were appropriate for blended biodiesel stocks (Table 3). Since CN increases with increasing fatty acid chain length and decreases with increasing degree of unsaturation, EPA (C20:5n3), which was the third most abundant fatty acid in all three treatments, decreased the estimated CN for *H. akashiwo*-derived FAMEs. EPA is an economically valuable omega-3 fatty acid, and it is commercially feasible to recover about 70% of the EPA fraction (Molina Grima et al., 2003). Based on this assumption, removal of EPA would increase the CN value to between 48-53 for all treatments, which is above the US standard for 100% biodiesel (Table 3). These estimated values are on par with the CN of soybean oil (51), currently used for biodiesel in the United States (Hu et al. 2008).

4. Conclusions

Overall, the fatty acid profiles reported in this study suggest that *H. akashiwo* is a good candidate for the production of biodiesel and EPA as a valuable co-product. Additionally, the quality of biodiesel acquired from *H. akashiwo* grown under fluctuating light conditions would be relatively stable. Cultures were not photoinhibited, nor did they reach light saturation at 1200 $\mu\text{moles quanta m}^{-2} \text{ s}^{-1}$, indicating that *H. akashiwo* would perform well in outdoor conditions. Our results also reinforce that carbohydrates are the preferred energy storage molecules in *H. akashiwo*, which may be a good source of fermentable carbon for bioethanol production.

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270 **References**

- 271 1. American Society for Testing Materials International, 2014. Standard specification for
272 biodiesel fuel blend stock (B100) for middle distillate fuels. Standard Designation
273 D6751-14.
- 274 2. Benemann, J., 2013. Microalgae for biofuels and animal feeds. *Energies* 6, 5869–5886.
- 275 3. Chizhov, A.O., Dell, A., Morris, H.R., Reason, A.J., Haslam, S.M., Mcdowell, R.A.,
276 Chizhov, O.S., Usov, A.I., 1998. Structural analysis of laminarans by MALDI and
277 FAB mass spectrometry. *Carbohydr. Res.* 310, 203–210.
- 278 4. Davis, R.E., Fishman, D.B., Frank, E.D., Johnson, M.C., Jones, S.B., Kinchin, C.M.,
279 Skaggs, R.L., Venteris, E.R., Wigmosta, M.S., 2014. Integrated evaluation of
280 cost, emissions, and resource potential for algal biofuels at the national scale.
281 *Environ. Sci. Technol.* 48, 6035–6042.
- 282 5. Davis, R., Fishman, D., Frank, E.D., Wigmosta, M.S., Aden, A., Coleman, A.M.,
283 Pienkos, P.T., Skaggs, R.J., Venteris, E.R., Wang, M.Q., June 2012. Renewable
284 diesel from algal lipids: An integrated baseline for cost, emissions, and resource
285 potential from a harmonized model. ANL/ESD/12-4; NREL/TP-5100-55431;
286 PNNL-21437. Argonne, IL: Argonne National Laboratory; Golden, CO: National
287 Renewable Energy Laboratory; Richland, WA: Pacific Northwest National
288 Laboratory.
- 289 6. Douskova, I., Doucha, J., Livansky, K., Machat, J., Novak, P., Umysova, D.,
290 Zachleder, V., Vitova, M., 2009. Simultaneous flue gas bioremediation and

291 reduction of microalgal biomass production costs. Appl. Microbiol. Biotechnol.
 292 82, 179–185.

293 7. Environmental Protection Agency, 2014. Air emission sources: Nitrogen Oxides
 294 <http://www.epa.gov/air/emissions/nox.htm> Retrieved 9/16/2010.

295 8. Environmental Protection Agency, 2015. 40 CFR part 60 Carbon pollution emission
 296 guidelines for existing stationary sources: Electric utility generating units; final
 297 rule. Federal Register 80(205), 64884.

298 9. Fuentes-Grünwald, C., Garcés, E., Alacid, E., Rossi, S., Camp, J., 2013. Biomass and
 299 lipid production of dinoflagellates and raphidophytes in indoor and outdoor
 300 photobioreactors. Mar. Biotechnol. 15, 37–47.

301 10. Fuentes-Grünwald, C., Garcés, E., Alacid, E., Sampedro, N., Rossi, S., Camp, J.,
 302 2012. Improvement of lipid production in the marine strains *Alexandrium*
 303 *minutum* and *Heterosigma akashiwo* by utilizing abiotic parameters. J. Ind.
 304 Microbiol. Biot. 39, 207–216.

305 11. Fuentes-Grünwald, C., Garcés, E., Rossi, S., Camp, J., 2009. Use of the
 306 dinoflagellate *Karlodinium veneficum* as a sustainable source of biodiesel
 307 production. J. Ind. Microbiol. Biot. 36, 1215–1224.

308 12. Guillard, R.R.L., 1975. Culture of phytoplankton for feeding marine invertebrates. In:
 309 *Culture of Marine Invertebrate Animals* (Smith, W.L., Chanley, M.H., eds), pp
 310 26–60. New York: Plenum Press.

311 13. Handy, S.M., Coyne, K.J., Portune, K.J., Demir, E., Doblin, M.A., Hare, C.E., Cary,
 312 S.C., Hutchins, D.A., 2005. Evaluating vertical migration behavior of harmful

313 raphidophytes in the delaware inland bays utilizing quantitative real-time PCR.
 314 Aquat. Microb. Ecol. 40, 121–132.

315 14. Hennige, S.J., Coyne, K.J., Macintyre, H., Liefer, J., Warner, M.E., 2013. The
 316 photobiology of *Heterosigma akashiwo*. Photoacclimation, diurnal periodicity,
 317 and its ability to rapidly exploit exposure to high light. J. Phycol. 49, 349–360.

318 15. Hu, Q., Sommerfeld, M., Jarvis, E., Ghirardi, M., Posewitz, M., Seibert, M., Darzins,
 319 A., 2008. Microalgal triacylglycerols as feedstocks for biofuel production:
 320 Perspectives and advances. Plant J. 54, 621–639.

321 16. Hutchins, D.A., Hare, C. E., Weaver, R. S., Zhang, Y., Firme, G. F., DiTullio, Alm,
 322 M.B., Riseman, S.F., Maucher, J.M., Geesey, M.R., Trick, C.G., Smith, G.J., Rue,
 323 E.L., Conn, R.J., Bruland, K.W., 2002. Phytoplankton iron limitation in the
 324 Humboldt current and Peru upwelling. Limnol. Oceanogr. 47, 997–1101.

325 17. Lei, A., Chen, H., Shen, G., Hu, Z., Chen, L., Wang, J., 2012. Expression of fatty acid
 326 synthesis genes and fatty acid accumulation in *Haematococcus pluvialis* under
 327 different stressors. Biotechnol. Biofuels 5, 1–11.

328 18. Li, Y., Han, D., Hu, G., Sommerfeld, M., Hu, Q., 2010. Inhibition of starch synthesis
 329 results in overproduction of lipids in *Chlamydomonas reinhardtii*. Biotechnol.
 330 Bioeng. 107, 258–268.

331 19. Molina Grima, E., Belarbi, E.-H., Acién Fernández, F.G., Robles Medina, A., Chisti,
 332 Y., 2003. Recovery of microalgal biomass and metabolites: Process options and
 333 economics. Biotechnol. Adv. 20, 491–515.

- 334 20. Nagarajan, S., Chou, S.K., Cao, S., Wu, C., Zhou, Z., 2013. An updated
335 comprehensive techno-economic analysis of algae biodiesel. *Bioresour. Technol.*
336 145, 150–156.
- 337 21. Rawat, I., Ranjith, K., R., Mutanda, T., Bux, F., 2013. Biodiesel from microalgae: A
338 critical evaluation from laboratory to large scale production. *Appl. Energy* 103,
339 444–467.
- 340 22. Richmond, A., Hu, Q. 2013 *Handbook of microalgal culture: Applied phycology and*
341 *biotechnology*, Second edition. Wiley-Blackwell: New York.
- 342 23. Solovchenko, A., Khozin-Goldberg, I., 2013. High-CO₂ tolerance in microalgae:
343 Possible mechanisms and implications for biotechnology and bioremediation.
344 *Biotechnol. Lett.* 35, 1745–1752.
- 345 24. Stewart, J.J., Bianco, C.M., Miller, K.R., Coyne, K.J., 2015. The marine microalga,
346 *Heterosigma akashiwo*, converts industrial waste gases into valuable biomass.
347 *Front. Energy Res.* 3, doi:10.3389/fenrg.2015.00012.
- 348 25. Stewart, J.J., Coyne, K.J., 2011. Analysis of raphidophyte assimilatory nitrate
349 reductase reveals unique domain architecture incorporating a 2/2 hemoglobin.
350 *Plant. Mol. Biol.* 77, 565–575.
- 351 26. Subramanian, S., Barry, A.N., Pieris, S., Sayre, R.T., 2013. Comparative energetics
352 and kinetics of autotrophic lipid and starch metabolism in chlorophytic
353 microalgae: Implications for biomass and biofuel production. *Biotechnol.*
354 *Biofuels* 6, 150.

- 355 27. Takahashi, H., Clowez, S., Wollman, F.A., Vallon, O., Rappaport, F., 2013. Cyclic
356 electron flow is redox-controlled but independent of state transition. Nat.
357 Commun. 4, 1954.
- 358 28. Tobin, E.D., Grunbaum, D., Patterson, J., Cattolico, R.A., 2013. Behavioral and
359 physiological changes during benthic-pelagic transition in the harmful alga,
360 *Heterosigma akashiwo*: Potential for rapid bloom formation. PLoS One 8,
361 e76663.
- 362 29. Warner, M.E., Madden, M.L., 2007. The impact of shifts to elevated irradiance on the
363 growth and photochemical activity of the harmful algae *Chattonella subsalsa* and
364 *Prorocentrum minimum* from Delaware. Harmful Algae 6, 332–342.
- 365 30. Zhang, Y., Fu, F.-X., Whereat, E., Coyne, K.J., Hutchins, D.A., 2006. Bottom-up
366 controls on a mixed-species hab assemblage: A comparison of sympatric
367 *Chattonella subsalsa* and *Heterosigma akashiwo* (Raphidophyceae) isolates from
368 the Delaware inland bays, USA. Harmful Algae 5, 310–320.
- 369

Figure Captions

Fig. 1. Electron transport rates for cultures of *H. akashiwo* previously acclimated to 160, 560, or 1200 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ after dark acclimation and a subsequent 5 min exposure to 150, 500 or 1200 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$.

Fig. 2. Carbohydrate, lipid, protein, and chl *a* contents of *H. akashiwo* acclimated to 160, 560, or 1200 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$.

Fig. 3. Fatty acid profiles of *H. akashiwo* acclimated to 160, 560, or 1200 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$.

Tables and Figures

Table 1

Growth rates, biomass, and cellular carbon and nitrogen contents in *H. akashiwo* acclimated to 160, 560, or 1200 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$.

	160	560	1200
Growth Rate (μ)	0.20 ± 0.04	0.22 ± 0.04	0.18 ± 0.02
Dry Weight (g/L)	0.27 ± 0.12	0.20 ± 0.11	0.26 ± 0.08
Carbon (pg/cell)	819 ± 40 (a)	500 ± 60 (b)	952 ± 270 (a)
Nitrogen (pg/cell)	174 ± 9 (a)	98 ± 18 (b)	176 ± 55 (a)
C/N (mol/mol)	5.48 ± 0.06 (a)	5.96 ± 0.6 (ab)	6.33 ± 0.2 (b)

*Values within rows followed by different letters are significantly different ($P < 0.05$, Tukey's HSD test)

Table 2

Fatty acid saturation type as a percentage of total fatty acid methyl esters in *H. akashiwo* acclimated to 160, 560, or 1200 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$.

	160	560	1200
Saturated	41 ± 5	46 ± 6	47 ± 1
Monounsaturated	15 ± 3 (a)	17 ± 1 (ab)	21 ± 3 (b)
Polyunsaturated	44 ± 5 (a)	37 ± 7 (ab)	32 ± 4 (b)

*Values within rows followed by different letters are significantly different ($P < 0.05$, Tukey's HSD test)

Table 3

Calculated parameters for potential biodiesel quality of FAMES derived from *H. akashiwo* acclimated to 160, 560, or 1200 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$.

	160	560	1200
Iodine Number	172 $\pm$ 18	148 $\pm$ 24	131 $\pm$ 11
Saponification Number	204 $\pm$ 2 (a)	207 $\pm$ 3 (ab)	210 $\pm$ 1 (b)
Cetane Number	34 $\pm$ 4	39 $\pm$ 5	42 $\pm$ 2
Cetane Number without 20:5n3*	48 $\pm$ 2	52 $\pm$ 3	53 $\pm$ 2

*Assuming 70% recovery efficiency of 20:5n3

**Values within rows followed by different letters are significantly different ($P < 0.05$, Tukey's HSD test)

Figure 1

Figure 2

Figure 3