Strategies of Resource Allocation by Diatoms under Dynamic Light

Nerissa Lynn Fisher

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School of Life Sciences
University of Technology Sydney

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Certificate of original authorship

I, Nerissa Lynn Fisher, declare that this thesis is submitted in fulfillment of the requirements for the award of Doctorate of Philosophy in the Faculty of Science at the University of Technology Sydney.

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Nerissa Lynn Fisher

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Table of Contents

List of Figures

Chapter 1

Figure 1.3. Energy budgets from nutrient limited cultures of *Dunaliella tertiolecta* and *Thalassiosira weissflogii* (left and middle plots) and light-limited cultures of *Thalassiosira pseudonana* (right plot) at a range of specific growth rates using carbon- and oxygen-based measurements. Components measured to build the energy budgets included gross primary production (GP_{O2} or GPP_{O2}), gross carbon production (GP_C), net primary production (NP_{O2} or $NP_{O2/C}$) and net carbon production (NP_C or NPP_C). Sub-divisions of energy allocation include light dependent respiration (LDR), nitrogen and sulfur (N+S) reduction, mitochondrial respiration, reductants from carbon catabolism which is equivalent to biosynthesis of macromolecules and net carbon production which is equivalent to biomass. Figures adapted from Halsey et al. 2013 (left and middle plots) and Fisher & Halsey 2016 (right plot).

Figure 1.4. Photosynthesis-irradiance (PE) curves showing two fundamental descriptors of photosynthetic status: E_k-dependent variation (A) and E_k-independent variation (B) when photosynthesis is normalised to chlorophyll (subscript b). The PE curve is characterised by a light-saturated maximum rate of photosynthesis (P_{max}), light-limiting slope (α) and light saturation index (E_k).

Figure 1.5. Phases of FRRf method measuring fluorescence transients. Roman numerals represent the different phases and the times found above correlate to the duration of each phase. Single turnover (ST) flashes (Phases I and V) encompass a series of high frequency (0.5-2 μs intervals) flashlets (80-120). Multiple turnover (MT) flashes (Phase III) is similar to ST with more flashlets (~4000) at lower frequency (20-

200 μs intervals). Following each turnover phase (II, IV, and VI), there is a relaxation protocol of 40-80 flashlets at intervals exponentially varying (50 μs–50 ms). Each phase provides information on various fluorescence transients: I and V give variable fluorescence (F_v) from initial (F_o) and maximal (F_m) fluorescence, functional absorption cross-section (σ_{PSII}), and energy transfer between PSII reaction centres, RCII; II and VI give the kinetics of Q_A re-oxidation; IV give kinetics of PQ pool re-oxidation; III gives fluorescence yield under MT conditions thereby allowing the effects of earlier MT excitations on photosynthetic parameters to be quantified (V and VI).

Chapter 2

Figure 2.1. Initial photophysiological monitoring of seven diatoms: *Phaeodactylum tricornutum* (orange inverted triangles), *Chaetoceros muelleri* (yellow circles), *Ditylum brightwellii* (green diamonds), *Thalassiosira rotula* (black Xs), *Thalassiosira pseudonana* (black squares), *Thalassiosira weissflogii* (blue triangles), and *Thalassisosira oceanica* (red circles). (A) Yield of non-photochemical quenching (YNPQ; see Eq 3) with increasing light intensity. (B) Dynamic non-photochemical quenching [1-Q] versus photochemical [1-C], where data points signify responses to stepped increases in light intensity starting from 0 (far right point) to 1304 μmol photons m^{-2} s^{-1} (far left point) for 4 min at each light intensity. Error bars represent the standard error of the mean of at least *n*=3 for independent biological replicates.

Figure 2.2. YNPQ vs. de-epoxidation state (DPS) of the XC for *T. weissflogii* (blue triangles), *T. oceanica* (red circles), and *T. pseudonana* (black squares) under 10 min exposure to growth irradiance (Ig, 85 μmol photons m^{-2} s^{-1}, solid symbols) and high

light (HL, 1200 µmol photons m^{-2} s^{-1}, open symbols). Dashed lines highlight the extent of changes observed between light treatments for each measured parameter. Data averaged from three independent replicates for DPS and at least four independent replicates for YNPQ with errors bars representing SE of the mean.

Figure 2.3. Representative photo-inhibition and recovery time courses for *T. oceanica* (To), *T. pseudonana* (Tp) and *T. weissflogii* (Tw). Black points show individual determinations of F_v'/F_m' or F_v/F_m from FRRf measurements of cultures with PSII repair active (absence of lincomycin). Dashed orange line connects F_v'/F_m' measures taken immediately after exposure to HL (1200 µmol photons m^{-2} s^{-1}) over 0 – 120 min or recovery light (15 µmol photons m^{-2} s^{-1}) at 150 and 180 min, influenced by combined effects of non-photochemical quenching induction and net photo-inactivation (if any). Solid green line connects F_v/F_m taken after 10 min of subsequent dark for HL time points, to allow relaxation of non-photochemical quenching, or taken immediately during the recovery light period. These points were used to fit the Kok model of PSII photo-inactivation countered by repair. Note the different patterns and amplitudes of short-term (10 min) relaxation of non-photochemical quenching, among the species (black dots). The dotted red line shows F_v/F_m data from separate lincomycin treated cultures to show the underlying photo-inactivation in the absence of counteracting repair.

Figure 2.4. Mean YNPQ and YNPQ relaxation amplitude for *T. weissflogii*, *T. oceanica* and *T. pseudonana* after 120 min high light exposure. (A) Mean values of YNPQ vs. the rate constant for PSII repair, k_{REC}, over 120 min HL exposure showing

nonphotochemical quenching measured immediately after light exposure (larger symbols) or after 10 min dark FRRf incubation (smaller symbols) for *T. weissflogii* (Tw, blue triangles), *T. oceanica* (To, red diamonds) and *T. pseudonana* (Tp, black squares). (B) The amplitude of YNPQ relaxation (also green arrows in (A)). Error bars show standard errors of the estimates for 3 or 4 independent biological replicates.

Figure 2.5. Proportions of total photochemical energy (GP_{O2}) allocated to various oxygen pathways over a 20 min incubation under growth irradiance (Ig) and high light (HL). Fractional percentages of GP_{O2} included net oxygen production (Net_{O2}, grey), dark respiration (R_{DARK}, black) and light dependent respiration (LDR, white) in *T. weissflogii*, *T. oceanica*, and *T. pseudonana* under Ig (85 µmol photons m^{-2} s^{-1}) and HL (1200 µmol photons m^{-2} s^{-1}). Data averaged from 2 or 3 independent biological replicates with error bars representing SE of the mean.

Figure 2.6. The yield of non-photochemical quenching (YNPQ) versus light dependent respiration (LDR) as a % of GP_{O2} for *T. weissflogii* (blue triangles), *T. oceanica* (red circles), and *T. pseudonana* (black squares) under 20 min exposure to growth irradiance (Ig, 85 µmol photons m^{-2} s^{-1}, solid data points) and high light (HL, 1200 µmol photons m^{-2} s^{-1}, open data points). Data averaged from 2 or 3 independent replicates for LDR and at least 4 independent replicates for YNPQ. Error bars represent SE of the mean.

Figure 2.7. Energy flux yields including YNPQ (dark blue), YNO (light blue) and YII, which was then further divided into fractions of LDR (white), R_{DARK} (balck) and Net_{O2} (grey), of *T. pseudonana*, *T. oceanica* and *T. weissflogii* under 85 µmol photons m^{-2} s^{-1}

(Ig) and 1200 μmol photons m^{-2} s^{-1} (HL). Data averaged from 3 independent biological replicates and error bars represent SE of the mean.

Figure 2.8. Summary of relative reliance (low to high; light grey to black) on various energy dissipation strategies when subject to transient HL including (i) de-epoxidation state (DPS) of xanthophyll cycle pigments, (ii) induction/relaxation of nonphotochemical quenching (parameterised as YNPQ), (iii) inactivation/repair of PSII and (iv) O_2 consuming pathways (LDR/R_{DARK}) for the three *Thalassiosira* diatom species examined here.

Chapter 3

Figure 3.1. Changes in $E_{k,YII}$ for *T. oceanica* (circles) and *T. weissflogii* (triangles) acclimated to a 12:12 L:D cycle under sinusoidal light with max irradiance of 400 μmol photons m^{-2} s^{-1} (grey solid line). $E_{k,YII}$ was calculated using FRRf-derived YII values collected from a FLC. The spectrally corrected light intensities for FRRf-incubated samples are shown for *T. oceanica* (dotted line) and *T. weissflogii* (dashed line). Sampling occurred along the photoperiod at 1, 3, 6, 9 and 11 hours after the onset of illumination. Error bars represent SE of the mean for at least 3 independent biological replicates.

Figure 3.2. Total respiration ($R_{TOTAL,}$ pmol O_2 [pg Chl *a* h]$^{-1}$) for *T. oceanica* and *T. weissflogii* acclimated to a sinusoidal light regime (maximum 400 μmol photons m^{-2} s^{-1}) at sample time points along the photoperiod. The light incubations representing

variations in LDR ($Sine_{\mu}$ – dotted white, and $Sine_{HL}$ – solid white) were plotted separately and both included the dark respiration (R_{DARK} – black) for each corresponding timepoint. Error bars represent SE of the mean for at least three independent replicates. Note that the R_{DARK} value at each timepoint does not change between light incubation treatments but LDR values do reflect the response to light intensity shifts between treatments ($Sine_{\mu}$ and $Sine_{HL}$) and the y-axis scale is different between species.

Figure 3.3. Respiratory components of gross oxygen production (GP_{O2}): (A,D) total respiration (R_{TOTAL}) as a percentage of gross O_2 production. GP_{O2} separated into the fraction of net oxygen production (Net_{O2}, grey), dark respiration (R_{DARK}, black) and light dependent respiration (LDR, white) under $Sine_{\mu}$ (B,D) and $Sine_{HL}$ (C,F) for *T. oceanica* (A-C) and *T. weissflogii* (D-F) sampled over the photoperiod. Error bars represent SE of the mean for at least three independent replicates.

Figure 3.4. Correlations between YII (dimensionless) and GP_{O2} (pmol [pg Chl *a* h]$^{-1}$) for *T. oceanica* (circles, solid lines) and *T. weissflogii* (inverted triangles, dashed lines) for incubations at $Sine_{\mu}$ and $Sine_{HL}$ over the integrated photoperiod. Lines of best fit were generated using a simple linear regression model where the shaded areas represent the 95% confidence intervals for significant correlations ($p < 0.05$) only. Model parameters are displayed in S3.2 Table.

Figure 3.5. Energy flux yields for the sum of YNPQ (teal), YNO (purple) and YII, which was then further divided using GP_{O2} fractions of LDR (white), R_{DARK} (black) and

Net_{O2} (grey) for *T. oceanica* and *T. weissflogii* exposed to $Sine_{\mu}$ and $Sine_{HL}$. Data averaged from at least 3 independent replicates with error bars representing SE of the mean.

Figure 3.6. Correlations between fluorescence- and oxygen-derived parameters for *T. oceanica* (circles) and *T. weissflogii* (inverted triangles) at growth irradiance ($Sine_{\mu}$) and 3x growth irradiance ($Sine_{HL}$) collated over a 12-h photoperiod. Correlations included R_{TOTAL} to YII (Panel A), YNPQ (Panel B) and YNO (Panel C). Data points represent three individual replicate measures for each sample timepoint (1, 3, 6, 9, 11 h). Regression lines were included for both *T. oceanica* (solid line) and *T. weissflogii* (dashed line) and 95% CI (grey shaded area) displayed only for significant correlations ($p < 0.05$). Model parameters are displayed in S3.2 Table.

Figure 3.7. Correlations between $E_{k,YII}$ and (A) R_{TOTAL}, (B) Net_{O2}, and (C,D) carbon (pg cell-1) for *T. oceanica* (circles) and *T. weissflogii* (inverted triangles). Regression lines with 95% CI (grey shaded area) are displayed only for significant correlations ($p < 0.05$). Model parameters are displayed in S3.2 Table.

Chapter 4

Figure 4.1. (A) PCA of relative metabolite abundances for constant light treatments (24:0 L:D) at high (yellow circles), medium (green circles) and low (blue circles) intensities. (B) PLS-DA of relative metabolite abundances for high and low constant (high and low) and pulse (high – orange triangles, low – purple triangles) light treatments. Light intensities for high, medium and low are 200, 60 and 5 μmol photons

m^{-2} s^{-1}, respectively. Data for relative metabolite abundances was normalised by internal standard then 'metabolite extract biomass'. Explained variances are shown as a percentage in brackets and shaded area is the 95% confidence region. Individual data points represent independent biological replicates (n=4).

Figure 4.2. Significant metabolites (green circles) identified by SAM between constant light treatments (high - HC, medium - MC, low - LC). Significant metabolites determined from an ANOVA are distinguished in bold followed by post-hoc correlations in italics for a significance level of $p < 0.05$.

Figure 4.3. Significant metabolites (green circles) identified by SAM between high and low constant and pulse light treatments (HC – high constant, HP – high pulse, LC – low constant, LP – low pulse). Significant metabolites are distinguished in bold followed by post-hoc analysis generated from an ANOVA ($p < 0.05$).

Figure 4.4. Summary metabolic pathway schematic of metabolites identified using GC-MS with overlaying plots of relative metabolite concentrations for high constant (HC, yellow bars), low constant (LC, blue bars), high pulse (HP, orange bars) and low pulse (LP, purple bars) light treatments.

Figure 4.5. Schematic of the general metabolic changes for significant metabolites in *T. pseudonana* grown under high vs low light intensity for both constant and pulse light dose treatments. Blue indicates upregulation of relative metabolite concentrations for corresponding processes under lower light acclimation and red correlates to processes

with associated significant metabolites that were upregulated under high light acclimation. Black indicates intermediary steps/processes that were not significantly up or down regulated according to the metabolites identified across all light treatments.

Chapter 5

Figure 5.1. Schematic of diatom responses to high light from species found in open ocean (*T. oceanica*) and estuary (*T. weissflogii*) in which coastal diatoms exhibit an intermediate response. The main flow of energy involves light energy harvested at photosystem II (PSII) where excitons are passed along the linear electron transport chain to PSI where NADPH is generated to fuel, in addition to ATP, the Calvin-Benson-Bassham (CBB) cycle. Products generated from the CBB cycle enter the cytoplasm (yellow box) to glycolysis which feeds into the tricarboxylic acid cycle (TCA) cycle in the mitochondria (red box) to build macromolecules to support growth. Alternatively, gluconeogenesis diverts energy away from TCA cycle to build carbon reserves. Sources of energy dissipation from high light before reaching PSII are the yield of constitutive losses via fluorescence and heat (YNO) and the yield of regulated thermal dissipation via nonphotochemical quenching (YNPQ) Once photolysis occurs at the PSII reaction centre, electrons can enter processes of light-dependent respiration (LDR) via oxidase activity within the chloroplast (green box) which can be a way to dissipate excess electrons or generate additional ATP. Additionally, mitochondrial respiration can supplement ATP demands via the catabolism of carbon molecules to supply to the CBB cycle or assimilation of nutrients (i.e. nitrogen, N). Nitrogen is particularly essential to build pigments and proteins that are fundamental components of the nonphotochemical quenching mechanism. The arrow thickness correlates to the

upregulation of pathways by these diatoms under high light as observed in Chapters 2 and 3.

Figure 5.2. Development of energy budget models using cellular currencies – carbon (green background), oxygen (blue background) and fluorescence (yellow background) – from (A) historical, (B) current to (C) proposed future models. Historical energy budget models typically include two cellular currencies and a separate biofractionation of macromolecules. Current energy budgets account for the three cellular currencies but does not include that deeper carbon insight gained from metabolomics. Future energy budgets models could integrate all cellular currencies including the information gained from further partitioning of carbon molecules (e.g. metabolites). Such comprehensive energy budgets will provide more accurate accounting of energy that ultimately is retained in biomass under various environmental stressors. Data adapted from (A) Fisher & Halsey (2016) and (B) Chapters 2 and 3 where sub-fractionations within cellular currencies are measurements that were collected from diatoms in response to light.

List of Tables

Chapter 2

low light recovery (15 μmol photons m^{-2} s^{-1}) (Fig 2.3). Values derived from curve fits (Kok 1956; Campbell & Serôdio 2020) of data from 3 independent replicates. Values in parentheses represent SE of the mean.

Chapter 3

Table 3.1. Exposure light intensities during MIMS analysis for each sampling time point. Incubation treatments either mimicked the growth light intensity ($Sine_{\mu}$,) or were 3.0-fold higher ($Sine_{HL}$). Spectral correction factors (SCF) were applied to account for the light source differences between MIMS (white LED) and FRRf (blue LED) incubations for *T. oceanica* and *T. weissflogii*.

Table 3.2. Cell culture and properties of *Thalassiosira oceanica* and *Thalassiosira weissflogii* during steady-state growth acclimated to a sinusoidal ($Sine_{\mu}$) light regime with a maximum irradiance of 400 μmol photons m^{-2} s^{-1} over a 12:12 L:D photoperiod at 20°C. Values in parentheses are standard error for at least 3 independent biological replicates at each sample time. A two-way ANOVA test comparing species, time and interactive (Species*Time) effects for each property are represented with significant *p*-values (< 0.05) in bold. The test results from a one-way ANOVA are shown using superscripted letters to identify the Bonferroni post-hoc analysis for significance between sample times (h). Letters indicate means that are statistically indistinguishable ($p < 0.05$; ANOVA).

Chapter 4

Table 4.1. Physiological characteristics of *Thalassiosira pseudonana* measured at the time of metabolomics sampling during steady-state acclimated growth to five different light regimes: Constant (high, medium, low), and Pulse (high, low) at 20^{o}C. Values in parentheses represent SE of the mean for at least 3 independent biological replicates for specific growth rate (d^{-1}), cell density (mL^{-1}), Chl *a* ($cell^{-1}$), carbon (pg $cell^{-1}$), nitrogen (pg $cell^{-1}$), C:N, F_v/F_m and σ_{PSII} (nm^2 $PSII^{-1}$). ANOVA or Kruskal-Wallis (KW) test results are presented using F or H-values, respectively, for constant light treatments followed by Tukey post-hoc analysis with differences between individual cell characteristics and light intensity designated by superscripted letters and Student's *t* – test using t-values for pulse light treatments with significant *p* values ($p < 0.05$) in bold. For 2-way ANOVA test results for light treatments of equal PFD (HC-HP, LC-LP) see S4.4 Table.

Table 4.2. Compilation of SAM-identified significant metabolites for the various comparisons of light treatments – constant only (yellow), pulse only (green) and constant+pulse treatments of equal average PFD (blue).

List of Supplementary Figures

Chapter 2

S2.1 Figure. Photophysiological assessment of seven diatoms. Conventional non-photochemical quenching (NPQ; see Eq. 2) capacities of *Phaeodactylum tricornutum* (orange inverted triangles), *Chaetoceros muelleri* (yellow circles), *Ditylum brightwellii* (green diamonds), *Thalassiosira rotula* (black Xs), *Thalassiosira pseudonana* (black squares), *Thalassiosira weissflogii* (blue triangles), and *Thalassisosira oceanica* (red circles) with increasing light intensity. Error bars represent the standard error of the mean of at least n=3 for independent biological replicates.

Chapter 3

S3.1 Figure. Schematic of experimental design including (1) culture growth light regime, (2) sample preparation of sub-culture aliquots inoculated with labelled oxygen ($^{18}O_2$), (3) light treatments used to measure oxygen consumption and production using membrane inlet mass spectrometry (MIMS) and (4) sampling time points across the photoperiod with corresponding incubation irradiances for MIMS treatment samples ($Sine_{\mu}$ and $Sine_{HL}$).

S3.2 Figure. Photon flux of light sources used for fluorescence (blue LED, black circles) and oxygen (white LED, grey squares) incubations and analyzed via FRRf and MIMS, respectively (left panel). Spectrally corrected fluorescence excitation detected at 730 nm of *T. weissflogii* (red dots) and *T. oceanica* (blue dots) weighting blue LED

light to white LED light over photosynthetically active radiation (PAR, 400-700 nm) wavelengths (right panel).

S3.3 Figure. Oxygen consumption in the dark and light by *T. oceanica* (circles) and *T. weissflogii* (triangles) acclimated to growth under 12 hours of sinusoidal light with a maximum intensity of 400 µmol photons m^{-2} s^{-1}. Dark respiration and LDR were measured over the photoperiod at 1, 3, 6, 9 and 11 hours. LDR measurements were completed at $Sine_{LL}$ and $Sine_{HL}$ light incubations for each timepoint as denoted in the "Light Treatment" panel. Remaining panels ('cell number', 'cell volume', 'carbon' and 'chlorophyll *a*') show oxygen consumption measurements normalised to the corresponding parameter. Error bars for 'cell number', 'carbon' and 'chlorophyll *a*' panel graphs represent SEM while the error associated with 'cell volume' normalisations were calculated using the percentage of uncertainty to get the propagation of error for averages from at least three independent biological replicates.

Chapter 4

S4.1 Figure. PCAs of the relative metabolite abundances for (A) pulse (12:12 L:D) treatments at high (HP, 200 µmol photons m^{-2} s^{-1}) and low (LP, 5 µmol photons m^{-2} s^{-1}) light intensities and (B) for constant and pulse (HC – yellow circles, HP – orange squares, LC – blue circles, LP – purple squares) light treatments. Explained variances are shown as a percentage in brackets and shaded area is the 95% confidence region of individual treatment groups. Individual data points represent independent biological replicates (n=4).

S4.2 Figure. Significant metabolites (green circles) identified by SAM between high and low pulse light treatments (HP – high pulse, LP – low pulse). Significant metabolites are distinguished in bold ($p < 0.05$) and a false detection rate (FDR) < 10%.

S4.3 Figure. Summary of annotated differentially expressed genes for those (A) downregulated in constant low light (LC, 5 µmol photons m^{-2} s^{-1}) relative to constant high light (HC, 200 µmol photons m^{-2} s^{-1}) and (B) upregulated in LC compared to HC. Differential expression was determined using the threshold $|FC| > 2$, $p < 0.05$ from three independent biological replicates.

S4.4 Figure. Differentially expressed genes for *T. pseudonana* grown at constant low light (LC, 5 µmol photons m^{-2} s^{-1}) compared to constant high light (HC, 200 µmol photons m^{-2} s^{-1}) conditions showing upregulated (green) and downregulated (red) genes in LC. Right panel figure shows the interplay between processes occurring in the plastid, peroxisome and mitochondrion and left panel figures shows more detailed interactions between glycolysis, fatty acid and branched chain amino acid (BCAA) degradation and TCA cycle where dashed lines represent the glyoxylate cycle two-step bypass. PEPCK = PEP carboxykinase; PPDK = pyruvate-phosphate dikinase; PK = pyruvate kinase; PC = pyruvate carboxylase; PEPC = PEP carboxylase; Pex1,5,19 = peroxins that import proteins into the peroxisomes.

List of Supplementary Tables

Chapter 2

S2.1 Table. Rates of oxygen production and consumption. MIMS analysis of gross oxygen production (GP_{O2}), light dependent respiration (LDR), dark respiration (R_{DARK}), and net oxygen production (Net_{O2}) for *T. weissflogii*, *T. oceanica*, and *T. pseudonana* under 20 min exposure to HL (1200 µmol photons m^{-2} s^{-1}). Data averaged from 3 independent replicates. Values in parentheses are SE of the mean. A 2-way ANOVA test comparing species and light treatment with oxygen measurements (GP_{O2}, Net_{O2}, LDR) and a 1-way ANOVA for R_{DARK} are presented with significant p-values (< 0.05) in bold. Superscripted asterisks (*) or letter identifies the Bonferroni post-hoc analysis for significance between species groups.

S2.2 Table. MIMS analysis of oxygen pathways as a percentage of gross oxygen production (% of GP_{O2}). Fractions (as a %) include light dependent respiration (LDR), dark respiration (R_{DARK}), and net oxygen production (Net_{O2}) for *T. weissflogii*, *T. oceanica*, and *T. pseudonana* under 20 min incubation at Ig (85 µmol photons m^{-2} s^{-1}) and HL (1200 µmol photons m^{-2} s^{-1}). Data averaged from 2 or 3 independent replicates. Values in parentheses are SE of the mean. A 2-way ANOVA comparing species and light treatment with oxygen measurements (R_{DARK}, Net_{O2}, LDR) are presented with significant p-values (< 0.05) in bold. Superscripted asterisks (*) identifies the Fisher's Tukey post-hoc analysis for significance between species groups.

Chapter 3

S3.1 Table. PE curve parameters for *T. oceanica* and *T. weissflogii* grown over 12:12 L:D photoperiod following a sinusoidal light regime with a maximum irradiance of 400 μmol photons m^{-2} s^{-1}. PE curves were collected after 1, 3, 6, 9 and 11 hours following the onset of illumination. YII_{max}, is the maximum fluorescence-derived photochemical conversion at PSII, α is the light-limiting slope, $E_{k,YII}$ is the light saturation index calculated as YII_{max}/α and R^2 represents the goodness of fit. Values in parentheses represent the SE of the mean for at least three independent replicates.

S3.2 Table. Correlation parameters for various comparisons between measures of oxygen (GP_{O2}, R_{TOTAL}, LDR, Net_{O2}), fluorescence (YII, YNPQ, YNO, $E_{k,YII}$) and biomass (carbon, pg $cell^{-1}$) combining replicate (n=3) measurements for all time points over the photoperiod for *T. oceanica* and *T. weissflogii* under $Sine_{\mu}$ and $Sine_{HL}$. Significant correlations ($p < 0.05$) shown in bold.

S3.3 Table. Rates of oxygen production and consumption. MIMS analysis of gross oxygen production (GP_{O2}), light dependent respiration (LDR), dark respiration (R_{DARK}), and net oxygen production (Net_{O2}) for *T. weissflogii* and *T. oceanica* grown under a sinusoidal light regime with a maximum of 400 μmol photons m^{-2} s^{-1} ($Sine_{\mu}$) over a 12:12 L:D cycle. Rates were also collected from a transient exposure to high light that was 3x $Sine_{\mu}$ ($Sine_{HL}$) at the sample time. Samples were collected during the photoperiod at 1, 3, 6, 9 and 11 hours. Data averaged from 3 independent replicates. Values in parentheses are SE of the mean. For direct comparison with oxygen consumption/production rates from Chapter 2 see S2.1 Table.

Chapter 4

S4.1 Table. Cell densities (mL^{-1}), volume of culture (mL) concentrated and preserved for metabolite extraction and the total cells (mL^{-1}) extracted for metabolomics analysis of technical replicates for three constant (high, medium, low) and two pulse (high, low) light treatments.

S4.2 Table. Fold change matrix of average total cell densities extracted for metabolomics analysis between light treatments (Constant – High [HC], Medium [MC], Low [LC] and Pulse – High [HP], Low [LP]) where n = 4 for independent biological replicates. Values in parentheses represent *p* values for independent t-tests.

S4.3 Table. Physiological characteristics of *Thalassiosira pseudonana* during steady-state acclimated growth to four light regimes: Constant (high, low), and Pulse (high, low) at 20°C. Values in parentheses represent SE of the mean for at least 3 independent biological replicates for specific growth rate (d^{-1}), Chl *a* ($cell^{-1}$), carbon ($pg\ cell^{-1}$), nitrogen ($pg\ cell^{-1}$), C:N, F_v/F_m and σ_{PSII} ($nm^2\ PSII^{-1}$). ANOVA or Kruskal-Wallis (KW) test results are presented using F or H-values, respectively, followed by Bonferroni or Dunnett T3 post-hoc analysis, respectively, with differences between individual cell characteristics and light treatment designated by superscripted letters and significant *p* values ($p < 0.05$) in bold.

S4.4 Table. Summary of differentially expressed genes involved in the glyoxylate cycle, TCA cycle, and related pathways. Genes with adjusted *p* value <0.05 in *T.*

pseudonana grown at constant low light (LC, 5 µmol photons m^{-2} s^{-1}) compared to constant high light (HC, 200 µmol photons m^{-2} s^{-1}) conditions.

List of Abbreviations

AA	Amino acids
ADP	Adenosine diphosphate
ANOVA	Analysis of variance
AOX	Mitochondrial alternative oxidase
Ar	Argon
ATP	Adenosine triphosphate
BCAA	Branched chain amino acid
β-Car	Beta carotene
C	Carbon
CBB cycle	Calvin-Bassham-Benson cycle
CCM	Carbon concentrating mechanism
CEF	Cyclic electron flow
Chl	Chlorophyll
CI	Confidence intervals
CO_2	Carbon dioxide
Cyt b_6f	Cytochrome b_6f
DCMU	3-(3,4-dichlorophenyl)-1,1-dimethylurea
Dd	Diadinoxanthin
DHA	Docosahexaenoic acid
DOC	Dissolved organic carbon
DPS	De-epoxidation state
Dt	Diatoxanthin
E	Irradiance
EGT	Endosymbiotic gene transfer
ETC	Electron transport chain
ETR/ETR_{PSII}	Electron transport rate from PSII
FC	Fold change
Fd	Ferredoxin
FDP	Flavodiiron protein
FDR	False discovery rate
FLC	Fluorescence light response curve
FNR	Fd-NADP+ oxidoreductase
FRRf	Fast repetition rate fluorometry
Fuc	Fucoxanthin
GAP	Glyceraldehyde phosphate
GC-MS	Gas chromatography mass spectrometry
GDC	Glycine decarboxylase p-protein
GF/F	Glass fiber filter
GP_C	Gross carbon production
GP_{O2}	Gross oxygen production
GPP	Gross primary production
H^+	proton
HC	High constant
$HgCl_2$	Mercuric chloride
HL	High light
HP	High pulse
HPLC	High-performance liquid chromatography

Ig	Growth irradiance
KCN	Potassium cyanide
KEGG	Kyoto encyclopedia of genes and genomes
KW	Kurskal-Wallis
L:D	Light:Dark
LC	Low constant
LC-MS	Liquid chromatography mass spectrometry
LDR	Light dependent respiration
LED	Light emitting diode
LEDR	Light enhanced dark respiration
LEF	Linear electron flow
LHC	Light harvesting complex
LHCX	Light harvesting complex proteins
LIFT	Light induced fluorescence transient
LL	Low light
LP	Low pulse
MAP	Mehler ascorbate peroxidase
MC	Medium constant
MeOH	Methanol
MIMS	Membrane inlet mass spectrophotometry
MOX	Midstream oxidase
MT	Multiple turnover
N/N_2	Nitrogen/nitrogen gas
NADPH	Nicotinamide adenine dinucleotide phosphate
Net_{O2}	Net oxygen production
NP_C	Net carbon production
NPP	Net primary production
NPQ	Nonphotochemical quenching
NPQ_{NSV}	Stern-Volmer nonphotochemical quenching
NR	Nitrate reductase
O_2	Oxygen
OAA	Oxaloacetate
OEC	Oxygen evolving complex
PAM	Pulse amplitude modulated
PAR	Photosynthetically active radiation
PC	Plastocyanin
PC	Pyruvate carboxylase
PCA	Principle component analysis
PE curve	Photosynthesis-irradiance curve
PEP	Phosphoenolpyruvate
PEPCK/PCK1	Phosphoenolpyruvate carboxykinase
PETC	Photosynthetic electron transport chain
Pex	Peroxin
PFD	Photon flux density
PGA	Phosphoglyceric acid
PGR5	Proton gradient regulation protein
PLS-DA	Partial least squares discriminant analysis
PK	Pyruvate kinase

POC	Particulate organic carbon
PON	Particulate organic nitrogen
PP	Primary productivity
PPDK	Pyruvate-phosphate dikinase
PQ	Plastoquinone
PSI/II	Photosystem I/II
PTOX	Plastid/plastoquinol terminal oxidase
PTS	Peroxisomal targeting signals
QMS	Quadrapole mass spectrometer
R_{DARK}	'Dark' (mitochondrial) respiration
R_{TOTAL}	Total respiration
RC (I/II)	Reaction centre (of PSI/PSII)
RNA	Ribonucleic acid
RuBisCO	Ribulose-1,5-bisphosphate carboxylase
S	Sulfur
SAM	Significance analysis of microarrays
SCF	Spectral correction factor
SE	Standard error
SHAM	Salicylhydroxamic acid
SHMT	Serine hydroxymethyltransferase
$Sine_{HL}$	Sinusoidal high light
$Sine_{\mu}$	Sinusoidal growth irradiance
ST	Single turnover
TCA cycle	Tricarboxylic acid cycle
XC	Xanthophyll cycle
YNPQ	Yield of regulated nonphotochemical quenching
YNO	Yield of constitutive losses via fluorescence and heat
YII	Yield of photochemical conversion at PSII

List of Symbols

μ	Specific growth rate (d^{-1})
[1-C]	Photochemical quenching
[1-Q]	Nonphotochemical quenching
α	Alpha; light limiting slope
σ_{PSII}	Functional absorption cross section of PSII
e^-	Electron
ϕ	Quantum yield
E_k	Light saturation index
$E_{k,YII}$	Light saturation index of photochemical conversion at PSII
F'	Steady-state fluorescence at any point
F_o	Minimum fluorescence from dark acclimated sample
F_m (F_m')	Maximum fluorescence from dark acclimated sample
F_v (F_v')	Variable fluorescence from dark acclimated sample
F_v/F_m	Maximum photochemical efficiency
F_o'	Minimum fluorescence under actinic light
F_m'	Maximum fluorescence under actinic light
F_v'	Variable fluorescence under actinic light
F_v'/F_m'	Maximum PSII efficiency under actinic light
k_{PI}	Photo-inactivation rate constant
k_{REC}	Recovery rate constant
P_{max}	Photosynthetic maximum

Thesis Abstract

Diatoms are the evolutionarily youngest phytoplankton group and considered to be the most productive across diverse ocean, coastal and freshwater environments. Based on their evolutionary history in diverse environments, diatoms have acquired unique diverse mechanisms to cope with fluctuating availability of resources required for cellular maintenance and growth. Yet how these mechanisms actually operate to moderate metabolic functioning by the energetic tracking of light energy to carbon capture – commonly measured as "emergent signatures" or photosynthesis rates via fluorescence, O_2 evolution and/or CO_2 uptake –remains somewhat of a black box.

This thesis addresses the response of diatoms to light, with particular emphasis on the gaps in current energy budgets that quantify trade-offs in O_2 evolution and carbon-assimilation. An initial assessment of a variety of diatom species revealed distinct categories of photo-protective capacities (i.e. nonphotochemical quenching) that correlated with ecological niche, i.e. taxa originating from estuarine, coastal and open ocean environments. Low capacity to dissipate light energy via nonphotochemical quenching by open ocean diatoms was compensated for by an upregulation of midstream oxidase activity highlighting a key trade-off between light harvesting and light utilization strategies. Diurnal monitoring of diatoms with divergent photo-protective capacities further revealed species-specific dynamic respiratory trends, whereby diatoms with high nonphotochemical quenching capacity exhibited more dynamic R_{DARK} while diatoms with low nonphotochemical quenching capacity exhibited more dynamic light-dependent respiration (LDR). Fluorescence-derived measures of photoacclimation ($E_{k,YII}$) were found to be significantly correlated to oxygen cycling and carbon retained as biomass. Subsequent metabolomic profiling provided deeper insight into these processes via the underlying light-driven metabolite

reorganisation. Using the model coastal diatom (*T. pseudonana*), high light metabolic profiles were reflective of pathways that support higher growth rate (e.g. glycolysis and TCA cycle) compared to low light metabolic profiles associated with carbon conserving pathways (e.g. gluconeogenesis and glyoxylate cycle).

Together these outcomes uncovered previously hidden dynamics of energy processing by diatoms – including dynamic respiration rates between taxa and with time of day, which also mapped differences in inherent metabolic pathways as well as "emergent" metabolic signatures (e.g. fluorescence, O_2 and CO_2 measures of primary productivity). Combining information from cellular currencies (fluorescence, oxygen and carbon) thus provides a more robust mechanistic understanding of metabolic processes. This thesis has created a foundation for future research to compile more comprehensive energy budgets and a framework for improved estimates of primary productivity models.

Thesis Structure and Declaration of Contribution

This thesis is comprised of three data chapters (Chapters 2 - 4) with each constructed around an independent experiment. All data chapters have been written in the form of a journal manuscript for peer-review. At the time of thesis submission, Chapter 2 has been published and Chapters 3 and 4 are in final draft for submission and ready for submission/peer review pending availability of funds. Each data chapter introduction is exhaustive thus, to avoid information redundancy, the thesis general introduction (Chapter 1) has focussed on topics not covered in as much depth within data chapter introductions to better develop the outlined aims of the thesis.

Chapter 2: Fisher N.L., Campbell D.A., Hughes D.J., U., Halsey, K.H., Ralph, P.J. and Suggett D.J. 2020. Divergence of energy strategies amongst diatoms. Published in *PLOS ONE*.

NLF, KHH, PJR, DJS designed the experiment. NLF conducted the experiment. NLF collected all samples and processed data for FRRf and MIMS measurements. UK performed HPLC data analysis. DAC created R script to process photo-inactivation and repair rates and provided figures. NLF wrote manuscript first draft. DAC, DJH, KHH, PJR, DJS provided substantial critical contributions and edits.

Chapter 3: Fisher N.L., Halsey K.H., Hughes D.J., Argyle P., Ralph P.J. and Suggett D.J. Contrasting dynamics of light-dependent and -independent respiration from two

Thalassiosira diatoms under diurnal light. Thesis chapter. Intended Journal, *Journal of Phycology*

NLF conceptualised and designed the experiment. KHH, DJH, DJS, NLF finalised the experimental design. NLF conducted the experiment. NLF collected all samples and processed data. NLF wrote manuscript first draft. PA assisted with statistical analyses. KHH, DJH, PJR, DJS provided substantial critical contributions and edits.

Chapter 4: Fisher N.L., Halsey K.H., Suggett D.J., Pombrol M., Ralph P.J., Lutz A., Sogin E.M. and Matthews J.L. Light-dependent metabolic phenotype of the model diatom *Thalassiosira pseudonana*. Submitted for peer review to *Journal of Experimental Botany*

NLF, KHH, PJR, DJS conceptualised and designed the experiment. NLF conducted the experiment. NLF collected and extracted all samples. AL provided protocols for metabolite extraction and initial processing of metabolomics samples. EMS processed metabolomics samples and resources associated with analysis. JLM identified metabolites from processed data and provided guidance for anlayzing metabolomics data. NLF analysed data and created metabolic pathways. MP and KHH provided transcriptomics data. NLF wrote manuscript first draft. KHH made substantial contributions to the written manuscript. JLM, KHH, PJR, DJS, AL, EMS provided critical contributions and edits.