Pre-Screening Technical Report

A meta-analytic review of species suitability and approaches to culture optimization for enhanced *in-vitro* lipid production in microalgal biomass at commercial scale.

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Executive Summary

The Pre-Screening component of the Cellana biofuels project was concerned with the identification of the top 150 microalgal species/strains meeting baseline physiological criteria for economically feasible microalgal feedstock production (Chisti 2007; Huntley and Redalje 2007). Baseline selection criteria included an average specific growth rate of at least 1 cell division per day (i.e. 1.4 $\mu.d^{-1}$) and a minimum total intracellular lipid content of 20% of cell dry weight (Chisti 2007). More than 10,000 strains (including duplicates) from twelve international culture collections were examined using as a reference the large corpus of published scientific literature available for microalgal growth, lipid productivities and environmental tolerances. From evidence provided here candidate species/strains were evaluated both qualitatively and, where adequate data was available, quantitatively, to determine those species best suited for mass cultivation under periodic extremes of temperature, irradiance and salinity.

First order removal of all freshwater and biotoxic species reduced this dataset by approximately half, while secondary removal of strains incapable of growth in high-density suspension culture i.e. benthic types that insist on being attached to something, reduced the remaining species set by approximately one third. In the latter case, exceptions were made for those species reporting both exceptionally high specific growth rates and/or lipid productivities and for which both planktonic i.e. aquatic suspension, and benthic stages to the life cycle were reported. From this reduced dataset, the environmental tolerances and acclimation capacities of target strains was rigorously evaluated at both the taxa and species levels and used to predictively infer species-specific physiological responses to periodically supra-optimal irradiance levels and temperatures such as are experienced at the current Kona, HI cultivation facility.

Species-specific nutrient and in particular nitrogen, preferences and the type(s) of major lipids produced by the remaining species/strain group were also examined to further refine pre-screen selections. Those displaying a preference for growth on ammonia and/or the synthesis of largely monounsaturated short carbon chain (i.e.C12 – C16) fatty acids with proportionally less than 1% double (or greater) carbon bonds were retained for further

screening. In all cases and where evidence existed for verification, all species/strains synthesizing proportional quantities of neutral Triacylglycerols (TAGs) above 15% of total intracellular fatty acids were unequivocally included for analysis. This caveat maximized chances of identifying those species/strains whose intracellular fatty acid profiles most closely match current requirements for meeting stringent regulatory standards for commercial biodiesel production.

Supplementary in-house isolations of novel, wild-type strains from local niche habitats scattered throughout the Hawaiian archipelago were also carried out to compensate for the widely reported loss of metabolic vigor in culture strains maintained too long in resource saturated conditions. Efforts here were focused on local niche habitats physiochemically analogous to those found at the current cultivation facility i.e. shallow, high salinity biomes, to identify naturally acclimatized strains capable of sustained growth under seasonally extreme environmental conditions. This proved an extremely productive exercise yielding >300 additional candidates from which ~90 fast growing oleaginous strains were identified and submitted to High Throughput Screening (HTS). The final number of strains submitted to HTS totaled 199, more than two-fold higher than the initial projected number. Of these, only strains obtained from culture collections are dealt with here and only those species for which adequate published data are available are assessed in detail.

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Introduction

Of primary concern in identifying optimal microalgal strains for industrial biodiesel feedstock production is the capacity of candidate species to maintain a specific growth rate >1.4μ.d^{-1} and internal lipid content of >20% total cell dry weight under environmental extremes of temperature, irradiance and salinity. At both current and proposed cultivation facilities, high mid-summer irradiances (max. 2134 μmol.m^{-2}.s^{-1}) and concomitantly high temperatures (>30°C) (Table 1) can generate steep salinity gradients, in turn reducing photosynthetic efficiency and depressing biomass yields to below the limits of economic feasibility (see Fig. 1). An in-depth assessment therefore of species-specific responses to environmental extremes and their influence on biomass and lipid productivity was central to the Pre-screening effort and provided essential information on which to qualify individual species suitable for High Throughput Screening (HTS)

Systematic screening of >10,000 culture strains (including duplicates) held by twelve international collections (Table 2) was conducted to identify species meeting the baseline criteria outlined above. From the outset, selections were confined to what are commonly termed euryphilic or extremophilic species, with the majority of strains selected originating from geographical regions physio-chemically analogous to those at the proposed culture facility (i.e. shallow, high salinity habitats at tropical to sub-tropical latitudes). Detailed, quantitative screening of this reduced sample set was then afforded by an extensive investigation of published scientific data and yielded a total of 27 individual species, primarily of the genera Bacillariophyta and Chlorophyta, potentially capable of meeting the requirements outlined. Multiple strains of selected species were purchased for further physiological and biochemical evaluation and details of individual culture strains purchased are provided in Table 3. The final number of strains submitted to HTS totaled 162, almost two-fold higher than the initial projected number. Of these, only strains obtained from culture collections are dealt with here and only those species for which adequate published data are available are assessed in detail (n = 24).

Table 1. List of culture collections screened for identification of thermotolerant, oleaginous microalgae.

Culture Collection	Strains screened
University of Hawaii at Manoa	2400
Center for Culture of Marine Phytoplankton (CCMP)	2500
University of Texas (UTEX)	1800
American Type Culture Collection (ATCC)	1200
Canadian Center for the Culture of Microorganisms	800
University of Toronto Culture Collection (UTCC)	650
CSIRO Collection of Living Microalgae (AUS)	1200
Culture Centre for Algae & Protozoa (UK)	1300
Plymouth Marine Collection (UK)	350
Sammlung von Algenkulturen (GER)	800
Roscoff Culture Collection (FRA)	650
Algoteca de Coimbra Culture Collection (POR)	700
Total	14350

Table 2. Monthly maximum, minimum and average temperatures and daily maximum and integrated PAR experienced at the Cellana, HI cultivation facility. Data obtained from Kona meteorological station.

Monthly Average	Jan	Feb	Mar	Apr	May	Jun	Jul
Max Temp (^{o}C)	28.1	27.9	29.0	28.9	28.7	29.7	30.8
Min Temp (^{o}C)	19.3	20.2	21.2	21.6	21.8	22.8	23.4
Ave Temp (^{o}C)	23.3	23.6	24.8	24.9	25.0	25.9	26.7
Daily Max PAR (μmol m^{-2} s^{-1})	1743	1836	2104	2106	2043	2134	2079
Daily Integrated PAR (mol m^{-2})	31.3	33.3	39.8	45.2	35.4	37.0	43.4

Materials

Foremost to the pre-screening process was the selection of species capable of meeting the stated target production rate of 15 $g.oil.m^{-2}.d^{-1}$. This was made possible by a comprehensive, quantitative assessment of published data on photosynthesis and growth, biomass accumulation and lipid production under extremes of temperature, irradiance and, where available, salinity. Preliminary work involved a systematic collation of over two thousand relevant publications from a diverse array of sources, including journals, books and academic dissertations. From this library, data was extracted either directly from text or from plots digitized using the freely downloadable software package, DataThief© (http://www.datathief.org/). Species and strain specific data on growth rate and lipid content was then computerized along with primary culture parameters i.e. nutrient, light, temperature and salinity, and where applicable, experimental control parameters such as specific nutrient limitation, UV light exposure, allelopathic compounds etc. Additional information on intracellular carbohydrate, protein and lipid concentrations and detailed fatty acid profiles were also procured for a small set of intensely studied oleaginous species traditionally used as aquaculture feedstock. This database comprised in excess of 5,000 individual data points and herein is termed the Cellana Microalgal Database (CMD). Further efforts to assess the specific acclimation responses of CMD species to environmental extremes were made in terms of reductions to photosynthetic, growth and lipid accumulation rates reported for certain species under high light and temperature regimes. Due to the complex and multi-faceted nature of microalgal physiology and the paucity of data available for species studied, species-specific analyses of these parameters was limited a total of twenty-four individual species from the diatom, chlorophyte and eustigmatophyte genera respectively.

Methods

1. Species/Strain Selection

Pre-screening of a >10,000 microalgal species from twelve international culture collections (Table 1) was conducted by means of a relative ranking scheme constructed from a pre-defined set of key physiological and biochemical criteria. Each criterion was weighted in

terms of its relative importance in maintaining biodiesel feedstock production costs below $0.90 liter of.finished product and limited species selection, in the first order to, fast-growing (1.4 – 2.8 $\mu.d^{-1}$), oleaginous (> 20% lipid total dry weight) and strictly marine species (Fig. 1). Secondary criteria relating to the environmental tolerances, harvestability and chemical suitability of intracellular lipid and fatty acid profiles for commercial biodiesel production allowed for the removal of less physiologically robust species and those exhibiting a tendency for carbohydrate rather than lipid accumulation under environmental stress. Concerns regarding feedstock toxicity further constrained species selection to those for which no current reports of *in vivo* toxin synthesis exist. With the exception of a small number reporting exceptionally high growth rates and/or lipid content, this resulted in the removal of most dinoflagellate and cyanobacterial species. A list of all ranking criteria applied, their weighted scores and source material used to determine occurrence in individual species of the physiochemical parameter of interest are provided in table 3.

The final scored sample set comprised a total of 74 morphologically distinct species representative of the bacillariophyte (diatom), chlorophyte (green algae), cryptophyte, eustigmatophyte, prasinophyte prymnesiophyte and xantophyte genera (Table 4). In excess of 50% of these were diatoms with eight species from the centric diatom genera *Chaetoceros, Skeletonema* and *Thalassiosira* ranked amongst the top ten. Numerous chlorophyte species, for example *Isochrysis galbana* and *Dunaliella tertiolecta,* also ranked highly while the eustigmatophyte *Nannochloropsis salina* emerged the highest ranked of the seventy-four species examined. From this reduced set, a total of twenty four diatom, two chlorophyte and one eustigmatophyte species were selected for HTS. Because of the marked tendency for carbohydrate rather than lipid accumulation under environmental stress, species from the genus *Nannochloropsis* were not selected despite their overall high ranking. Diatoms on the other hand, do not display such strong tendency and exhibit relatively high lipid productivities when compared to other taxa. In addition, the extraordinary species diversity found within the group identified it as a good source of candidate strains for HTS. Details of multiple strain purchases of the top ranking predominantly centric, diatom species are provided in Table 5. Additional novel isolations of wild-type strains sampled from local Hawaiian habitats are not dealt with here however a map of study sample locations is provided in Fig. 2.

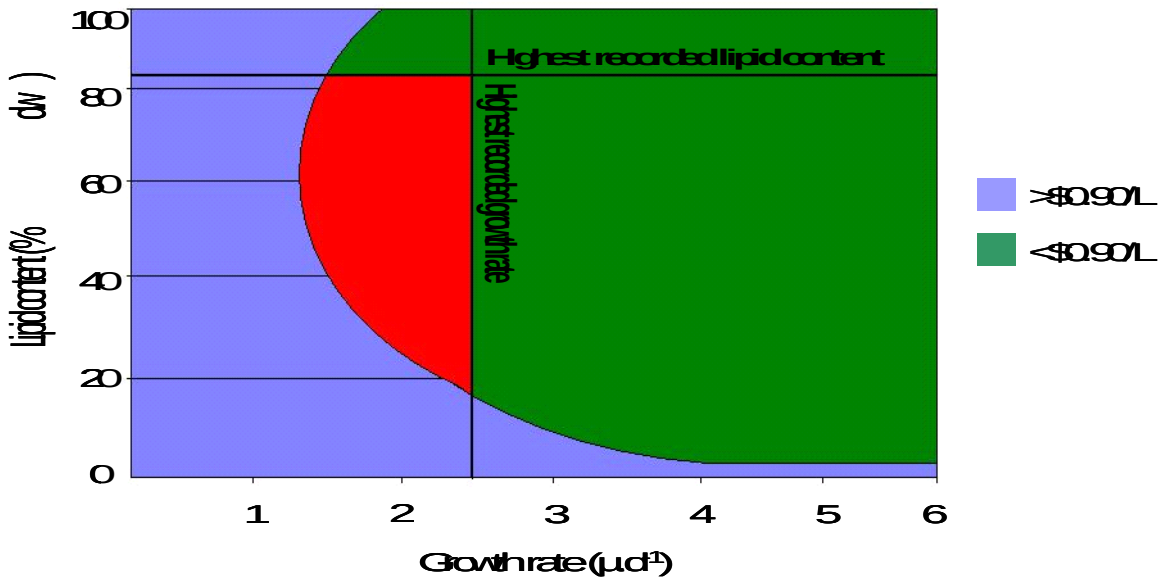

Fig. 1. Plot illustrating critical physiological caveats (growth rate vs. total cellular lipid) dictating the economic feasibility of microalgal biodiesel production. Calculations assume crude oil > $100 bbl. Adapted from Chisti 2007.

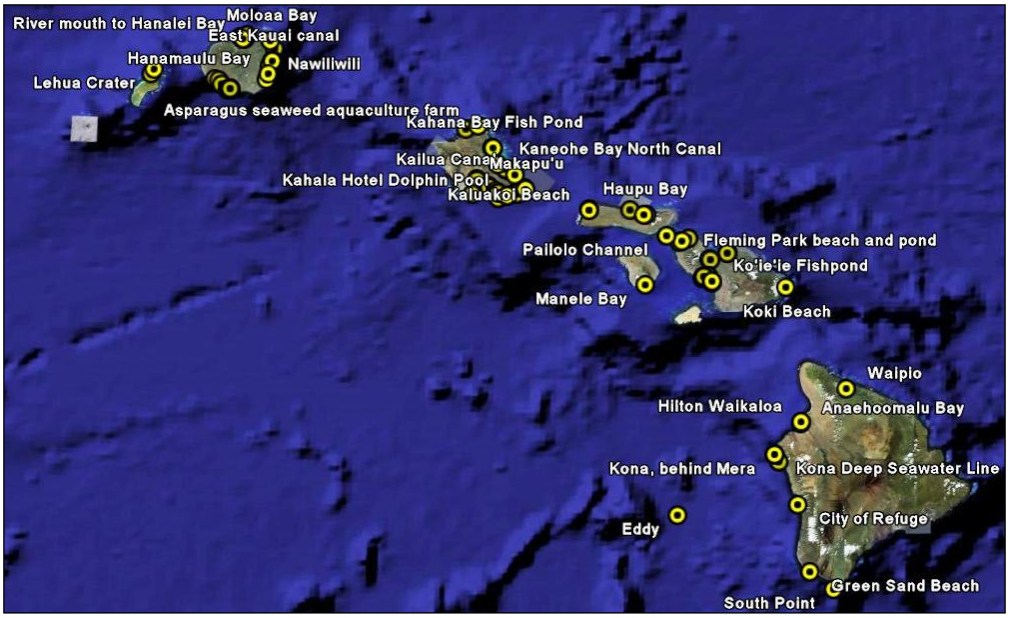

Fig 2. Map showing geographical locations throughout the Hawaiian archipelago sampled as part of the novel isolations adjunct of the Pre-screening process.

Table 3. Table outlining physiochemical ranking criteria, criteria scores and means by which criteria scores were derived. CMD = Cellana Microalgae Database.

Ranking Criteria	Sub-criteria	Max Score (%)	Derivation
Lipid Productivity	Max. recorded Growth Rate		CMD
	x	30	
	Max. recorded Lipid Content		
Ease of Cultivation	Growth on Ammonia	10	CMD (see Table 24)
	Tolerance of Environment		CMD
	Phototolerance	6.66	
	Thermotolerance	6.66	
	Halotolerance	6.66	
	Harvestability		CMD
	Chain-forming	3.33	
	Unicellular	0	
	Benthic	-3.33	
	Large cell volume	3.33	
	Rapid floculator	3.33	
Suitability for Biodiesel	Fatty Acid Profile		CMD
Feedstock	Neutral Lipids (>15% TAG)	20	
	16:0+18:0 <5% TFA	2.5	
	Cn:x, x>3<1% TFA	2.5	
	12:1 – 22:1 (%TFA)	2.5	
	C12 – C16 (%TFA)	2.5	
	Maximum Score	100	

Table 4. Ranking criteria and final scores used to evaluate microalgal species suitability for commercial biodiesel production. Top performing centric diatom species (*n* = 24) were identified and multiple strains (*n* = 72) ordered from CCMP and CSIRO collections (see Table 4) while an additional set of novel, wild-type strains (*n* = 90) were distributed among partner laboratories for incipient biochemical characterization.

		Lipid content		Growth rate		Lipid productivity		Ease of cultivation		Suitability for Biodiesel Feedstock					
Class	**Species**	**Max recorded**	**Average**	**Max recorded**	**Average**	**Lipid content x Growth rate**	**Growth on NH4+**	**Tolerance of environment**	**Harvestabilty**	**Neutral lipids**	**16:0 + 18:0**	**Cn:x, x>3**	**Mono C12 - C22**	**C12 - C16**	**Final Score**
Eust	Nannochloropsis salina	50.0	18.8	1.3	1.3	17.6	10.0	20.0	0.0	20.0	0.0	0.0	2.5	2.5	72.6
Cos	Chaetoceros simplex	42.6	31.7	2.6	2.6	30.0	10.0	20.0	3.3	ND	2.5	0.0	0.0	2.5	68.3
Prym	Isochrysis galbana	42.1	21.7	2.0	0.8	22.8	0.0	20.0	0.0	20.0	0.0	0.0	2.5	2.5	67.8
Cos	Chaetoceros sp. 'tenuissimus-like'	25.4	18.3	1.7	1.7	11.6	10.0	20.0	3.3	20.0	0.0	0.0	0.0	2.5	67.4
Cos	Thalassiosira fluviatilis	68.1	44.2	1.8	1.0	33.6	10.0	20.0	3.3	ND	0.0	0.0	0.0	0.0	66.9
Cos	Thalassiosira pseudonana	31.0	21.3	3.4	1.9	28.5	10.0	20.0	3.3	ND	0.0	0.0	2.5	2.5	66.8
Cos	Chaetoceros muelleri	26.1	20.1	4.0	4.0	28.3	10.0	20.0	3.3	ND	0.0	0.0	2.5	2.5	66.6
Cos	Chaetoceros calcitrans	26.9	18.7	3.4	3.4	24.8	10.0	20.0	3.3	ND	2.5	0.0	2.5	2.5	65.6
Cos	Skeletonema costatum	30.3	15.4	2.6	1.1	21.3	10.0	20.0	3.3	ND	2.5	2.5	2.5	2.5	64.6
Cos	Thalassiosira oceanica	46.0	42.7	1.9	1.0	23.9	10.0	20.0	3.3	ND	0.0	0.0	2.5	2.5	62.2
Cos	Cyclotella cryptica	36.8	29.9	2.1	1.8	20.7	10.0	20.0	3.3	ND	0.0	2.5	2.5	2.5	61.5
Eust	Nannochloris sp.	42.4	30.2	2.5	2.5	28.7	10.0	20.0	0.0	ND	0.0	0.0	2.5	0.0	61.2
Prym	Emiliania huxleyi	23.0	18.0	2.2	2.2	13.7	10.0	16.7	0.0	20.0	0.0	0.0	0.0	0.0	60.4
Chloro	Dunaliella tertiolecta	36.9	12.9	2.5	2.5	25.0	10.0	20.0	0.0	ND	0.0	2.5	0.0	2.5	60.0
Cos	Thalassiosira floridiana	35.0	35.0	2.1	2.0	19.4	10.0	20.0	3.3	ND	0.0	0.0	2.5	2.5	57.7
Bac	Nitzschia closterium	27.8	18.3	2.3	0.4	17.6	10.0	20.0	0.0	ND	2.5	0.0	2.5	2.5	55.1
Cos	Chaetoceros neogracile	25.4	18.3	2.0	2.0	13.8	10.0	20.0	3.3	ND	2.5	0.0	2.5	2.5	54.6
Pav	Pavlova lutheri	30.0	18.6	2.4	0.8	19.5	10.0	20.0	0.0	ND	2.5	0.0	0.0	2.5	54.5
Cos	Chaetoceros cf. wighami	23.0	12.0	3.1	3.1	19.3	10.0	16.7	3.3	ND	0.0	0.0	2.5	2.5	54.3
Crypto	Cryptomonas sp	22.0	22.0	0.7	0.4	4.1	10.0	20.0	0.0	20.0	0.0	0.0	0.0	0.0	54.1
Cos	Skeletonema tropicum	20.0	13.2	2.6	1.1	14.1	10.0	20.0	3.3	ND	2.5	0.0	0.0	2.5	52.4
Cos	Chaetoceros sp.	25.4	18.3	2.0	2.0	13.8	10.0	20.0	3.3	ND	0.0	0.0	2.5	2.5	52.1
Bac	Amphora sp.	19.3	19.3	1.9	1.6	9.9	0.0	16.7	0.0	20.0	0.0	0.0	2.5	2.5	51.6
Cos	Thalasiossira weissflogii	24.0	23.1	2.4	2.4	15.6	10.0	15.1	3.3	ND	0.0	2.5	2.5	2.5	51.5
Cos	Ditylium brightwellii	38.0	30.8	2.1	0.9	21.3	10.0	20.0	0.0	ND	ND	ND	ND	ND	51.3
Cos	Extobucellus spinifer	33.4	25.7	1.9	1.9	17.0	10.0	20.0	3.3	ND	ND	ND	ND	ND	50.3
Cos	Cyclotella sp	54.0	54.0	1.2	0.9	16.9	10.0	20.0	3.3	ND	ND	ND	ND	ND	50.2
Cos	Biddulphia aurita	32.6	18.4	1.6	1.6	14.1	10.0	20.0	3.3	ND	0.0	0.0	0.0	2.5	49.9
Unk	Ankistrodesmus sp.	38.1	30.8	2.0	2.0	20.6	10.0	13.3	3.3	ND	0.0	0.0	2.5	0.0	49.8
Cos	Cylindrotheca fusiformis	26.3	20.2	2.0	2.0	14.2	10.0	20.0	0.0	ND	0.0	0.0	2.5	2.5	49.2
Cos	Chaetoceros gracilis	12.8	10.0	2.8	2.8	9.7	10.0	20.0	3.3	ND	0.0	0.0	2.5	2.5	48.0
Pav	Pavlova sp.	27.2	25.2	2.4	0.8	17.7	10.0	20.0	0.0	ND	0.0	0.0	0.0	0.0	47.7
Pras	Tetraselmis suecica	23.4	13.1	2.0	1.4	12.7	10.0	20.0	0.0	ND	0.0	0.0	2.5	2.5	47.7
Cos	Chaetoceros affinis	25.4	18.3	2.0	2.0	13.8	10.0	20.0	3.3	ND	0.0	0.0	0.0	0.0	47.1
Cos	Lauderia annulata	19.0	19.0	2.5	1.2	12.9	10.0	20.0	3.3	ND	ND	ND	ND	ND	46.2
Chloro	Chlorella minutissima	23.2	12.9	2.4	2.4	15.1	10.0	20.0	0.0	ND	0.0	0.0	0.0	0.0	45.1
Cos	Odontella mobilensis	17.4	16.3	2.5	1.1	11.7	10.0	20.0	3.3	ND	ND	ND	ND	ND	45.0
Chloro	Chlorella sp.	25.5	19.0	1.8	1.8	12.4	10.0	20.0	0.0	ND	0.0	2.5	0.0	0.0	44.9
Cos	Asterionella glacialis	11.6	11.6	3.6	3.6	11.3	10.0	15.1	3.3	ND	0.0	0.0	2.5	2.5	44.7
Eust	Nannochloropsis sp.	47.7	32.4	0.7	0.3	9.0	10.0	20.0	0.0	ND	0.0	0.0	2.5	2.5	44.0
Prym	Hymenomonas carterae	20.0	17.2	1.9	1.9	10.3	10.0	20.0	3.3	ND	0.0	0.0	0.0	0.0	43.6
Cos	Melosira *cf* nummuloides	18.5	18.5	1.6	1.6	8.0	10.0	20.0	0.0	ND	0.0	0.0	2.5	2.5	43.0
Cos	Odontella sp.	17.4	16.3	2.5	2.5	11.8	10.0	20.0	0.0	ND	ND	ND	ND	ND	41.8
Chloro	Dunaliella salina	28.1	18.6	2.5	2.5	19.0	0.0	20.0	0.0	ND	0.0	0.0	2.5	0.0	41.5
Eust	Ellipsoidion sp.	33.3	25.4	0.4	0.3	3.2	10.0	20.0	3.3	ND	0.0	0.0	2.5	2.5	41.5
Cos	Dunaliella primolecta	26.2	21.5	1.6	0.4	11.4	10.0	20.0	0.0	ND	0.0	0.0	0.0	0.0	41.4
Prym	Isochrysis sp.	33.4	21.5	1.8	1.8	16.3	0.0	20.0	0.0	ND	2.5	0.0	2.5	0.0	41.3
Cos	Biddulphia sinensis	9.6	9.6	1.0	1.0	2.6	10.0	20.0	3.3	ND	0.0	0.0	2.5	2.5	40.9

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Table 4. continued:

		Lipid content		Growth rate		Lipid productivity	Ease of cultivation			Suitability for Biodiesel Feedstock					
Class	Species	Max recorded	Average	Max recorded	Average	Lipid content x Growth rate	Growth on NH4+	Tolerance of environment	Harvestabilty	Neutral lipids	16:0 + 18:0	Cn:x, x>3	Mono C12 - C22	C12 - C16	Final Score
Cos	Thalassiosira rotula	8.5	ND	1.0	0.6	2.3	10.0	20.0	3.3	ND	0.0	0.0	2.5	2.5	40.6
Eust	Nannochloropsis oculata	33.7	20.7	0.9	0.6	8.3	10.0	16.7	0.0	ND	0.0	0.0	2.5	2.5	40.0
Cos	Bacteriastrum hyalina	8.5	ND	2.7	2.2	6.2	10.0	20.0	3.3	ND	ND	ND	ND	ND	39.5
Cos	Leptocylindrus danicus	8.5	ND	2.7	1.8	6.1	10.0	20.0	3.3	ND	ND	ND	ND	ND	39.4
Bac	Nitzschia frustulum	33.5	28.5	0.8	0.8	7.3	10.0	16.7	0.0	ND	0.0	0.0	2.5	2.5	38.9
Pras	Tetraselmis tetrathele	19.2	16.3	1.7	1.7	8.8	10.0	20.0	0.0	ND	0.0	0.0	0.0	0.0	38.8
Bac	Nitzschia inconspicua	20.5	15.7	0.6	0.4	3.2	10.0	20.0	0.0	ND	0.0	0.0	2.5	2.5	38.2
Cyan	Synechococcus subsalsa	8.8	6.7	1.0	1.0	2.4	10.0	20.0	3.3	ND	0.0	0.0	0.0	2.5	38.2
Bac	Navicula jeffreyi	24.2	13.8	2.2	1.9	14.4	10.0	13.3	0.0	ND	0.0	0.0	0.0	0.0	37.7
Bac	Nitzschia laevis	35.7	35.7	0.8	0.8	7.7	10.0	20.0	0.0	ND	0.0	0.0	0.0	0.0	37.7
Bac	Nitzschia longissima	21.0	21.0	0.4	0.0	2.3	10.0	20.0	0.0	ND	0.0	0.0	2.5	2.5	37.3
Chloro	Nannochloris atomus	21.0	21.0	0.8	0.4	4.5	10.0	20.0	0.0	ND	0.0	2.5	0.0	0.0	37.0
Cos	Minutocellus polymorphus	8.5	ND	1.5	ND	3.4	10.0	20.0	3.3	ND	ND	ND	ND	ND	36.7
Cos	Stephanopyxis palmeriana	8.5	8.5	0.8	0.5	1.8	10.0	20.0	3.3	ND	ND	ND	ND	ND	35.1
Bac	Nitzschia paleacea	25.9	15.3	0.3	0.2	1.8	10.0	20.0	0.0	ND	0.0	0.0	0.0	2.5	34.3
Chloro	Dunaliella bardawil	8.9	8.9	1.8	1.8	4.3	10.0	20.0	0.0	ND	0.0	0.0	0.0	0.0	34.3
Pav	Pavlova salina	30.5	21.3	0.5	0.3	4.1	10.0	20.0	0.0	ND	0.0	0.0	0.0	0.0	34.1
Cos	Rhizosolenia setigera	8.5	ND	0.6	0.2	1.5	10.0	20.0	0.0	ND	0.0	0.0	0.0	2.5	34.0
Chloro	Botryococcus braunii	18.7	18.7	1.1	1.1	5.6	0.0	20.0	3.3	ND	0.0	2.5	2.5	0.0	33.9
Prym	Prymnesium parvum	37.9	32.0	1.1	0.9	11.3	0.0	20.0	0.0	ND	0.0	0.0	2.5	0.0	33.8
Ped	Pedinomonas minor	21.1	21.1	0.3	0.2	1.7	10.0	20.0	0.0	ND	ND	ND	ND	ND	31.7
Pras	Tetraselmis sp	10.3	13.8	0.2	0.8	0.5	10.0	20.0	0.0	ND	0.0	0.0	0.0	0.0	30.5
Prym	Isochrysis T-ISO	28.5	24.5	0.5	0.3	3.7	0.0	20.0	0.0	ND	0.0	0.0	0.0	0.0	23.7
Prym	Isochrysis aff galbana	20.0	22.3	0.6	0.4	3.2	0.0	20.0	0.0	ND	0.0	0.0	0.0	0.0	23.2
Crypto	Rhodomonas salina	22.0	14.9	0.2	0.4	1.2	0.0	20.0	0.0	ND	0.0	0.0	0.0	0.0	21.2
Crypto	Rhodomonas lens	13.3	13.3	1.2	1.2	4.3	0.0	15.1	0.0	ND	0.0	0.0	0.0	0.0	19.4

Table 5. Geographic origins, cell dimensions (µm) (diameter x length) and temperature (°C) (T) tolerances of microalgal species/strains undergoing HTS

Species	Collection	Strain	Geographic Origin	Dimensions	T - tolerance
Bacteriastrum hyalinum	CCMP	141	Gulf of Mexico	13-30 x 15-54	22-26
Chaetoceros affinis	CCMP CCMP CSIRO	159 160 CS-78	Gulf of Mexico Bay of Marseilles, France Port Hacking, NSW	5-10 x 19-25 5-12 x 6-19	18-26 22-26
Chaetoceros cf neogracile	CCMP CCMP CCMP	1425 1317 1318	Turks and Caicos Islands, British West Indies Three miles west of Scripps, LaJolla, CA Costa Rica, Dome, Station 49	0-1 x 10-15 3-4 x 8-11 4-10 x 4-8	18-22 22-26 17-26
Chaetoceros cf tenuissimus	CSIRO	CS-365/1	ND	ND	ND
Chaetoceros galvestonensis	CCMP	186	off St.Petersburg, Florida	3-5 x 3-5	22-26
Chaetoceros muelleri	CCMP CCMP CSIRO	194 1316 CS-176	saline crater lake, Galapagos Islands, Ecuador Oceanic Institute, Hawaii Oceanic Institute, Hawaii	5-10 x 5-10 4-10 x 4-9 5-8	22-26 15-30 ND
Chaetoceros simplex	CCMP	200	Persian Gulf	3-6 x 5-9	22-26
Ditylum brightwellii	CCMP CCMP CCMP CSIRO CSIRO CSIRO	358 360 361 CS-74 CS-131 CS-734/01	Gulf of Mexico Malborough Sound, New Zealand Scripps Institute of Oceanography, La Jolla, CA Port Hacking, NSW Port Hacking, NSW Cornelian Bay, Tasmania	12-38 x 40-81 19-23 x 23-100 25-48 x 30-125 ND ND ND	22-26 22-26 22-26 ND ND ND
Extubocellulus spinifera	CCMP CCMP CSIRO	393 396 CS-136	Falmouth Great Pond, Falmouth, MA Puerto Penasco, Sonora, Mexico 100m depth station, Port Hacking, NSW	3-4 x 35-55 2-3 x 4-7 2-3 x 4-7	4-32 9-32 ND

Leptocylindrus danicus	CCMP CCMP	470 1856	Morro Bay, California 25.0000N 90.0000W	2-3 x 3-5 12-18 x 54-81	22-26 18-26
Minutocellus polymorphus	CCMP CCMP CCMP CCMP CCMP CSIRO	497 498 499 500 501 CS-3	Southeast of Bermuda Surinam Chain Cruise 48 Sandy Hook, New Jersey Sargasso Sea West Sayville, Great Salt Bay, Long Island, NY Port Hacking, NSW	0-0x4-8 3-4x3-7 3-6x3-6 4-4x5-23 2-3x3-4 ND	15-32 11-32 11-32 15-32 11-32 ND
Odontella mobiliensis	CCMP CCMP CCMP CSIRO CSIRO	596 597 598 CS-133 CS-82	Scripps Institute of Oceanography, La Jolla, CA Gulf of Mexico Surinam Chain Cruise 48 Great Barrier Reef, Qld Port Hacking, NSW	15-50 x 25-120 24-70 x 35-90 15-35 x 40-140 ND ND	22-26 22-26 11-16 ND ND
Rhizosolenia setigera	CCMP	1694	Gulf of Oman, Arabian Sea, Station OM-3	3-12 x 130-440	18-26
Skeletonema dhornii	CCMP CCMP	779 789	Bass Strait, Australia unknown	2-8 x 7-19 6-10 x 19	22-26 22-26
Skeletonema grethae	CCMP	775	Gulf of Mexico	4-5 x 4-8	22-26
Skeletonema japonicum	CCMP CCMP CCMP	784 1281 1283	Uncle Sam Bank, Baja California, Mexico near Santa Cruz Island, California near Santa Cruz Island, California	5-9 x 5-12 6-10 x 6-12 6-10 x 6-12	22-26 18-22 18-22
Skeletonema marinnoii	CCMP	1332	Milford, Connecticut (tank)	6-8 x 6-14	11-30
Skeletonema tropicum	CCMP CCMP CCMP CCMP CCMP	778 788 2070 2157 2802	Tortuguero River, Costa Rica Galveston Channel, Texas Las Perlas Islands, Gulf of Panama, Panama 1/2 mile off Samba River, Darien, Panama Montevideo, Uruguay	8-10 x 6-9 8-10 x 5-10 6-11 x 4-6 2-10 x 6-10 4-8 x 6-10	12-26 22-26 18-22 18-22 18-22
Stephanopyxis palmeriana	CCMP	814	Gulf of Mexico	48-56 x 71-150	22-26
Streptotheca tamesis	CSIRO CSIRO CSIRO	CS-129 CS-130 CS-81	Port Hacking, NSW Port Hacking, NSW Port Hacking, NSW	ND ND ND	ND ND ND

Thalassiosira floridana	CCMP	985	Pompano Beach, Florida	5-9 x 5-12	18-26
Thalassiosira fluviatilis	WHOI				
Thalassiosira oceanica	CCMP CCMP CCMP CCMP CCMP CCMP CCMP CCMP CCMP CSIRO	998 999 1000 1001 1002 1003 1004 1005 1006 CS-67	continental slope continental slope continental slope continental slope continental slope Sargasso Sea Gulf Stream Warm Core Ring 81D Sargasso Sea Bermuda South West Arm, Port Hacking, NSW	6-8 x 8-14 4-8 x 6-21 4-10 x 6-10 4-13 x 6-10 0.5-4 x 5-10 4-6 x 5-8 5-10 x 6-20 6-10 x 4-12 10-10 x 5-10 9-19	18-26 22-26 22-26 22-26 22-26 22-26 22-26 22-26 22-26 ND
Thalassiosira pseaudonana	CCMP CCMP CSIRO CSIRO	1335 1013 CS-173 CS-20	Moriches Bay, Forge River, Long Island, NY Conwy, Gwynedd, Wales, United Kingdom Moriches Bay, Forge River, Long Island, NY Swan River Estuary, WA	4-5 x 4-6 3-4 x 4-6 4-5 5-5	4-25 11-16 ND ND
Thalassiosira rotula	CSIRO CSIRO CSIRO	CS-32 CS-344 CS-77	La Jolla, California Jervis Bay, NSW Port Hacking, NSW	11-32 x 17-21 ND 50	
Thalassiosira weissflogii	CCMP CCMP CCMP CCMP	1050 1051 1336 1587	Del Mar Slough, California King Kalakaua's Fishpond, Kahaluu, Hawaii Gardiners Island, Long Island, New York Jakarta Harbor, Indonesia	14-20 x 16-26 8-15 x 10-20 10-12 x 12-22 8-10 x 14-18	22-26 11-32 11-22 18-22
Dunaliella sp.	Unknown	Unknown	Unknown	4-13 x 5-15	22-26
Tetraselmis sp.	Unknown	Unknown	Unknown	8-13 x 10-15	22-26
Isochrysis sp	Unknown	Unknown	Unknown	4-6 x 4-8	17-30

2. Data Analysis

Statistical methods used included ordinary Least Squares Regression (LSR) and an additional quantile regression as described by Koenker and Bassett (1978). This latter method allows relationships to be inferred from the edges of scatter plots where, for example, the 99th quantile describes a line below which 99% of observations can be found. This method is based on Least Absolute Deviations (LAD) regressions which uses the median rather than the mean to fit regression lines. Therefore, the LAD technique is less sensitive to extreme outlier values when compared to ordinary LSR. The quantile of interest is estimated using an optimization function that minimizes the sum of weighted LADs and the solution to the minimization problem achieved using an algorithm such as the simplex method (Cade and Noon 2003). The quantile regression technique is appropriate for analysis of highly variable data sets and calculations were carried out using the "quantreg" library tool for the freely available statistical software package R (http://www.r-project.org).

When estimating extreme quantiles (e.g. *tau* = 99) a large sample set is required to provide a reasonable density of observations near the edge of the data envelope (Cade et al. 1999; Bissinger et al. 2008). To ensure reliable estimates at the 99th quantile therefore, a large dataset is required, and it has been suggested $n > 5/(1 - q)$ (where n is the sample size and q is the quantile of interest i.e. *tau* = 99, $n > 500$) (Rogers 1992). For this study, $n = 607$ with data obtained from studies that measured growth rates at only one temperature and those that collected growth rates over a number of different temperatures to a maximum of 35°C in batch, continuous and chemostat culture and for various daylength and photon flux density combinations (Table 5). Both the 95% Confidence Intervals (CI) and associated statistics were then used to determine a single function for predicting temperature related maximum specific growth rate (μ_{max}). For this, growth rate data was linearized by log-transformation prior to analysis and then back-transformed to allow calculation of the growth function. Q_{10} values were also calculated for eight centric diatom, two chlorophyte and one prymnesiophyte species used in this data set. In addition, Ordinary LSR was used to assess species-specific variation in μ_{max} as a function of temperature and provided additional species level information on the thermotolerant capacities of species under consideration.

Analysis of variation in intracellular lipid concentrations as a function of biotic (i.e. culture age and nutrient status) and abiotic (i.e. temperature and irradiance) factors was also investigated based on information extracted from the primary literature. Cursory examination of inter and intra strain variation in species-specific fatty acid profiles was also possible for eight centric diatoms and was assessed again as a function of temperature and irradiance levels in culture. To allow for objective comparison of the results of these pre-screening analyses, sequestered data was binned according to the different measurement units used and for practically all parameters of interest the high degree of variation encountered is expected to have limited the statistical strength of some analyses. For this reason, results were interpreted cautiously and with due consideration to the enormous inter and intra-specific variation characteristic of microalgal physiology (Dickson et al. 1969; Goldman and Mann 1980; Saavedra and Voltolina 1994; Sushchik et al. 2003).

Table 6. Microalgae species used to compile the Cellana Microalgal Growth Database (*n* = # of data points)

Species	Study	Temperature (° C)	*n*
Bacillariophyta			
Actinocyclus octonarius	Baars 1981	20	2
Actinocyclus octonarius	Baars 1981	26	1
Actinocyclus octonarius	Baars 1981	30	1
Actinoptychus senarius	Baars 1981	20	1
Actinoptychus senarius	Baars 1981	30	1
Amphora coffeaformis	Renaud etal 99	25	1
Amphora sp.	Sheehan et al 98	30	1
Asterionella glacialis	Morales-Loo & goutx 90	19	1
Asterionella glacialis	Brand & Guillard 81	24	8
Asterionella kariana	Baars 1981	20	2
Bacteriastrum delicatulum	Brand & Guillard 81	24	8
Biddulphia aurita	Baars 1981	20	1
Biddulphia regia	Baars 1981	20	2
Biddulphia regia	Baars 1981	26	1
Biddulphia sinensis	Baars 1981	20	2
Biddulphia sinensis	Baars 1981	26	1
Biddulphia sinensis	Baars 1981	30	1
Biddulphia sp.	Brand & Guillard 81	24	8
Chaetoceros calcitrans	Thompson et al 92b	10	1
Chaetoceros calcitrans	Thompson et al 92b	15	1
Chaetoceros calcitrans	Thompson et al 90	18	6
Chaetoceros calcitrans	Robert & His 87; Thompson et al 92b	20	2
Chaetoceros calcitrans	Robert et al 04	23	1
Chaetoceros calcitrans	Thompson et al 92b	25	2
Chaetoceros calcitrans f. pumila	Robert & His 87	20	1
Chaetoceros calcitrans f. pumilum	Robert et al 04	23	1
Chaetoceros cf. wighami	de Castro Araujo & Garcia 05	20	4
Chaetoceros cf. wighami	de Castro Araujo & Garcia 05	25	4
Chaetoceros cf. wighami	de Castro Araujo & Garcia 05	30	4
Chaetoceros debilis	Baars 1981	20	2
Chaetoceros gracilis	Thompson et al 92b	10	1
Chaetoceros gracilis	Thompson et al 92b	15	1

Chaetoceros gracilis	Thompson et al 91; Levasseur et al 93	18	12
Chaetoceros gracilis	Mortensen et al 88; Robert & His 87; Thompson et al 92; Lombardi & Wangersky 95	20	4
Chaetoceros gracilis	Mortensen et al 88	22	4
Chaetoceros gracilis	Robert et al 04; Mortensen et al 88	23	2
Chaetoceros gracilis	Thomas & Dodson 72; Taguchi et al 87	25	23
Chaetoceros gracilis	Mortensen et al 88	26	1
Chaetoceros gracilis	Mortensen et al 88	28	1
Chaetoceros gracilis	Mortensen et al 88	30	1
Chaetoceros muellerii	Liang et al 06; Giordano et al 01	18	3
Chaetoceros muellerii	Martinez-Fernandez et al 06	26	1
Chaetoceros muellerii	Johansen et al 90	30	7
Chaetoceros muellerii	Sheehan et al 98; Johansen et al 90	35	2
Chaetoceros pseudocurvisetus	Kuwata et al 93	24	3
Chaetoceros simplex	Thompson et al 92b	10	1
Chaetoceros simplex	Thompson et al 92b	15	1
Chaetoceros simplex	Thompson et al 90	18	6
Chaetoceros simplex	Thompson et al 92b	20	1
Chaetoceros simplex	Thompson et al 92b	25	2
Chaetoceros sp.	Chan 78	21	3
Chaetoceros sp.	Sanchez-Saavedra & Voltolina 96	23	3
Chaetoceros sp.	Reitan et al 94	24	2
Chaetoceros sp.	Renaud et al 99	25	2
Chaetoceros sp.	Martinez-Fernandez et al 06	26	1
Chaetoceros sp.	Renaud et al 02	27	1
Chaetoceros sp.	Renaud et al 02	30	1
Chaetoceros sp.	Renaud et al 02	35	1
Chaetoceros sp. 'minus'	Robert et al 04	23	2
Corethron criophilum	Brand & Guillard 81	24	8
Coscinodiscus granii	Baars 1981	20	2
Coscinodiscus granii	Baars 1981	26	1
Coscinodiscus granii	Baars 1981	30	1
Coscinodiscus jonesianus var. commutata	Baars 1981	20	1
Coscinodiscus jonesianus var. commutata	Baars 1981	26	1
Coscinodiscus jonesianus var. commutata	Baars 1981	30	1
Coscinodiscus pavillardii	Baars 1981	20	1

Coscinodiscus pavillardii	Baars 1981	30	1
Coscinodiscus radiatus	Baars 1981	20	2
Coscinodiscus radiatus	Baars 1981	26	1
Coscinodiscus radiatus	Baars 1981	30	1
Coscinodiscus sp.	Brand & Guillard 81	24	8
Cyclotella sp.	Taguchi et al 87	25	7
Cylindrotheca fusiformis	Chan 78	21	3
Cylindrotheca fusiformis	Liang et al 05	22	3
Ditylum brightwelli	Strickland et al 69	15	2
Ditylum brightwelli	Thompson etal 91	18	1
Ditylum brightwelli	Baars 1981	20	2
Ditylum brightwelli	Brand & Guillard 81	24	8
Ditylum brightwelli	Baars 1981	30	1
Fragilaria pinnata	Renaud et al 99	25	1
Guinardia flaccida	Baars 1981	20	2
Hantzschia sp.	Taguchi et al 87	25	8
Hemiaulus hauckii	Brand & Guillard 81	24	8
Lauderia annulata	Baars 1981	20	2
Lauderia annulata	Baars 1981	26	1
Navicula comoides	Baars 1981	20	1
Navicula jeffreyi	Mansour et al 05	20	1
Navicula sp.	Baars 1981	20	2
Navicula sp.	Mansour et al 05	20	1
Navicula sp.	Sheehan et al 98	30	1
Nitzschia closterium	Maddux & Jones 64	16	10
Nitzschia closterium	Morales-Loo & Goutx 90	19	1
Nitzschia closterium	Renaud et al 95	20	1
Nitzschia closterium	Maddux & Jones 64	23	2
Nitzschia closterium	Renaud et al 95	25	1
Nitzschia closterium	Renaud et al 95	30	1
Nitzschia closterium	Maddux & Jones 64	31	1
Nitzschia closterium	Renaud et al 95	35	1
Nitzschia frustulum	Renaud & Parry 94	25	6
Nitzschia laevis	Chen et al 2007	23	1
Nitzschia laevis	Wen & Chen 00	25	1
Nitzschia paleacea	Renaud et al 95	10	1

Nitzschia paleacea	Renaud et al 95	15	1
Nitzschia paleacea	Renaud et al 95	20	1
Nitzschia paleacea	Renaud et al 95	25	1
Nitzschia sp	Renaud et al 99; Ohta et al 92	25	2
Phaeodactylum tricornutum	Kudo et al 00; Thompson et al 92b	10	3
Phaeodactylum tricornutum	Thompson et al 92b	15	1
Phaeodactylum tricornutum	Thompson et al 90	18	12
Phaeodactylum tricornutum	Contreras et al 98 Kudo et al 00	20	8
Phaeodactylum tricornutum	Reitan et al 94	24	2
Phaeodactylum tricornutum	Fawley 84; Thompson et al 92b	25	6
Rhizosolenia alata var. indica	Baars 1981	20	2
Rhizosolenia alata var. indica	Baars 1981	26	1
Rhizosolenia fragilissima	Ignatiades & Smayda 70	18	12
Rhizosolenia fragilissima	Ignatiades & Smayda 70	25	12
Rhizosolenia imbricata	Baars 1981	20	2
Rhizosolenia imbricata	Baars 1981	30	1
Rhizosolenia robusta	Baars 1981	20	2
Rhizosolenia robusta	Baars 1981	26	1
Rhizosolenia setigera	Baars 1981	20	4
Skeletonema costatum	Sanchez et al 95; Yoder 1979	10	2
Skeletonema costatum	Sanchez et al 95	15	10
Skeletonema costatum	Yoder 1979	16	2
Skeletonema costatum	Shaw et al 89	18	4
Skeletonema costatum	Morales-Loo & Goutx 90	19	1
Skeletonema costatum	Sanchez et al 95; Blanchemain & Grizeau 96; Baars 1981	20	13
Skeletonema costatum	Yoder 1979	22	2
Skeletonema costatum	Robert et al 04	23	4
Skeletonema costatum	Sanchez et al 95	25	2
Skeletonema costatum	Sanchez et al 95	30	1
Skeletonema sp	Renaud etal 99	25	2
Stephanopyxis palmeriana	Baars 1981	20	2
Stephanopyxis palmeriana	Baars 1981	26	1
Streptotheca tamesis	Brand & Guillard 81	24	4
Synedra tabulata	Baars 1981	20	1
Thalassiosira andamanica	Gedde 99	19	1
Thalassiosira andamanica	Gedde 99	24	1

Thalassiosira andamanica	Gedde 99	30	1
Thalassiosira decipiens	Baars 1981	20	1
Thalassiosira eccentrica	Baars 1981	20	2
Thalassiosira eccentrica	Chan 78	21	3
Thalassiosira eccentrica	Baars 1981	26	1
Thalassiosira eccentrica	Baars 1981	30	1
Thalassiosira floridiana	Chan 78	21	3
Thalassiosira hendeyi	Baars 1981	20	1
Thalassiosira nordenskioldii	Durbin 1974	15	12
Thalassiosira pseudonana	Stramski et al 02; Thompson et al 92b	10	2
Thalassiosira pseudonana	Stramski et al 02	15	2
Thalassiosira pseudonana	Thompson etal 91; Levasseur et al 93	18	11
Thalassiosira pseudonana	Mansour et al 05; Stramski et al 02; Brown et al 96	20	6
Thalassiosira pseudonana	Thompson et al 96; Parker & Armhurst 05	22	6
Thalassiosira pseudonana	Robert et al 04; Sanchez-Saavedra & Voltolina 96	23	4
Thalassiosira pseudonana	Brand & Guillard 81	24	8
Thalassiosira pseudonana	Stramski et al 02; Thompson et al 92; Thompson & Harrison 92	25	8
Thalassiosira rotula	Baars 1981	20	2
Thalassiosira rotula	Baars 1981	26	1
Thalassiosira sp.	Brand & Guillard 81	24	6
Thalassiosira weissflogii	Jonasdottir 94	16	6
Thalassiosira weissflogii	Milligan & Harrison 00	19	2
Thalassiosira weissflogii	Strzepek & Price 00	20	6
Thalassiosira weissflogii	Strzepek & Price 00	24	2
Thalassiosira weissflogii	Ishida et al 00	25	4
Thalassiosira weissflogii	Strzepek & Price 00	27	2
Thalassiosira weissflogii	Strzepek & Price 00	29	2
Unidentified diatom	Renaud et al 99	25	1
Chlorophyta			
Ankistrodesmus sp.	Thompson et al 92; Uriarte et al 93; James et al 89	25	3
Botryococcus braunii	Uriarte et al 93	25	3
Chlorella sp.	Thompson et al 92	15	1
Chlorella sp.	Thompson et al 90	20	3
Chlorella sp.	Thompson et al 90	25	2
Chlorella sp.	Thompson et al 90	30	1

Chlorella sp.	Thompson et al 92	35	1
Chlorella vulgaris	Thompson et al 91	25	1
Dunaliella bardawil	Siron et al 89	25	1
Dunaliella primolecta	Siron et al 89; Levasseur et al 93	15	4
Dunaliella primolecta	Levasseur et al 93	25	1
Dunaliella salina	Levasseur et al 93; Morales-Loo & Goutx 90	25	3
Dunaliella salina	James etal 89; Brown 82	30	4
Dunaliella tertiolecta	Thompson et al 92b	10	1
Dunaliella tertiolecta	Lombardi & Wangersky 95	15	1
Dunaliella tertiolecta	Ohta et al 92; James etal 89; Yu et al 07; Gordillo et al 98	18	14
Dunaliella tertiolecta	Ben-Amotz et al 85	19	1
Dunaliella tertiolecta	Ben-Amotz et al 85	20	2
Dunaliella tertiolecta	Thompson et al 92; Ben-Amotz et al 85	25	3
Dunaliella viridis	Ben-Amotz et al 85	25	8
Nannochloris bacillaris	Giordano & Bowes 97	20	3
Nannochloris sp.	James et al 89	25	1
Micromonas pusilla	Thompson et al 91	18	1
Micromonas pusilla	Martinez-Fernandez et al 06	26	1
Nephroselmis astigmata	Ponis et al 06B	23	1
Nephroselmis sp	Renaud et al 99	25	1
Prasinophyte	Martinez-Fernandez et al 06	26	1
Tetraselmis sp.	Molina Grima et al 91	15	1
Tetraselmis sp.	Maddux & Jones 64	16	8
Tetraselmis sp.	Molina Grima et al 91; Abu-Rezq et al 99	20	3
Tetraselmis sp.	Brown et al 98	22	1
Tetraselmis sp.	Abu-Rezq et al 99; Maddux & Jones 64	23	3
Tetraselmis sp.	Reitan et al 94	24	2
Tetraselmis sp.	Renaud et al 99; Molina Grima et al 91	25	3
Tetraselmis sp.	Molina Grima et al 91	28	1
Tetraselmis sp.	Maddux & Jones 64	31	1
Tetraselmis suecica	Robert & His 87	20	1
Tetraselmis suecica	Robert et al 04	23	1
Tetraselmis tetrathele	Robert & His 87	20	1

Results and Discussion

Marked relationships between cell size and photosynthetic and nutrient uptake rates have been observed at both the generic and specific and pheno- and genotypic levels in microalgae (Finkel et al. 2001; Frenette et al. 1996; Marannon et al. 2007; Pedro et al. 2005). For example, Geider et al (1986) hypothesized that light saturated photosynthetic rates vary as a function of cell-size and regarded these as a species-specific constant. Such relationships become more pronounced under conditions of abiotic stress and commonly confer a competitive advantage to smaller cells relative to their larger counterparts in both nutrient saturated and limited conditions. The relatively higher surface area to volume ratios of smaller cells is known to provide a greater relative area over which nutrients can be absorbed while some investigators have reported on higher ratios of chlorophyll *a* (Chl.*a*) to carbon (C) in smaller cells grown under high irradiance. In a mass culture scenario where, rapid biomass turnover is necessary to maximize available photobioreactor and pond space, the comparatively faster growth rates of smaller cells, particularly under abiotic stress, is a highly desirable trait.

In natural systems, the ecological advantages of smaller cell size are manifest as the annual explosion of diatom growth at the onset of spring with sustained diatom dominance in the upper ocean layers under turbulent, well-mixed conditions. Such turbulence effectively maximizes cell exposure time to both incidence irradiance and dissolved nutrients and is essential for maximizing growth under artificial cultivation conditions (Lewis et al. 1984; Gervais et al. 1997). The adaptation of diatoms for growth in turbulent conditions (Thomas and Gibson 1990; Peters 2006) in combination with their capacity for rapid growth under abiotic stress (Fisher 1977; Vardi et al. 2006) and the low reported incidence of toxicity among species (Bates 2000) distinguished this taxonomic class as a promising source of candidate species for pre-screening. This distinction was borne out by the high percentage of diatom species (>70%) found in the upper half of the ranked species set (Table 4). In addition, because the extraordinary diversity of this class remains largely unexplored in terms of its species-specific lipid and fatty acid composition, considerable scope exists for the

isolation and identification of several new oleaginous "*superbugs"* capable of meeting or indeed exceeding the requirements for economically feasible biodiesel production.

1. Photosynthetic efficiency and growth at high irradiance

As photoautotrophic organisms, microalgae depend primarily on an adequate supply of light in the form of Photosynthetically Active Radiation (PAR) (400 - 700 nm) to maintain net growth. Photosynthetic efficiency in resource saturated conditions is most commonly expressed as a Poisson function of irradiance or the P-E curve.

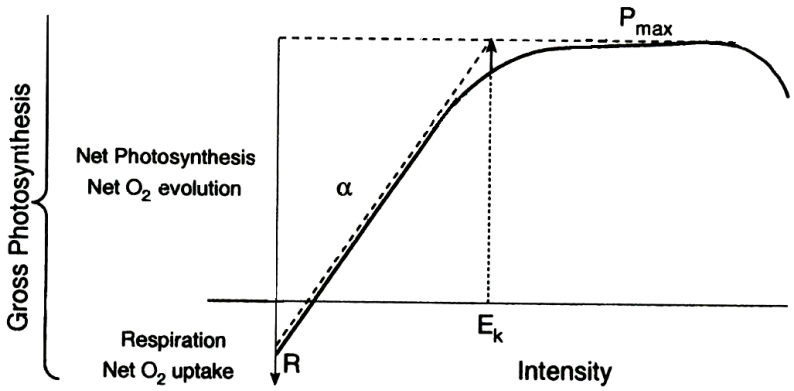


Fig. 3. A typical P-E curve. At low irradiances, photosynthesis is approximately a linear function of irradiance (symbol). At supra-optimal levels, photosynthesis decreases at a rate inversely proportional to symbol. Light-saturated photosynthesis rate (at irradiance E_k) is denoted by P_{max}. Adapted from Falkowski and Raven 2007.

This light dependent relationship is described in the first order, by the equation:

$$P_E = E_a \Phi_E \qquad \text{(eq. 1)}$$

where:

P_E = photosynthetic rate at any incidence irradiance, E.

E_a = light absorbed by the organism (quanta.m^{-2}.s^{-1})

Φ_E = quantum yield at irradiance E.

A derived biomass specific alternative to the P-E curve is often used to calculate light-saturated rates of photosynthetic carbon (C) fixation and for our purposes serves to better explain light utilization in terms of biomass accumulation. Here, C-specific rates of photosynthesis are expressed as the product of the absorption cross-section of the thylakoid membrane (on a unit chlorophyll basis), the ratio of cellular chlorophyll *a* to C and the incident photon flux density reaching the photosynthetic apparatus. The C-specific photosynthetic rate is defined by the following equation:

$$P^c(I) = Z\ Q_p\ a^{chl}\ \Phi\ I \qquad \text{(eq. 2)}$$

where:

$P^c(I)$ = gross carbon specific photosynthetic rate.

Z = dimensionless constant equal to the atomic mass of C (0.012 mg.C.µmol.C).

Q_p = quantum efficiency of photosynthesis (mol C:mol photons).

a^{chl} = chlorophyll *a* specific absorption cross section (m^{-2}.mg.Chl*a*).

Φ = Chl.*a*:C (mg.Chl*a*:mg.C).

I = Photon Flux Density (PFD) (µmol.photons.m^{-2}.s^{-1}).

An inverse relationship between Φ and irradiance is apparent as decreasing values of a^{chl} at high irradiances (Geider and Osborne 1987). This results from what is commonly termed the pigment "package effect" whereby decreases in intracellular ratios of light-harvesting photosynthetic pigments, primarily Chl. *a*, to non-photosynthetic pigments, for example xantophyll, occur as irradiance levels increase (Long et al. 1994). The ecological function of the "package effect" is to protect intra-thylakoidal light harvesting complexes from surplus chemical energy thus preventing cellular damage and photoinhibition under high irradiance (Falkowski and Raven 1997). From an industrial perspective, this can have the undesirable side effect of decreasing photosynthetic and consequently growth rates as irradiance increases above photosynthetically saturating levels.

When biomass is normalized to Chl.*a* yields, little phenotypic variation (<20%) in a^{chl} or the initial light-limited slope of the P-E curve is observed (MacIntyre et al 2002). However at photosynthetically saturating irradiances, significant taxa-specific decreases in Φ are found to occur with an inverse allometric dimension to the relationship reported by several authors (Agusti 1991; Banse 1976; Blasco et al. 1982; Finkel 2001; Geider et al 1986). These decreases typically occur in following taxonomic order:

chlorophytes > diatoms > dinoflagellates.

Based on this observed pattern, published data pertaining to three diatom and one chlorophyte species of interest was adapted to examine the hypothesis that values of Φ, and therefore a^{chl}, vary as a function of both irradiance and cell size. Cell volume data measured at five experimental irradiance levels (25, 40, 90, 190, 330 and 750 $\mu.E.m^{-2}.s^{-1}$) for the diatoms *Chaetoceros gracilis, Thalassiosira weissflogii*, an unidentified *Coscinodiscus* sp. and the chlorophyte *Dunaliella tertiolecta* was abstracted from Fujiki and Taguchi (2002) and converted to intracellular C concentration according to the equation given by Mullin et al. (1966).

$$Log_{10}C = 0.76\ log_{10}V - 0.29 \qquad \text{(eq. 3)}$$

where:
C = cell C (picograms)
V = cell volume (μ^3)
95% CL = 0.76 ± 0.05; St. Error = 0.20.

Values of Φ were then derived using data provided on intracellular Chl. *a* concentrations measured under each experimental irradiance and for each species under study. Ordinary LSR showed a statistically significant (P<0.05) inverse relationship between Φ and irradiance for all species examined (Figs. 4 & 5). Greatest variation was observed for the considerably

larger *Coscinodiscus* sp. at a maximum study irradiance of 750 µ.E.m^{-2}.s^{-1} and was considered a partial artifact of the unique optical properties reported for species of the genus (de Stefano 2007). Moreover this result was considered a product of the previously posited inverse relationship between cell volume and intracellular Φ as irradiance increases and was evidenced by the return of a significant least squares regression coefficient (r^2 = 0.8) for the same data set (Fig. 6).

Fujiki and Taguchi (2002) noted a species-specific pigment "package effect" that varied, in all species studied, as an inverse proportion of cell optical thickness at low irradiances. Optical thickness (ρ') is a dimensionless parameter defined by the equation:

$$\rho' = d.a_{cm} \qquad \text{(eq. 4)}$$

where:

d = diameter (m) of a sphere equivalent to the cell volume.

a_{cm} = absorption coefficient (m^{-1}) i.e. product of cellular Chl. *a* per unit volume and the *unpackaged* Chl. *a* specific absorption coefficient (a^{chl*}).

In turn, ρ' is related to both the photosynthetic absorption efficiency, Q_a, and the pigment packaging parameter, Q_a*, by the following equations:

$$Q_a = 1 + (2e^{-\rho'}/\rho') + 2(e^{-\rho'} - 1/\rho'^2) \qquad \text{(eq. 5)}$$

and:

$$Q_a^* = (3/2)(Q_a/\rho') \qquad \text{(eq. 6)}$$

Studies show that ρ increases with increasing cell volume (Figs. 7 & 8) while both these parameters vary independently as inverse functions of irradiance (Figs. 9 to 12) (MacIntyre 2002; Fujiki and Taguchi 2002 Thompson et al 1991). In the latter case, cell volume decreases occurred at sub-saturating irradiances (i.e. ≥400 µ.E.m^{-2}.s^{-1}) for all but the largest *Coscinodiscus* species examined (Fig. 12.a.) where a strong negative relationship between

irradiance and cell volume (r^2 = 0.9) was apparent up to 800 $\mu.E.m^{-2}.s^{-1}$. However, because Φ decreases with increasing irradiance (Figs. 13 & 14) and absolute species-specific proportions of cellular Chl. *a* (on a molecular weight basis) appear relatively conservative, decreases in Φ as irradiance increases are expected to be comparatively smaller in small rather than large cells. Thus, despite an increased pigment "package effect" at high irradiances, the limiting effects of this on rates of photosynthesis and growth are expected to be comparatively smaller in smaller species.

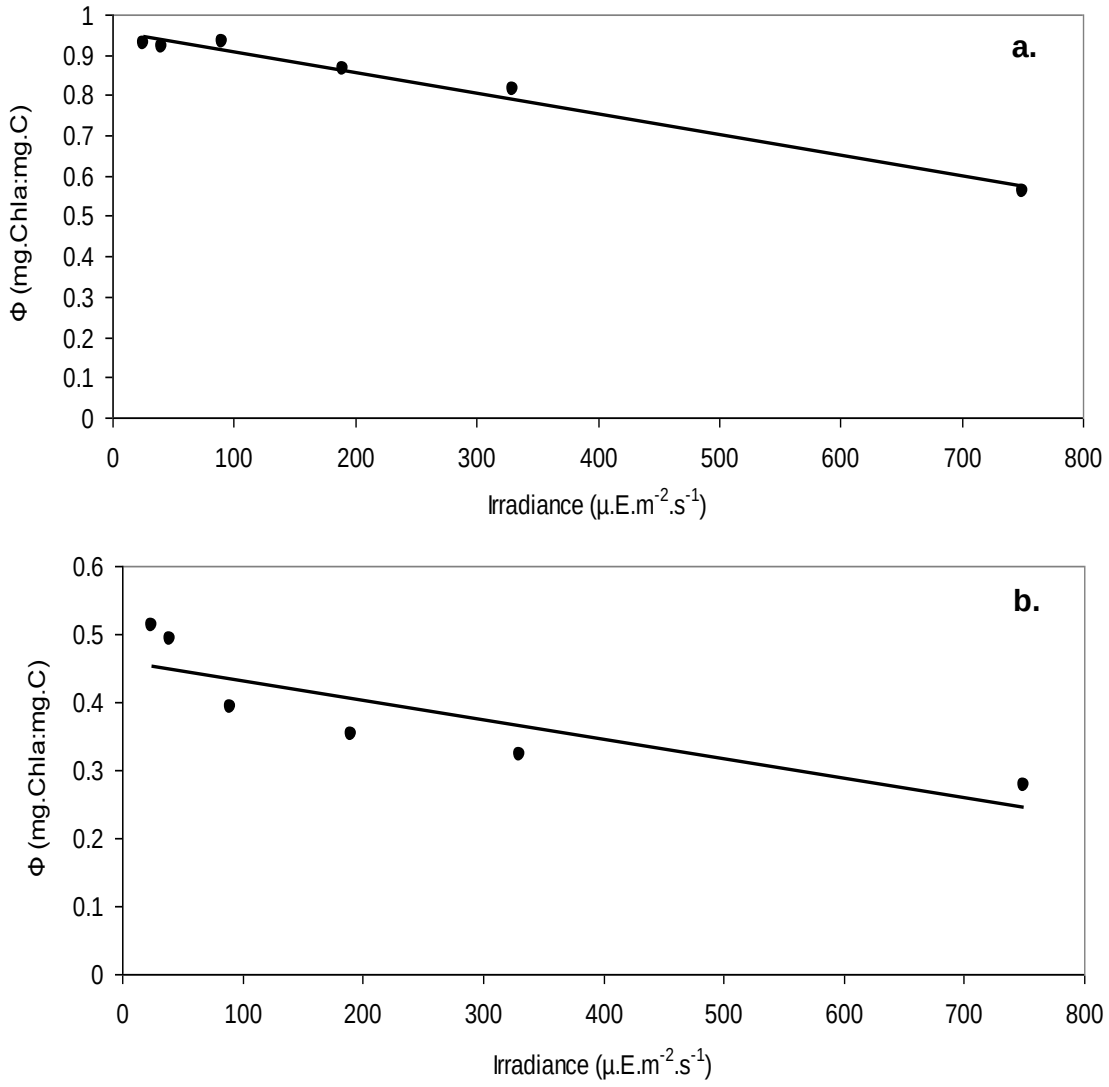
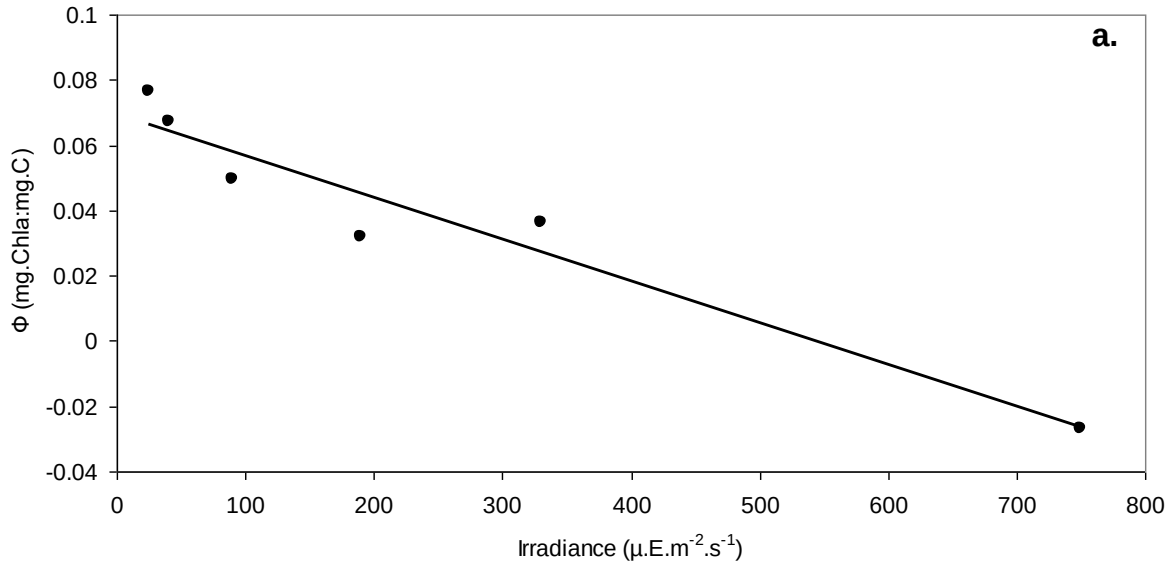
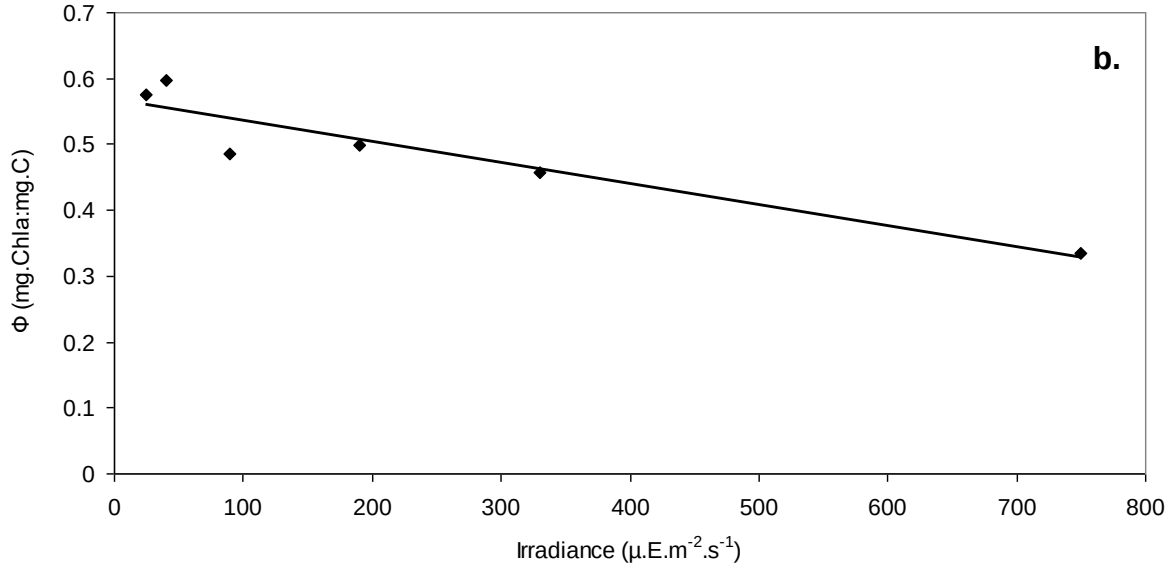


Fig. 4. Ordinary LSR of Φ (Chl.*a*:C) as a function of irradiance in a) *Chaetoceros gracilis* (r^2 = 0.98) and b) *Thalassiosira weissfloggii* (r^2 = 0.71) . Adapted from Fujiki and Taguchi 2002.





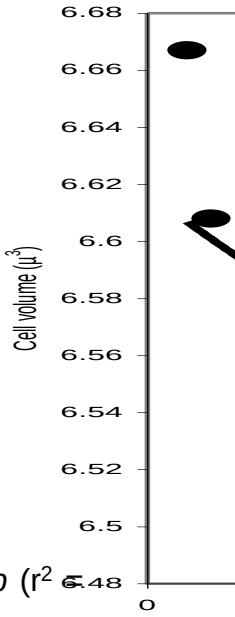


Fig. 5. Ordinary LSR of Φ (Chl.*a*:C) as a function of irradiance in a) *Coscinodiscus sp* (r^2 = 0.94) and b) *Dunaliella tertiolecta* (r^2 = 0.88) . Adapted from Fujiki and Taguchi 2002.

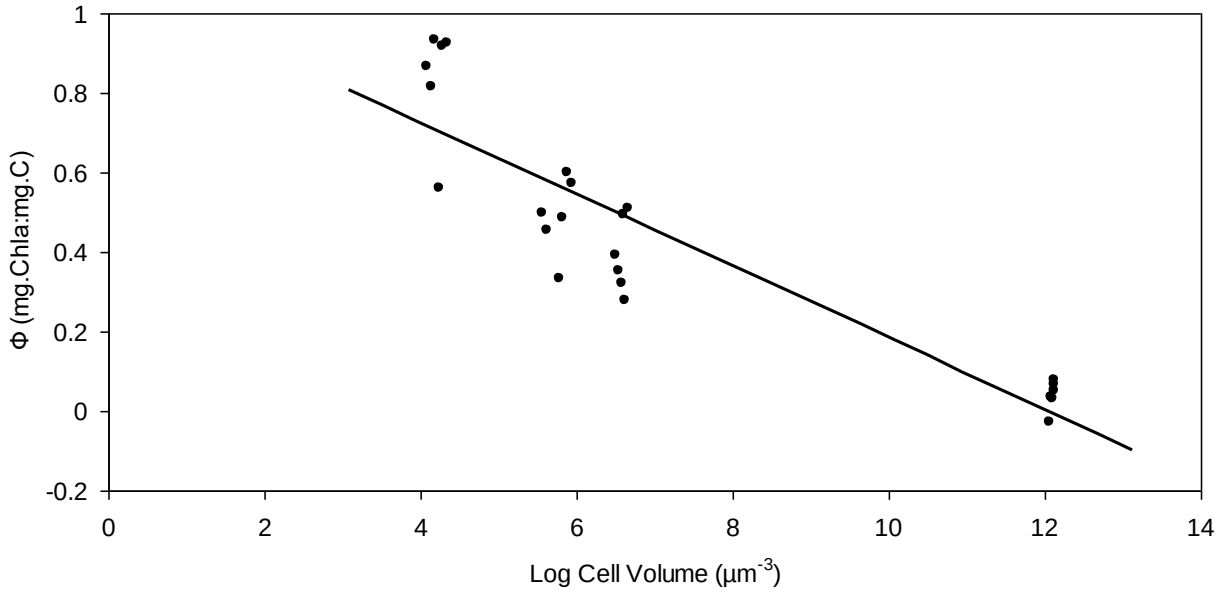


Fig. 6. Ordinary LSR of Φ against cell volume under increasing irradiance (25 – 750 µmol.photons.m^{-2}.s^{-1}) in one chlorophyte and three diatom species (r^2 = 0.8; P<0.05). Adapted from Fujiki and Taguchi 2002.

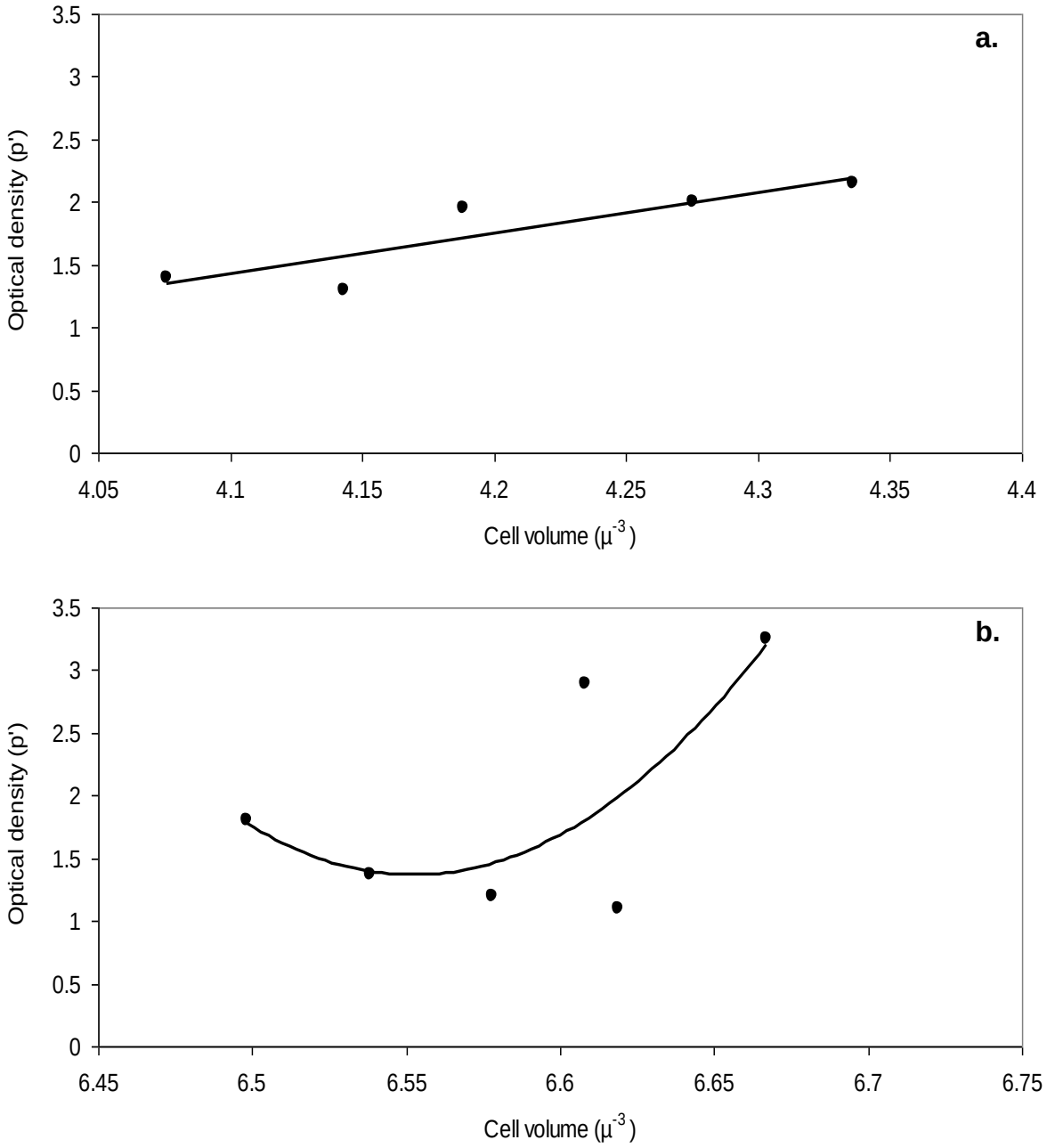


Fig. 7. Ordinary LSR of cell volume vs. optical density (ρ') in a) *Chaetoceros gracilis* (r^2 = 0.8) and b) *Thalassiosira weissflogii* (r^2 = 0.5). Adapted from Fujiki and Taguchi 2002.

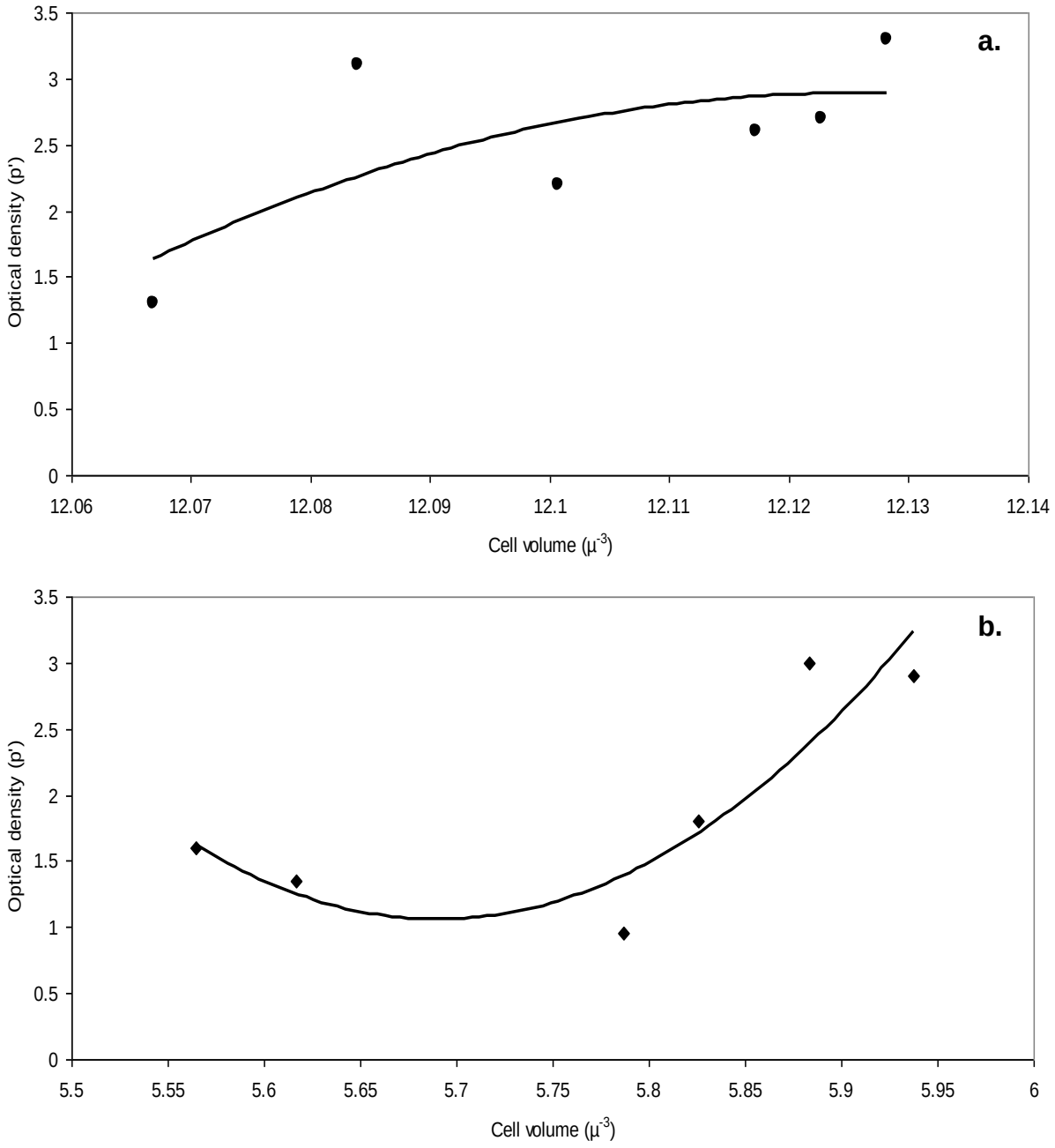


Fig. 8. Ordinary LSR of cell volume vs. optical density (ρ') in a) an unidentified *Coscinodiscus* sp. (r^2 = 0.5) and b) *Dunaliella tertiolecta* (r^2 = 0.8). In all cases P<0.05. Adapted from Fujiki and Taguchi 2002.

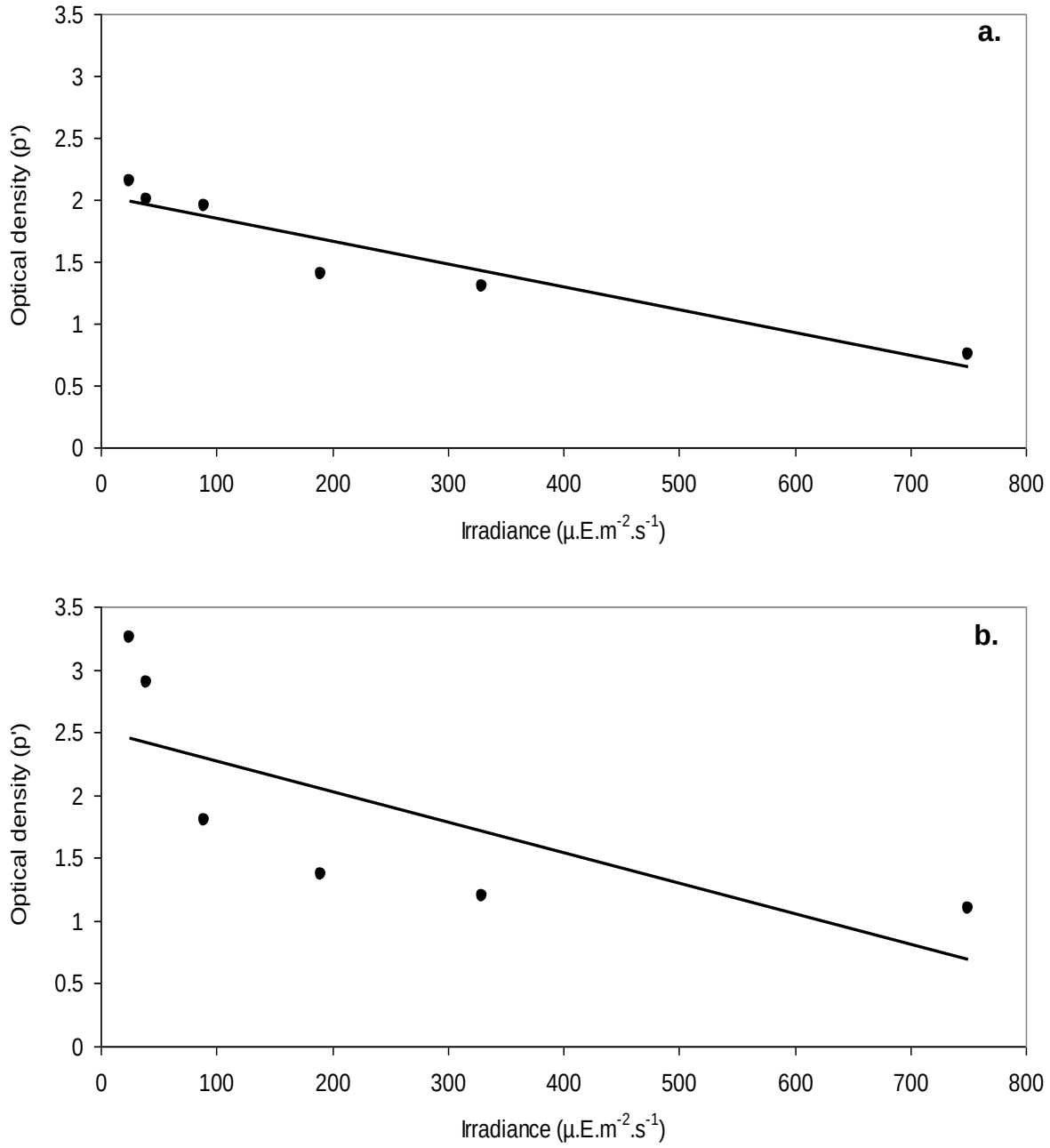


Fig 9. Ordinary LSR showing the inverse relationship between optical density (p') and irradiance in a) *Chaetoceros gracilis* (r^2 = 0.9) b) *Thalassiosira weissflogii* (r^2 = 0.6). P<0.05. Adapted from Fujiki and Taguchi 2002.

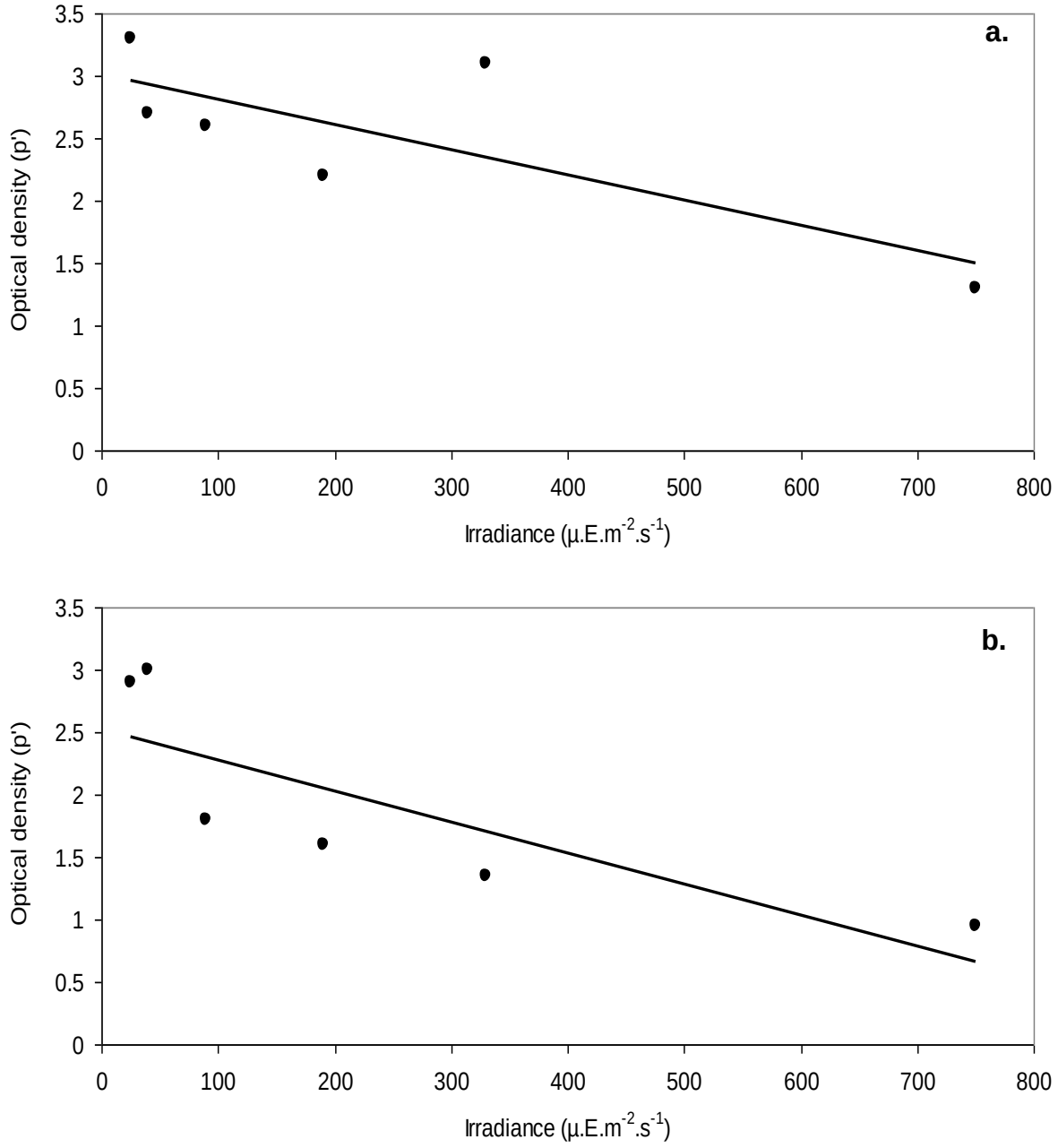


Fig 10. Ordinary LSR showing the inverse relationship between optical density (p') and irradiance in a) an unidentified *Coscinodiscus* sp. (r^2 = 0.6) and b) *Dunaliella tertiolecta* (r^2 = 0.6). P<0.05. Adapted from Fujiki and Taguchi 2002.

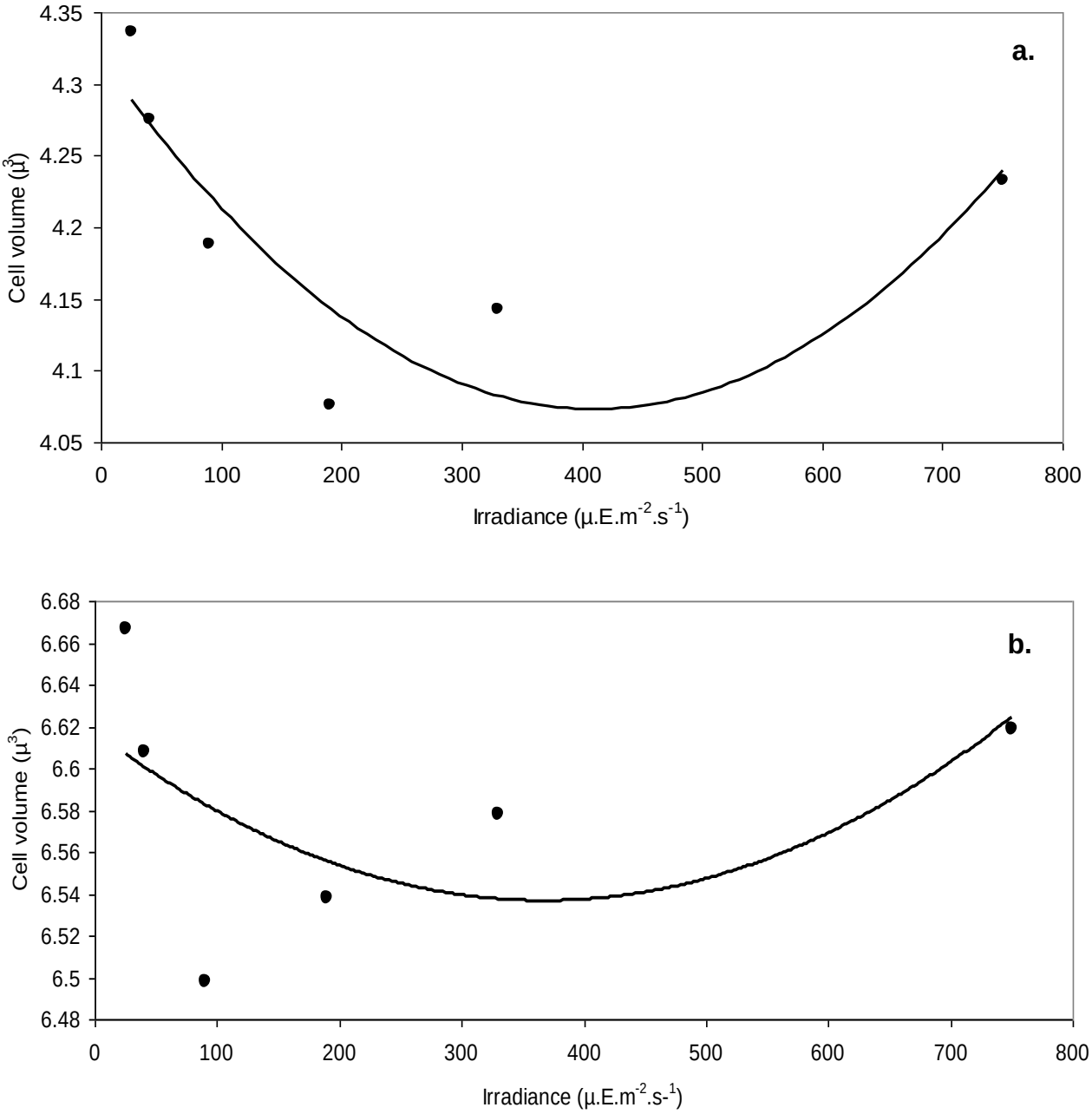


Fig. 11. Ordinary LSR of relationship between cell volume and irradiance in a) *Chaetoceros gracilis* (r^2 = 0.7) and b) *Thalassiosira weissfloggii* (r^2 = 0.3) (P<0.05). Adapted from Fujiki and Taguchi 2002.

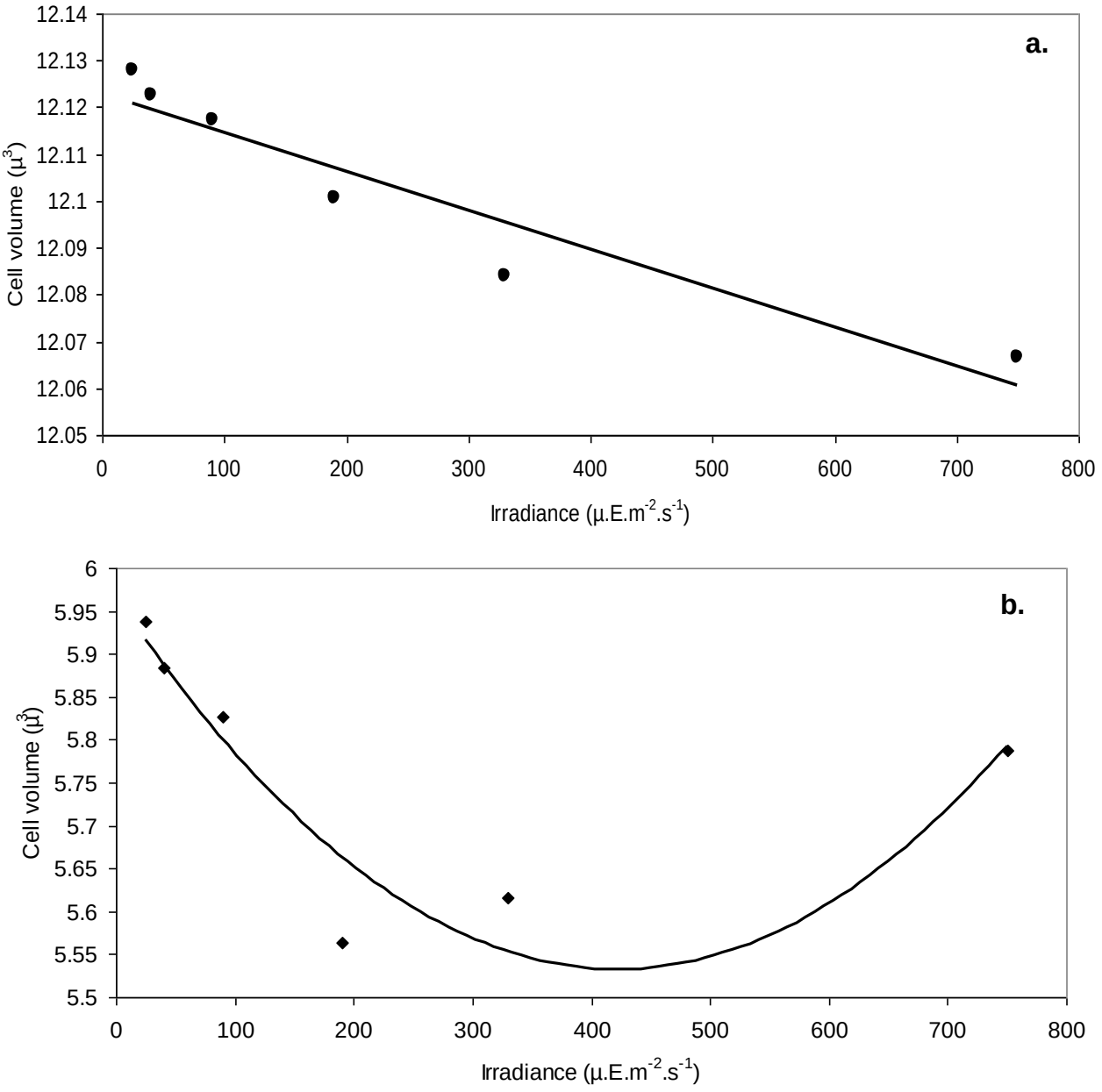


Fig. 12. Ordinary LSR of relationship between cell volume and irradiance in a) an unidentified *Coscinodiscus* sp. (r^2 = 0.9) and b) the chlorophyte, *Dunaliella tertiolecta* (r^2 = 0.9) ($P<0.05$). Adapted from Fujiki and Taguchi 2002.

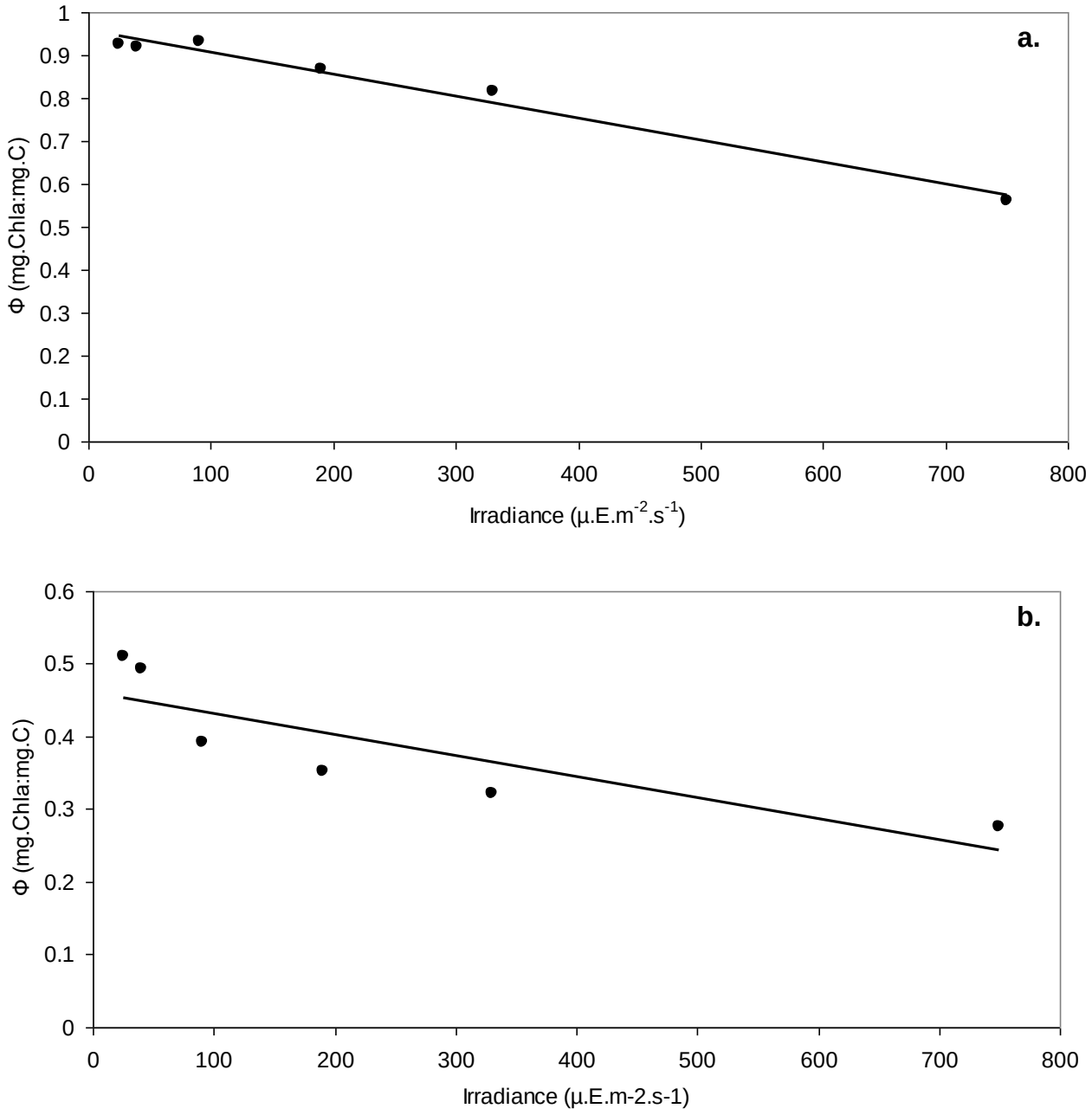


Fig. 13. Ordinary LSR of relationship between Φ and irradiance in a) *Chaetoceros gracilis* (r^2 = 0.98) and b) *Thalassiosira weissfloggii* (r^2 = 0.7) (P<0.05). Adapted from Fujiki and Taguchi 2002.

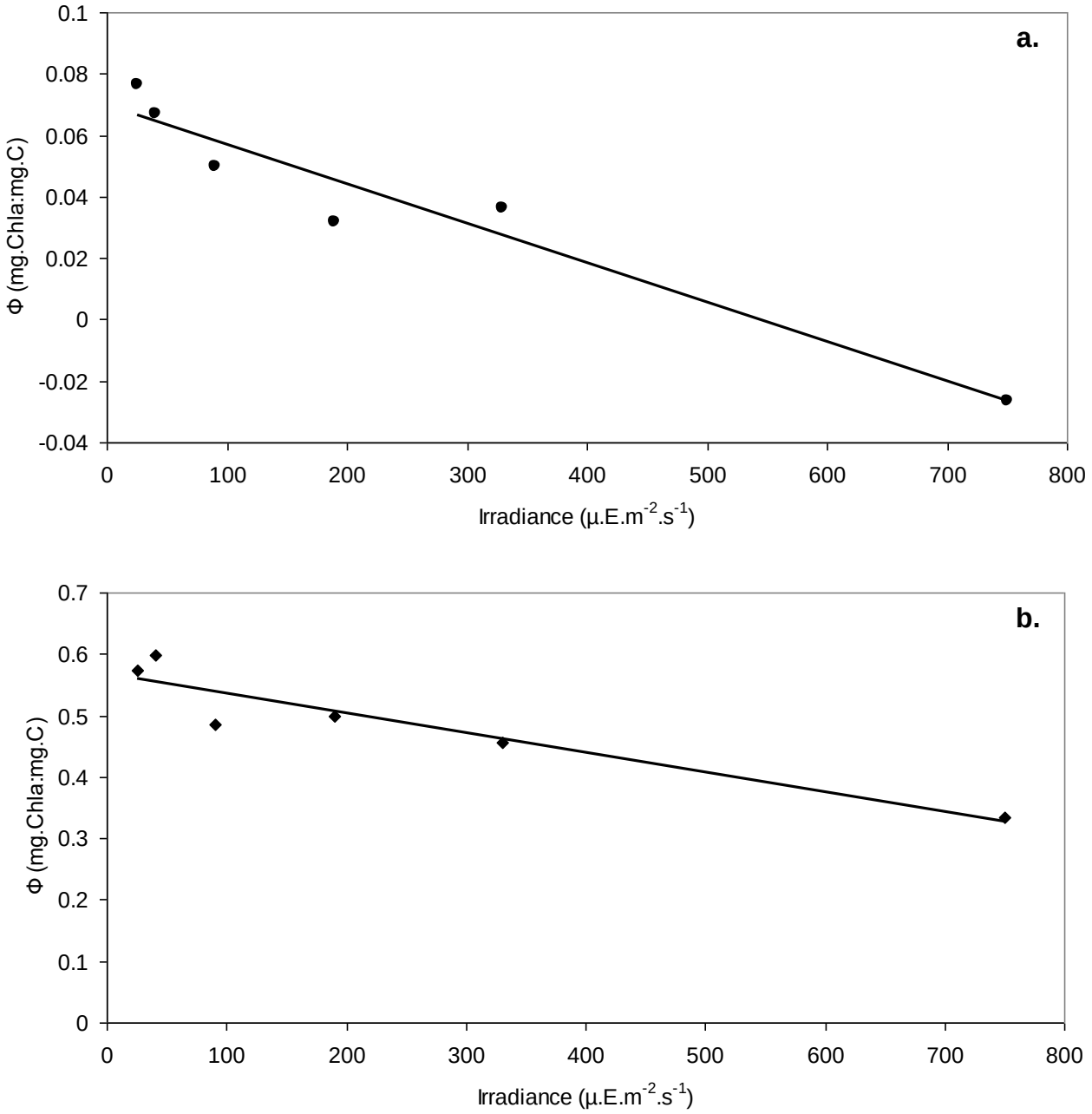


Fig. 14. Ordinary LSR of relationship between Φ and irradiance in a) an unidentified *Coscinodiscus* sp. (r^2 = 0.9) and b) the chlorophyte, *Dunaliella tertiolecta* (r^2 = 0.9) (P<0.05). Adapted from Fujiki and Taguchi 2002.

Far from responding statically to different light levels, microalgae have multiple mechanisms of acclimation, which in turn can affect photosynthetic rates and efficiencies (Falkowski 1991;

Geider 1997). Inter-specific variation in the relative importance of these mechanisms however are common and a good example of this was observed in this study as changes in the effects of irradiance on cell ρ' at low and high irradiances in *T. weissflogii* and *Coscinodiscus* sp respectively (Fig. 7.b. & 8.a.). In the latter case, this was again considered a product of the unique optical properties of *Coscinodiscus* species' frustules, (de Stefano 2007) while in the former, variation was thought to result from the substantial genetic diversity reported within the genus *Thalassiosira* (Armbrust and Galindo 2001; Armbrust et al. 2004). Generally speaking however, the strong allometric nature of both Φ and Q_a* suggests a greater capacity of small microalgal species to withstand and acclimate to periodic extremes in incoming irradiance. This hypothesis is supported by frequent reports of numerical dominance by small diatom and chlorophyte species in natural phytoplankton assemblages in tropical and sub-tropical coastal waters (Hallegraeff 1984; Tiselius and Kuylenstierna 1996) and supports the selection of multiple species from these genera for HTS and industrial scale feedstock production.

The high degree of inter and intra specific variability reported for rates of microalgal photosynthesis is naturally apparent as changes in specific growth rate ($\mu.d^{-1}$) under increasing irradiance (Post 1985). Such changes were examined as part of the pre-screening process using pooled species data obtained from the primary literature. Sufficient data for analysis was obtained for the centric diatom genera *Chaetoceros* and *Thalassiosira* and a single centric species, *Skeletonema costatum*. Given the requirements for statistical robustness, all taxonomically relevant data was included to maintain a reasonably large sample size however culture conditions varied considerably between studies. For all species examined, ordinary LSR predicted a generally upward trend in $\mu.d^{-1}$ as irradiance increased to a maximum of 1000 $\mu.E.m^{-2}.s^{-1}$ (Fig. 15). The low regression coefficients returned for all species however served to highlight the complex species-specific nature of the photoacclimatory response. For the genus *Chaetoceros* and the species *Skeletonema*

costatum, only data from within the ranges 0 - 800 µ.E.m^{-2}.s^{-1} and 0 - 500 µ.E.m^{-2}.s^{-1} respectively was available. Nevertheless, specific growth rates for both *Chaetoceros* and *Thalassiosira* species at irradiances exceeding 400 and 300 µ.E.m^{-2}.s^{-1} exceeded the critical pre-screening limit of 1.4 µ.d^{-1} in ~1/2 of the data points available. This would suggest that growth could be sustained above this level at high irradiances particularly in thermotolerant tropical strains, and supports the selection of several species strains for HTS.

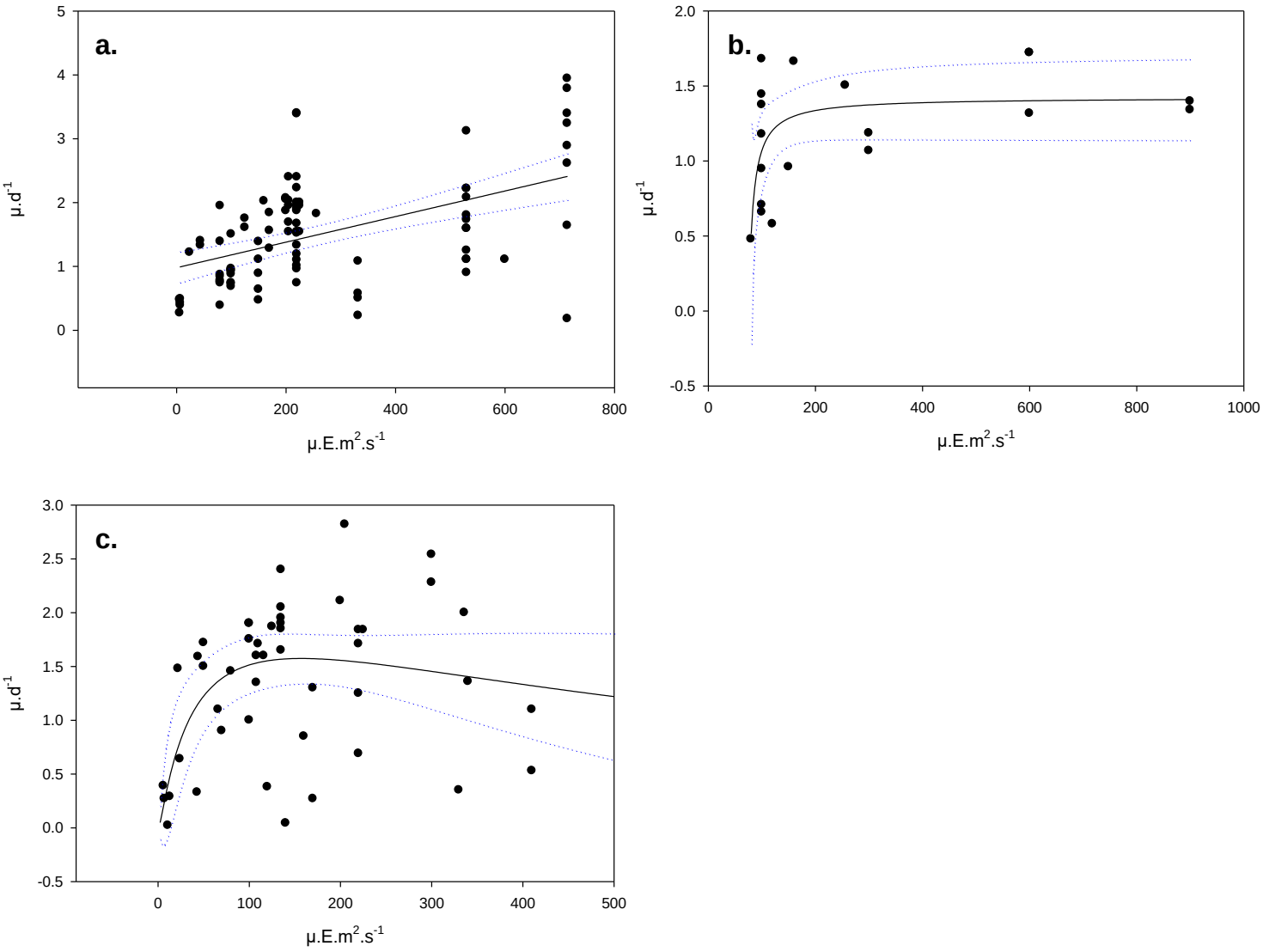


Fig. 15. Ordinary LSR of specific growth rate as a function of irradiance in a) select *Chaetoceros* species (r^2 = 0.25) b) *Skeletonema costatum* (r^2 = 0.07) and c) select *Thalassiosira* species. (r^2 = 0.26). P < 0.0001. Dashed lines: 95% confidence intervals.

Table 7. List of references from which data for growth v's light plots were obtained.

Class (ITIS scheme)	Species	Strain	Reference
Coscinodiscophyceae	*Chaetoceros calcitrans*	Argenton	McGinnis et al 97
		Unknown	Sanchez-Saavedra & Voltolina 96
		Unknown	Thompson et al 90
		NEPCC590	Thompson et al 91
		NEPCC590	Thompson et al 92a
	Chaetoceros calcitrans f. *pumilum*	Argenton	Robert et al 04
	Chaetoceros cf. *wighami*	Unknown	Chan 78
	Chaetoceros gracilis	Unknown	Kuwata et al 93
		CHAET-1	Levasseur et al 93
		CCAP1010/3	Robert et al 04
		CCAP1010/3	Robert et al 04
		Unknown	Thompson et al 92b
		NEPCC645	Taguchi et al 87
		Unknown	Thompson et al 93
		Unknown	Thompson et al 90
		NEPCC645	Thompson et al 91
		NEPCC645	Thompson et al 92a
	Chaetoceros muellerii	Unknown	Mortensen et al 88
		CS176	Enright et al 86
	Chaetoceros pseudocurvisetus	Wild strain	Giordano et al 01
	Chaetoceros simplex	Unknown	de Castro Araujo & Garcia 05
		NEPCC591	Thompson et al 92b
		Unknown	Thompson et al 93
		Unknown	Thompson et al 90
		NEPCC591	Thompson et al 91
		NEPCC591	Thompson et al 92a
	Chaetoceros sp.	CS256	Robert et al 04
		CH-X-1	Robert et al 04
	Chaetoceros sp. *'minus'*	Argenton	Renaud et al 02
	Chaetoceros sp. *'tenuissimus-like'*	Argenton	Robert et al 04
	Skeletonema costatum	Unknown	Blanchemain & Grizeau 99
		Unknown	Blanchemain et al 94
		Unknown	Chan 78
		CF1	Lourenco et al 02
		GOC36	Renaud etal 99
		Unknown	Sakshaug & Andresen 86
		SK-C-2	Sanchez-Saavedra & Voltolina 96
	Skeletonema sp.	CS-252	Mansour et al 05
	Thalassiosira andamanica	C	Gedde 99
	Thalassiosira eccentrica	Unknown	Chan 78
	Thalassiosira pseudonana	CS-173	Brown et al 96
		3H	Levasseur et al 93
		CS-20	Mansour et al 05
		CCMP1335	Parker & Armbrust 05

	Argenton	Robert et al 04
	TH-P-1	Sanchez-Saavedra & Voltolina 96
	CCMP1335	Stramski et al 02
Thalassiosira pseudonana	NEPCC58	Thompson & Harrison 92
	3H	Thompson et al 90
	Unknown	Thompson et al 91
	3H	Thompson et al 92b
	NEPCC58	Thompson et al 96
Thalassiosira weissflogii	Actin	Milligan & Harrison 00
	CCMP1336	Strzepek & Price 00

2. Photosynthetic efficiency and growth at high temperature

Of particular relevance to the pre-screening process was an investigation of microalgal photosynthetic efficiencies and specific growth rates at high temperatures. The relative abundance of data available on this topic facilitated a rigorous statistical evaluation of the thermotolerant capacities of select microalgal species and results of these analyses indicate a considerable capacity for sustained growth at local maxima >30°C by several pre-screen species. From seminal work by Eppley (1972) it is known that maximum specific microalgal growth rate (μ_{max}) increases as an exponential function of temperature within the range 0 – 40ºC.

$$\mu_{max} = 0.59e^{0.0633T} \qquad \text{(eq. 7)}$$

Eppley's calculations were based on growth measurements from both field and laboratory observations and included measurements for microalgal classes that, due to their comparatively slower growth rates, were not relevant to the pre-screening effort. For this reason, an independent evaluation of growth rate in diatom and chlorophyte species undergoing screening and as a function of temperature was conducted using measurements taken from laboratory culture studies only. This set of measurements, referred to as the Cellana Microalgal Database (CMD), consisted of specific growth rate measurements (n = 607) obtained from batch, continuous and chemostat culture where cultures were maintained at both one temperature and a number of different temperatures and under different daylengths and photon flux densities. Linearized, log transformed growth rate measurements were plotted as a function of temperature (Fig. 16) using the R statistical software package

and the 99th quantile slope parameter estimates (Table 6) used to predict the rate at which μ_{max} increased with increasing temperature (to a maximum of 35°C). Back transformation of slope parameters from the 99th quantile allowed calculation of a single temperature function described by the following equation:

$$\mu_{max} = 0.97e^{0.05001T} \quad \text{(eq. 8)}$$

where:

0.97 = the exponent of the slope intercept

0.05001 = the coefficient of temperature related growth rate.

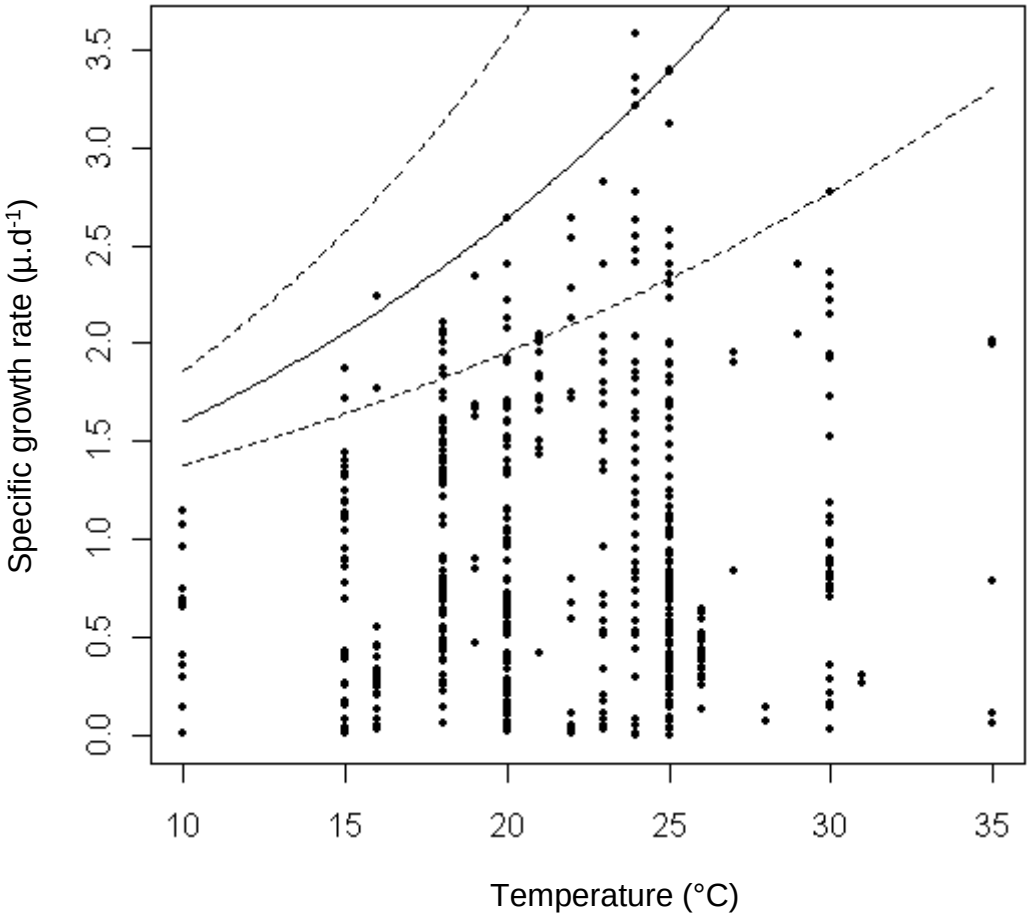


Fig. 16. LAD regression plot showing the 99th quantile and associated ±95% CI calculated from the Cellana Microalgal Database (CMD).

Table 8. Slope parameter estimates and associated statistics at the 99th quantile from the CMD. Parameter values calculated to a confidence level of 0.05.

Data set	*n*	*n*>5(1 - q)	Estimate	-95%CI	+95%CI
CMD	607	500	0.05001	0.0242	0.0637

This model predicts that for diatom and chlorophyte species grown in the range 0 - 35° C, μ_{max} will increase exponentially at a rate of 0.97 for every 0.05 degree rise in temperature ($p<0.05$). The CMD maximum recorded growth rate of 3.4 $\mu.d^{-1}$ reported for *Chaetoceros calcitrans* (strain NEPCC590) occurred at an ambient temperature of 25° C and correlated well with that predicted from the model (i.e. 25.8° C). Above this threshold, growth rates generally decreased to an average of 0.9 $\mu.d^{-1}$. In the case of five centric diatom species of interest to the pre-screening process, at temperatures above 25.8° C specific growth rates were maintained ≥1.4 $\mu.d^{-1}$ (Fig. 17) and augurs well for optimization of growth in these species at temperatures > 35° C.

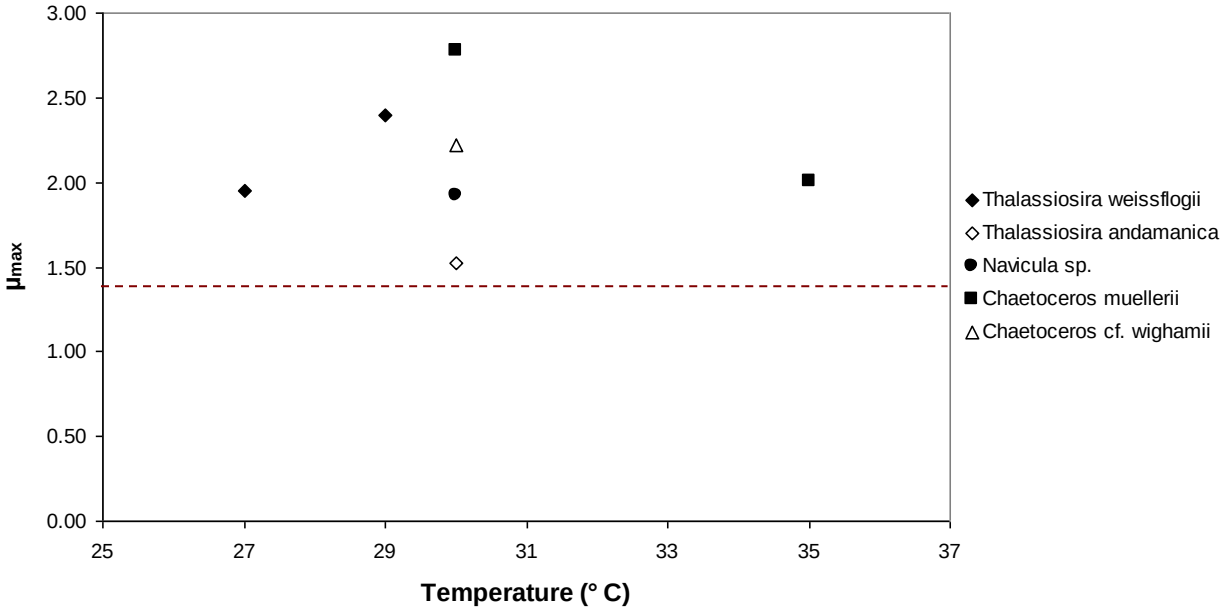


Fig. 17. Simple scatter plot showing growth rates > 1.4 $\mu.d^{-1}$ under increasing temperature for five centric diatom species of interest to the pre-screening process.

Comparison of the CMD model function with Eppley's equation for which the slope intercept (0.59) was considerably lower strongly suggests a relatively greater capacity for sustained growth by diatom and chlorophyte species at higher temperatures. This also accords with the findings of several other investigators (Bissinger et al 2008; Furnas 1990) where a large percentage of smaller diatom and chlorophyte species are often found associated with the upper envelope of temperature related growth scatters. This would suggest, as is the case with species-specific photosynthetic rates, that smaller cells possess a greater capacity for thermal acclimation and growth at high temperatures relative to their larger counterparts. To examine this relationship more closely, coefficients of temperature related rates of response (Q_{10}) in specific growth rates were calculated for eight centric diatoms, two chlorophytes and one prymnesiophyte and these are presented in table 7.

Q_{10} represents the factor by which the rate (R) of a reaction increases for every 10-degree rise in the temperature (T) and these values are commonly used to measure primary productivity rates in natural phytoplankton assemblages (Lamoureux et al. 1999). Q_{10} values are calculated using the following equation:

$$Q_{10} = (R_2/R_1)^{(10/(T_2 - T_1)} \qquad \text{(eq. 9)}$$

where:

Q_{10} = temperature coefficient (unitless)

R_2 = rate of reaction at temperature T_2

R_1 = rate of reaction at temperature T_1

If the rate of the reaction is completely temperature independent, it can be seen from the equation above that the resulting Q_{10} will be 1.0. However, if the reaction rate increases with increasing temperature, Q_{10} will be greater than 1. Thus, the more temperature dependent a process is, the higher its Q_{10} value. For the CMD a Q_{10} maximum of 1.7 was returned for two species of interest to pre-screening, namely the diatom, *Thalassiosira weissflogii* and an unidentified chlorophyte, *Dunaliella* sp. A Q_{10} minimum of 0.7 was returned for the

prymnesiophyte, *Isochrysis* sp. while Q_{10} values in excess of 1 were returned for the centric diatoms *Chaetoceros neogracile, C. simplex, C. tenuissimus, Skeletonema costatum* and *Thalassiosira pseudonanna* and for pooled species data in the chlorophyte *Tetraselmis.* With the exception of one species, namely *D. brightwelli*, a strong temperature dependency to μ_{max} was observed. This dependency was considerably greater in smaller compared to larger species (i.e. Q_{10} for larger *D. brightwellii* = 0.9) in accordance with previous results where photosynthetic rates, and therefore growth rates, were found to vary as a function of cell size.

Table 9. Q_{10} values and ordinary LSR statistics associated with temperature related average growth rate scatter plots.

Class	Species	Q_{10} Value	T–range (°C)	r^2	slope	y-intercept
Cos	*C. muellerii*	1.0	15 – 30	0.97	-54.90	2.1
Cos	*C. neogracile*	1.2	18 – 35	0.46	-42.10	1.7
Cos	*C. simplex*	1.3	10 – 36	0.71	-23.60	1.8
Cos	*C. tenuissimus*	1.4	10 – 25	0.84	-19.80	0.8
Cos	*D. brightwellii*	0.9	10 – 25	0.34	-10.00	1.0
Cos	*S. costatum*	1.5	10 – 22	0.73	-18.20	1.4
Cos	*T. pseudonanna*	1.1	10 – 25	0.92	-22.71	1.6
Cos	*T. weissflogii*	1.7	10 – 29	0.81	-80.79	3.8
Chloro	*Dunaliella* species	1.7	10 – 25	0.24	0.05	0.2
Chloro	*Tetraselmis* species	1.4	15 – 28	0.65	0.08	0.9
Prymn	*Isochrysis* species	0.7	15 - 22	0.82	0.03	0.2

Simple LSR of temperature related growth in four *Chaetoceros*, two *Thalassiosira*, five *Dunaliella*, three *Isochrysis* and three *Tetraselmis* species in addition to two individual diatom species, *Ditylum brightwelli* and *Skeletonema costatum*, were also generated to determine the degree of deviation between studies of growth at high temperatures. Graphical results are presented below (Figs. 18 to 23) and associated regression coefficients can be found in table 7 above. With the exception of the relatively larger species, *D. brightwelli*, regression coefficients exceeded 0.7 ($P<0.05$) in all centric diatom species examined. In the

case of the chlorophyte genera (Dunaliella and Tetraselmis) however, r^2 values remained comparatively low (0.2 and 0.6 respectively) while a strong dependency was apparent for species of the prasinophyte genus *Isochrysis* (r^2 = 0.8).

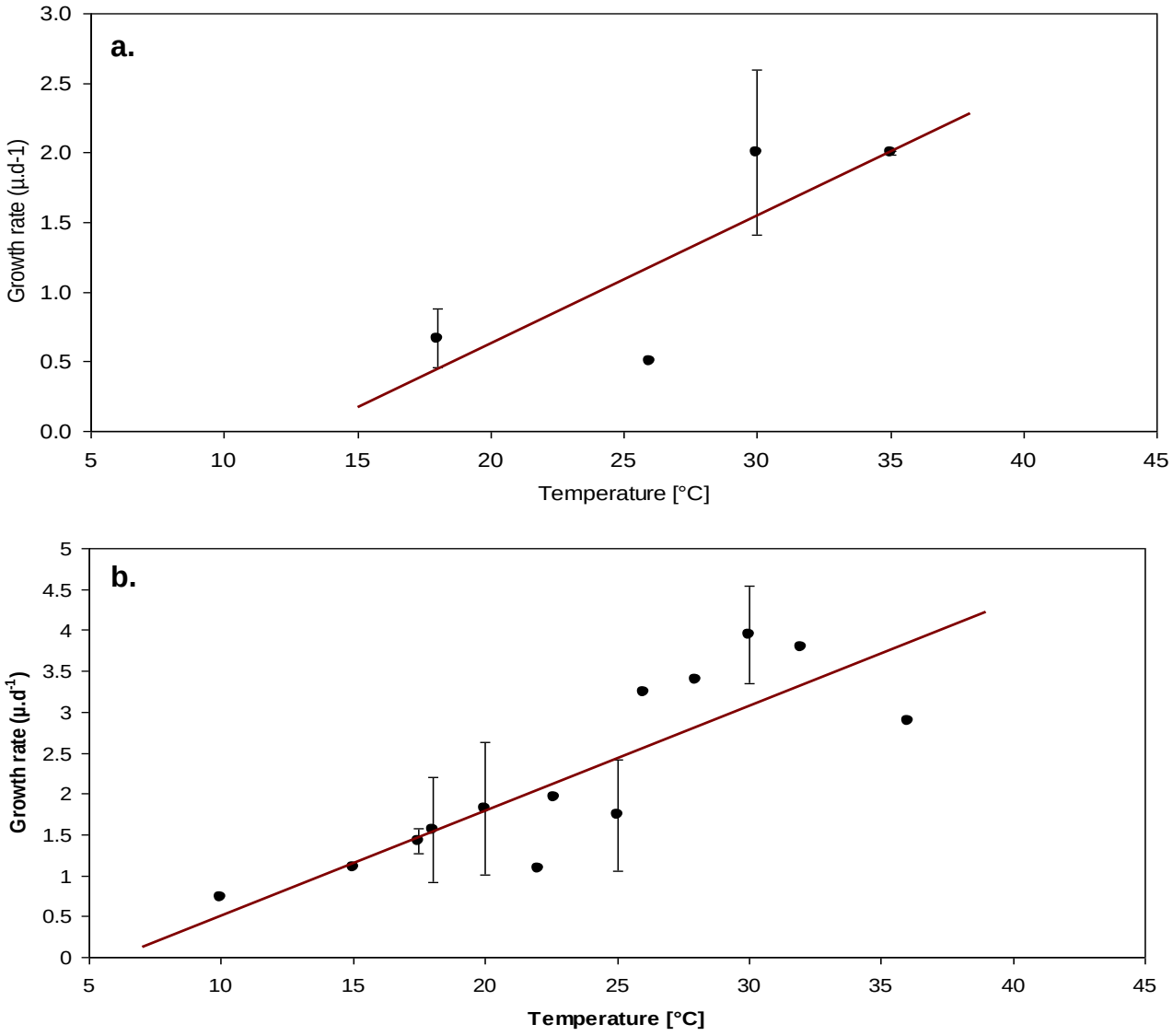


Fig. 18. Ordinary LSR of temperature related average growth rate in a) *Chaetoceros muellerii* (r^2 = 0.7) and b) *Chaetoceros neogracile* (r^2 = 0.7). P<0.05.

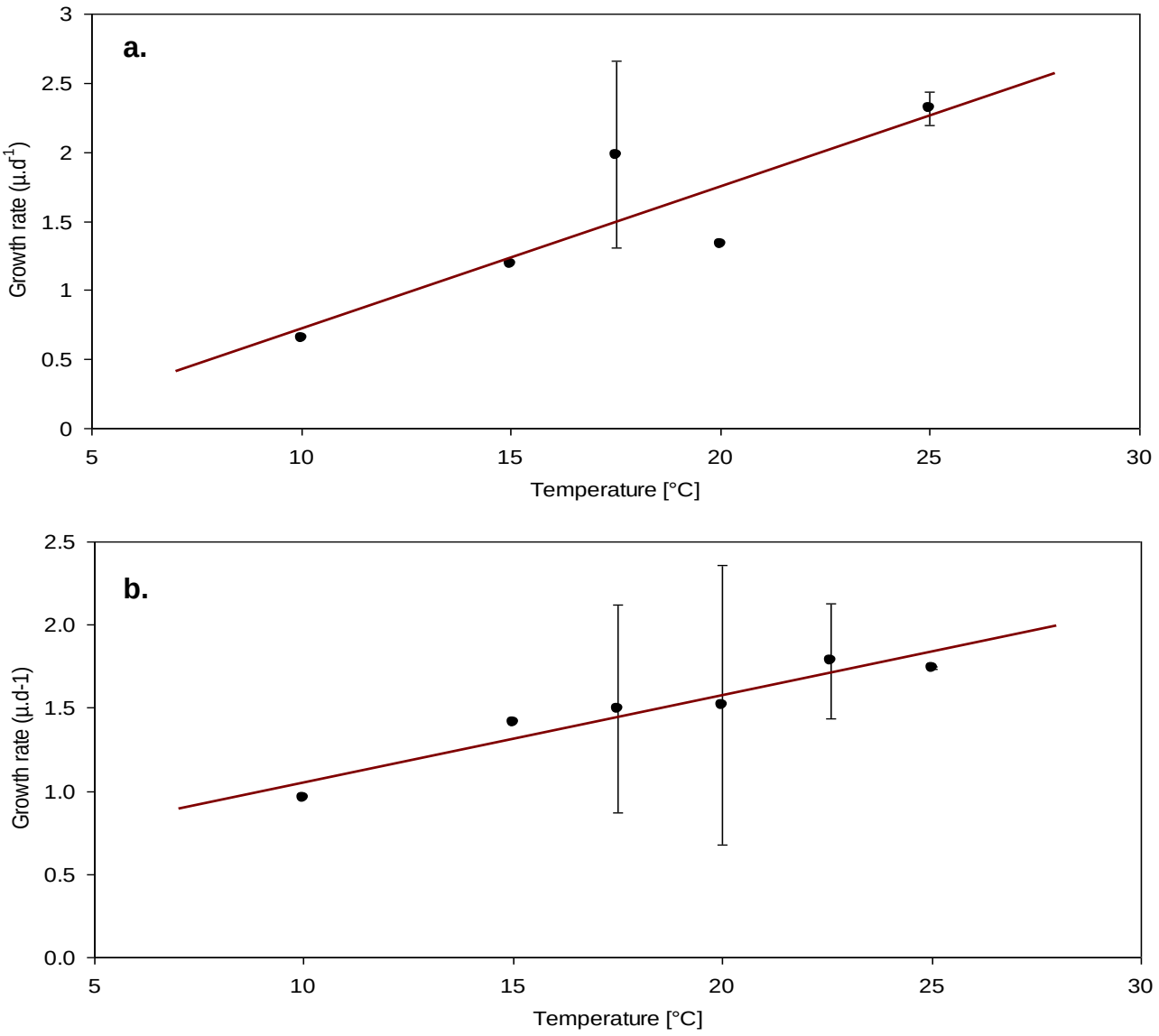


Fig. 19. Ordinary LSR of temperature related average growth rate in a) *Chaetoceros simplex* (r^2 = 0.8) and b) *Chaetoceros tenuissimus* (r^2 = 0.9). P<0.05.

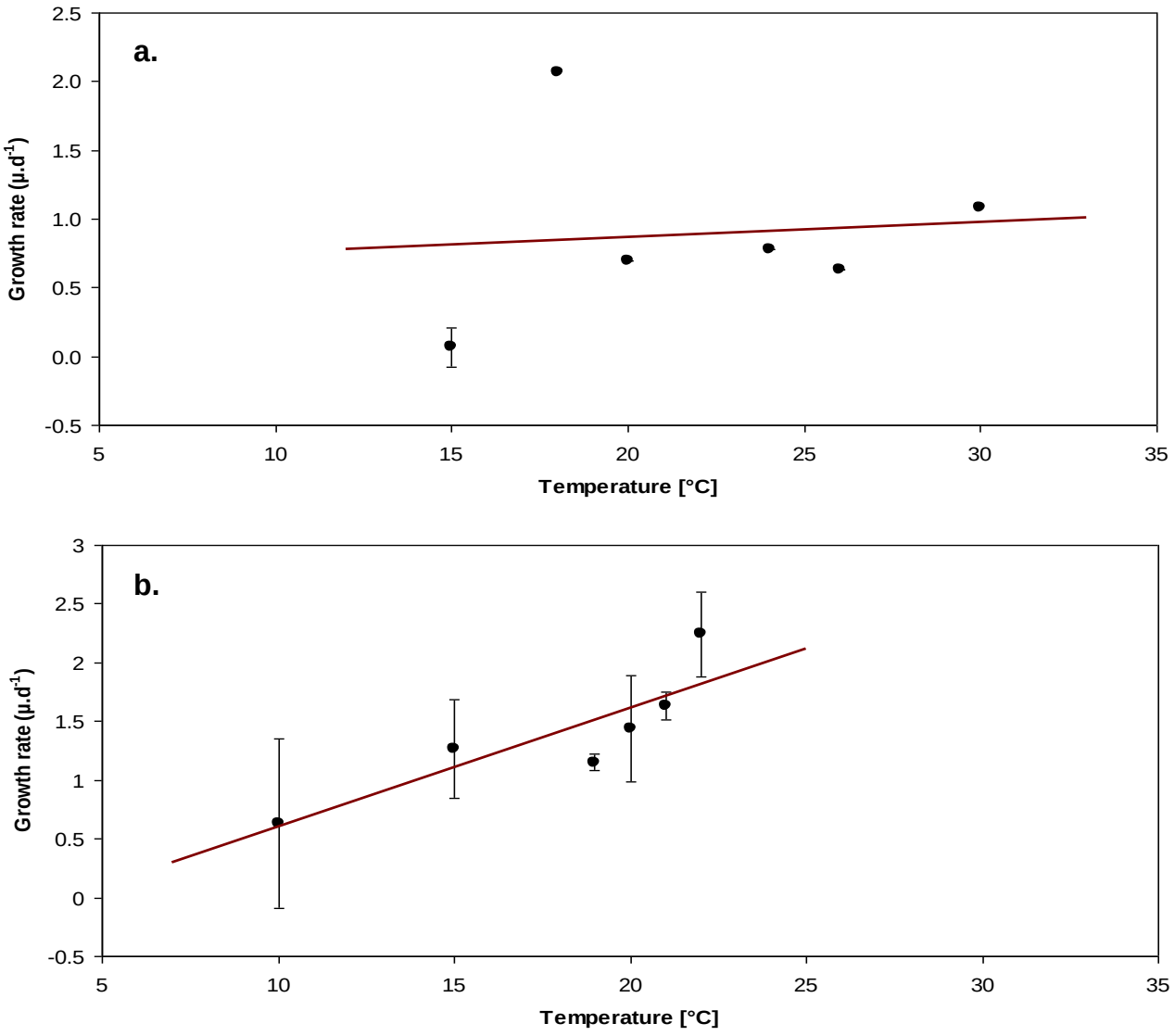


Fig. 20. Ordinary LSR of temperature related average growth rate in a) *Ditylum brightwellii* (r^2 = 0.008) and b) *Skeletonema costatum* (r^2 = 0.7). P<0.05.

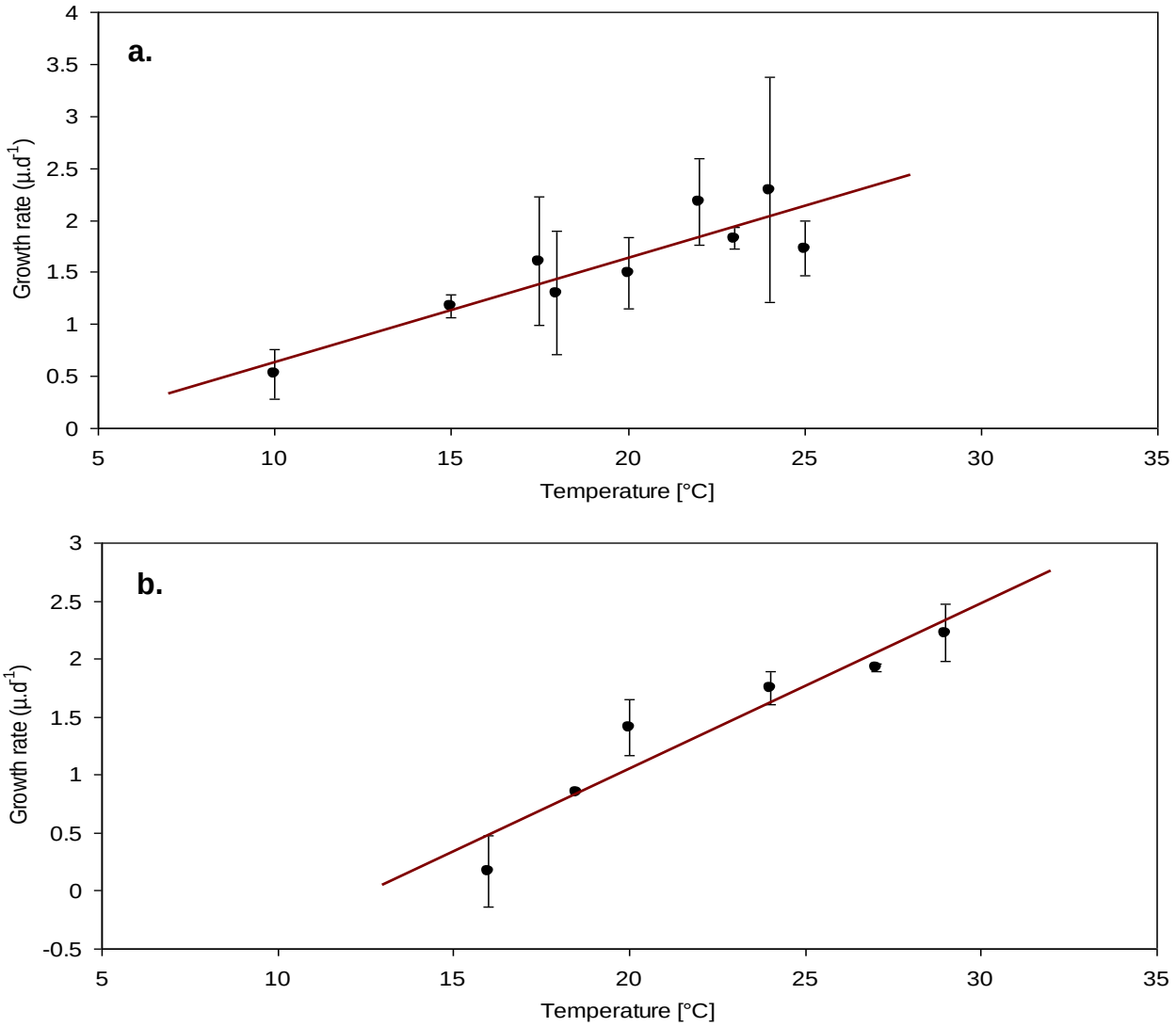


Fig. 21. Ordinary LSR of temperature related average growth rate in a) *Thalassiosira pseudonanna* (r^2 = 0.8) and b) *Thalassiosira weissflogii* (r^2 = 0.9). P<0.05.

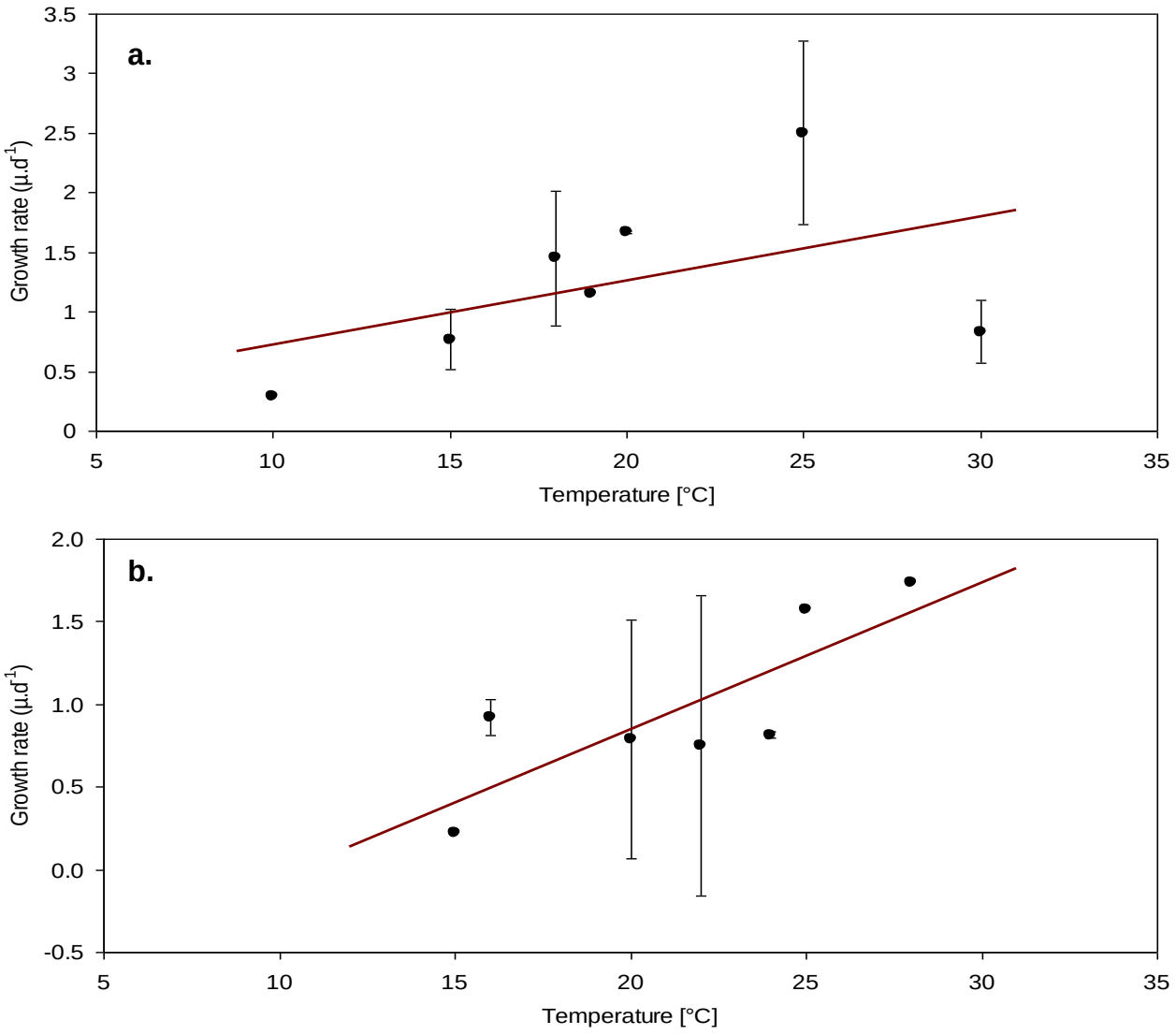


Fig. 22. Ordinary LSR of temperature related average growth rate in pooled species data for the chlorophyte genera a) *Dunaliella* (r^2 = 0.2) and b) *Tetraselmis* (r^2 = 0.6). P<0.05.

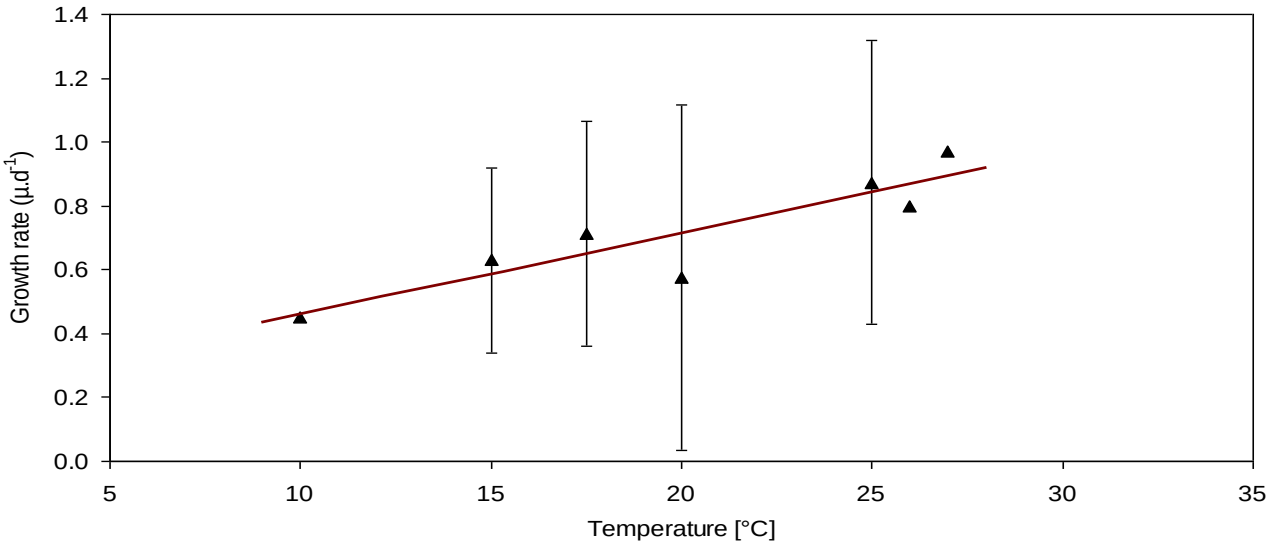


Fig. 23. Ordinary LSR of temperature related average growth rate in pooled species data for the prymnesiophyte genus *Isochyrsis* (r^2 = 0.8).

In addition to direct limitation of growth rate via reductions in metabolic process rates above a given temperature, secondary inhibition is also known to occur where for example, reduced rates of activity in thermosensitive processes such as i*n vivo* ATP generation (Raven 1988) and intra-thylakoidal RuBisCo activity (Salvucci 2004) reduce the quantum efficiency of cellular photosynthesis, inhibiting carbon fixation and thus growth at high temperatures. The selection therefore of predominantly smaller thermally acclimated tropical and subtropical species as opposed to the comparatively larger dinoflagellate species maximizes the odds of growth being maintained above the target threshold of 1.4 µ.d^{-1} and serves to illustrate the scientific rationale used to identify those species best suited for sustained productivity under mass cultivation conditions from an initial pool of >10,000 possible candidates.

Table 10. List of references from which data for growth v's temperature plots were obtained.

Class (ITIS scheme)	Species	Strain	Temperature (°C)	Reference
Coscinodiscophyceae	*Chaetoceros muellerii*	CS176	18	Giordano et al 01
		CS176	18	Liang et al 06
		CS176	26	Martinez-fernandez et al 06
		CHAET57	30	Johansen et al 90
		CHAET59	30	Johansen et al 90
		CHAET58	30	Johansen et al 90
		CHAET63	30	Johansen et al 90
		CHAET61	30	Johansen et al 90
		CHAET6	30	Johansen et al 90
		CHAET9	30	Johansen et al 90
		CHAET10	35	Johansen et al 90
		CHAET14	35	Sheehan et al 98
	Chaetoceros neogracile	Unknown	18	Levasseur et al 93
		Unknown	20	Lombardi & Wangersky 95
		CHAET-1	20	Mortensen et al 88
		CHAET-1	26	Mortensen et al 88
		CHAET-1	28	Mortensen et al 88
		CHAET-1	30	Mortensen et al 88
		CHAET-1	32	Mortensen et al 88
		CHAET-1	36	Mortensen et al 88
		Unknown	20	Robert & His 87
		CCAP1010/3	22.6	Robert et al 04
		Unknown	25	Taguchi et al 87
		Unknown	25	Thomas & Dodson 72
		Unknown	17.5	Thompson et al 90
		NEPCC645	10	Thompson et al 92b
		NEPCC645	15	Thompson et al 92b
		NEPCC645	17.5	Thompson et al 92b
		NEPCC645	20	Thompson et al 92b
		NEPCC645	25	Thompson et al 92b
	Chaetoceros simplex	NEPCC591	**10**	Thompson et al 92b
		NEPCC591	**15**	Thompson et al 92b
		Unknown	17.5	Thompson et al 90
		Unknown	17.5	Thompson et al 91
		NEPCC591	**17.5**	Thompson et al 92b
		NEPCC591	**20**	Thompson et al 92b
		NEPCC591	**25**	Thompson et al 92b
	Chaetoceros tenuissimus	NEPCC590	10	Thompson et al 92b
		NEPCC590	15	Thompson et al 92b
		Unknown	17.5	Thompson et al 90
		NEPCC590	17.5	Thompson et al 92b

		Unknown	17.5	Thompson et al 91
		Unknown	20	Robert & His 87
		NEPCC590	20	Thompson et al 92b
	Chaetoceros tenuissimus	Argenton	22.6	Robert et al 04
		NEPCC590	25	Thompson et al 92b
	Ditylum brightwellii	Unknown	14.5	Strickland et al 69
		Unknown	17.5	Thompson et al 91
		Unknown	20	Baars 1981
		DB	24	Brand & Guillard 81
		Unknown	26	Baars 1981
		Unknown	30	Baars 1981
	Skeletonema costatum	SK6C	**10**	Yoder 1979
		SK6C	**16**	Yoder 1979
		Unknown	19	Morales-Loo & Goutx 90
		Unknown	20	Blanchemain & Grizeau 96
		Unknown	20	Baars 1981
		Unknown	21	Chan 78
		Unknown	21	Chan 78
		SK6C	**22**	Yoder 1979
		CCAP1077	22.6	Robert et al 04
		SK-C-2	23	Sanchez-Saavedra & Voltolina 96
		SKEL	23	Shifrin & Chisholm 81
		GOC36	25	Renaud etal 99
		Unknown	30	Saanchez 95
	Thalassiosira pseudonana	CCMP1335	10	Stramski et al 02
		3H	**10**	Thompson et al 92b
		CCMP1335	15	Stramski et al 02
		3H	**15**	Thompson et al 92b
		3H	17.5	Thompson et al 90
			17.5	Thompson et al 91
		3H	**17.5**	Thompson et al 92b
		3H	18	Levasseur et al 93
		CCMP1335	18	Thompson 99
		CS-173	20	Brown et al 96
		CS-20	20	Mansour et al 05
		3H	**20**	Thompson et al 92b
		CCMP1335	22	Parker & Armbrust 05
		NEPCC58	22	Thompson et al 96
		CCAP1085/3	22.6	Robert et al 04
		TH-P-1	23	Sanchez-Saavedra & Voltolina 96
		3H	24	Brand & Guillard 81
		CCMP1335	25	Stramski et al 02
		3H	**25**	Thompson et al 92b
		NEPCC58	**25**	Thompson & Harrison 92

	Thalassiosira weissflogii	Actin	18.5	Milligan & Harrison 00
		CCMP1336	**20**	Strzepek & Price 00
		CCMP1336	**24**	Strzepek & Price 00
	Thalassiosira weissflogii	CCMP1336	**27**	Strzepek & Price 00
		CCMP1336	**29**	Strzepek & Price 00
Chlorophyceae	*Dunaliella bardawil*	ATCC30861	25	Ben-Amotz et al 85
	Dunaliella primolecta	Unknown	15	Uriarte et al 93
	Dunaliella salina	UTEX200	25	Ben-Amotz et al 85
	Dunaliella tertiolecta	NEPCC1	10	Thompson et al 92b
		NEPCC1	15	Thompson et al 92b
		Unknown	17.5	Thompson etal 90
		Unknown	17.5	Thompson et al 91
		NEPCC1	17.5	Thompson et al 92b
		Unknown	18	Levasseur et al 93
		Unknown	18	Siron et al 89
		Unknown	19	Morales-Loo & Goutx 90
		Unknown	20	Lombardi & Wangersky 95
		NEPCC1	20	Thompson et al 92b
		Unknown	25	de Lorenzo et al 04
		NEPCC1	25	Thompson et al 92b
	Dunaliella viridis	Fuente de Piedra, S Spain	25	Gordillo et al 98
Prasinophyceae	*Tetraselmis* sp.	CS-362	22	Brown et al 98
		Unknown	20	Abu-Rezq et al 99
		Unknown	23	Abu-Rezq et al 99
		BT-2	16	Maddux & Jones 64
		BT-2	23	Maddux & Jones 64
		BT-2	23	Maddux & Jones 64
		BT-2	31	Maddux & Jones 64
		Unknown	15	Molina Grima et al 91
		Unknown	20	Molina Grima et al 91
		Unknown	25	Molina Grima et al 91
		Unknown	28	Molina Grima et al 91
		Unknown	24	Reitan et al 94
		NT18	25	Renaud et al 99
		TEQL01	25	Renaud et al 99
	Tetraselmis suecica	Unknown	20	Robert & His 87
		CCAP66/4	23	Robert et al 04
Prymnesiophyceae	*Isochrysis aff. galbana*	T-ISO/AC-102	22.5	Ponis et al 06B
		T-ISO	20	Robert & His 87
	Isochrysis galbana	T-ISO	25	Brown et al 93
		T-ISO	22	Brown et al 93
		T-ISO	20	Lombardi & Wangersky 95

		Unknown	20	Phatarpekar et al 00
		T-ISO	24	Reitan et al 94
		Unknown	20	Robert & His 87
Prymnesiophyceae	*Isochrysis galbana*	CCAP927	22.6	Robert et al 04
		T-ISO	17.5	Thompson et al 90
		T-ISO	17.5	Thompson et al 91
		NEPCC601	10	Thompson etal 92b
		NEPCC601	15	Thompson et al 92b
		NEPCC601	17.5	Thompson et al 92b
		NEPCC601	20	Thompson et al 92b
		NEPCC601	25	Thompson et al 92b
	Isochrysis sp.	Unknown	20	Abu-Rezq et al 99
		Unknown	22.5	Abu-Rezq et al 99
		Unknown	25	Abu-Rezq et al 99
		UTEX2307	25	Ben-amotz etal 85
		CS177	26	Martinez-Fernandez et al 06
		CS177	25	Renaud & Parry 94
		T-ISO	25	Renaud et al 02
		T-ISO	27	Renaud et al 02
		PS11	10	Renaud et al 95
		PS11	15	Renaud et al 95
		PS11	20	Renaud et al 95
		PS11	25	Renaud et al 95
		NT14	25	Renaud et al 99
	Isochrysis T-ISO	CS177	10	Renaud et al 95
		CS177	15	Renaud et al 95
		CS177	20	Renaud et al 95
		CS177	25	Renaud et al 95
		Unknown	17.5	Thompson et al 91

3. Growth and intracellular lipid accumulation

With the assistance of key essential elements, namely nitrogen (N), phosphorous (P) and in the case of diatoms, silicon (Si), carbon (C) fixed during photosynthesis is metabolized to form the structural and functional proteins, carbohydrates and, lipids necessary for microalgal cell maintenance and vegetative growth. The requirement by microalgal cells for these key nutrients is most commonly described by the stoichiometric molecular ratio known as the Redfield Ratio.

C:Si:N:P = 106:15:16:1.

Under nutrient replete conditions where irradiance is at photosynthetically saturating levels, microalgal cells synthesize the structural proteins, lipids and amino acids necessary for cell growth. In contrast, during periods of N, Si and in some cases, P, deficiency, photosynthetically fixed carbon is diverted from the metabolic pathway leading to structural element synthesis to that synthesizing primary storage materials such as neutral lipids or carbohydrates. Microalgal lipids are synthesized from free fatty acids and divided into two primary classes, the structural polar lipids such as phospholipids and glycolipids, and the neutral storage lipids, such as mono, di and tri acyl glycerols. Polar lipids are an essential component of cell membranes and constitute approximately 5-20% of cell dry weight (Hu et al. 2008) Neutral lipids on the other hand, occur freely in the cell cytoplasm and consist primarily of long chain (C18-28) largely poly and mono unsaturated fatty acids that serve as an energy repository for cells during times of environmental or biochemical stress. Of primary interest here were the neutral triacylglycerols (TAGs), and in particular those comprised of shorter chain length (C12-16) monounsaturated fatty acids important in meeting industrial biodiesel fuel standards (Table 8). Therefore, both total and neutral lipid content and individual fatty acid profiles of several species of interest to the pre-screening process were quantitatively investigated to determine those species best suited to meeting these standards.

Table 11. Chemical properties important in meeting quality control standards for commercial biodiesel production. TFA = Total Fatty Acid. Cn = carbon chain length. x = double bonds.

TAG Concentration	C16:0 + C18:0	Cn:x (%TFA)	Mono C12 -16 %TFA	Total C12 -16 (%TFA)
15 %TFA	Max 5% TFA	Max x>3<1%	High	High

Cellular N depletion is the most common environmental factor leading to neutral lipid synthesis in microalgae (Larson and Rees 1996; Fidalgo et al. 1998; Ahlgren and Hyenstrand 2003; Zhila et al 2005). As the primary growth limiting nutrient, this also creates a trade-off

between high growth rate and high final biomass yields under N sufficient conditions and high total lipid yields under conditions of N deficiency. Nitrogen typically constitutes 7-10% of dry weight of exponentially growing microalgal (Hu 2004) and cell N must fall below a threshold level before active growth ceases and lipid accumulation begins. Considerable variation in the rate of cellular N depletion under conditions of nutrient stress however is known to occur both within and between species (Dortch 1982; Dortch 1984; Dortch, Thompson et al. 1991). This is primarily due to the relatively greater capacity for some species to accumulate N reserves under nutrient replete conditions which can then be utilized during times of nutrient stress. Generally speaking however, intracellular N-depletion begins upon cessation of exponential growth as cells enter the stationary phase of growth under batch culture conditions. Using pooled species data from the CMD, variations in the average lipid content (% dw) of cells maintained in N replete and N depleted cultures for species of the diatom and chlorophyte taxa and a third group comprising oleaginous species from various other taxa were investigated (Figs. 24 & 25). Substantial differences in total intracellular lipid yields were observed with an average of 19.5% more lipid in stressed compared to unstressed cells. A similar intra-specific pattern was also evident among ten strains of *Chaetoceros muellerii* (Fig. 26) with lipid concentrations on average two fold higher in stressed compared unstressed cells. This was also the case for this species under conditions of Si deficiency although total lipid concentrations in Si deficient cells remained ~ 5% lower than those reported under N stress.

An unusual albeit weak (r^2 = 0.3), trend of increasing ratios of intracellular lipid to carbohydrate was also observed for the chlorophyte species *Dunaliella primolecta* and *D. tertiolecta* under N-replete conditions (Fig. 27.b.) and underlines the high degree of variability in species specific responses to environmental stress. For example, under simultaneous conditions of N-depletion and supra-optimal irradiance, the preferential accumulation of carbohydrate rather than neutral lipid as the primary storage material has been reported for a number of microalgal species (Roessler 1988).

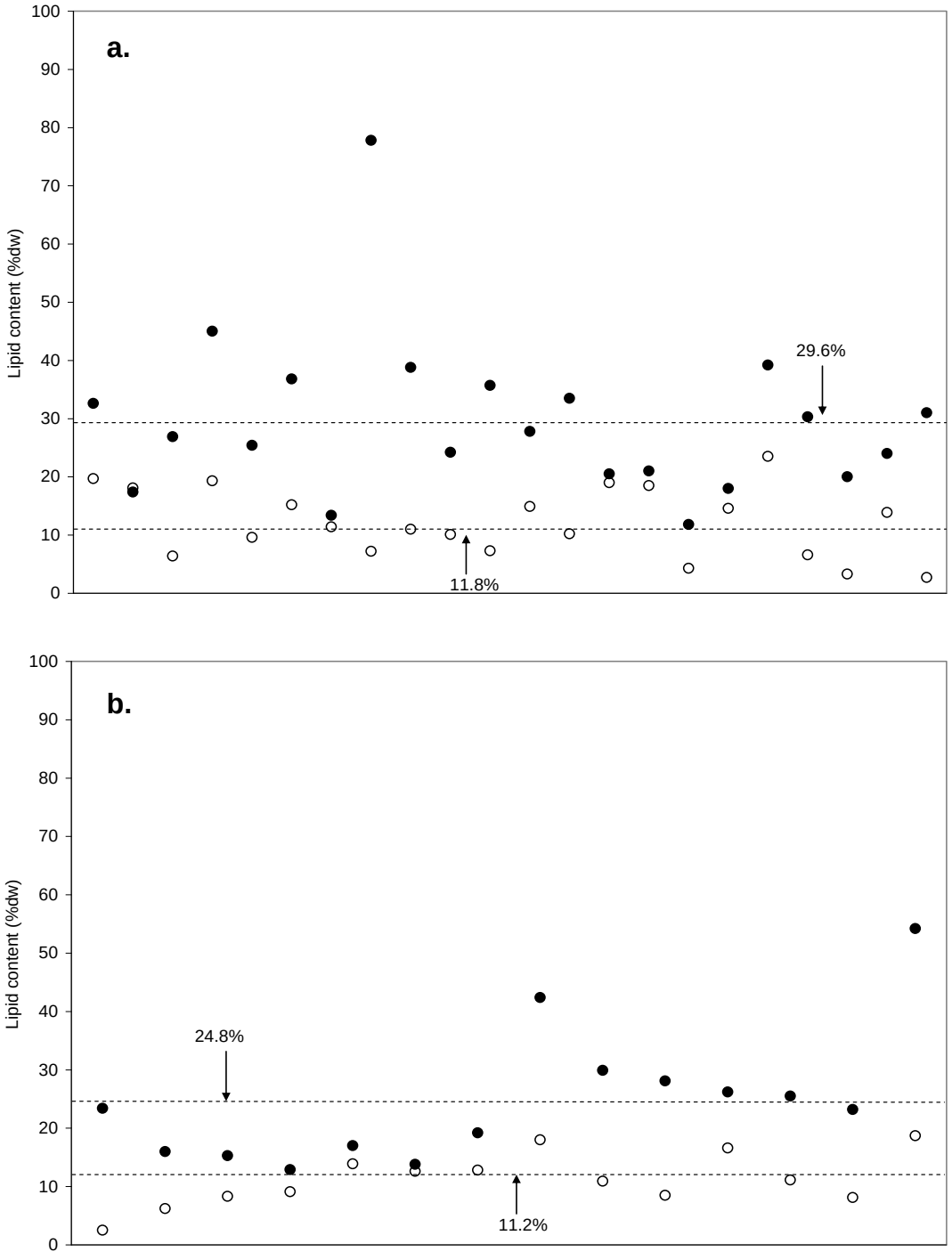


Fig. 24. Total cellular lipid content in various classes of microalgae under normal and stressed growth conditions. (a) Diatoms and (b) Chlorophytes. Closed circles: data obtained under nitrogen-depleted or other stress conditions. Dashed lines: maxima under stress and minima under normal growth conditions.

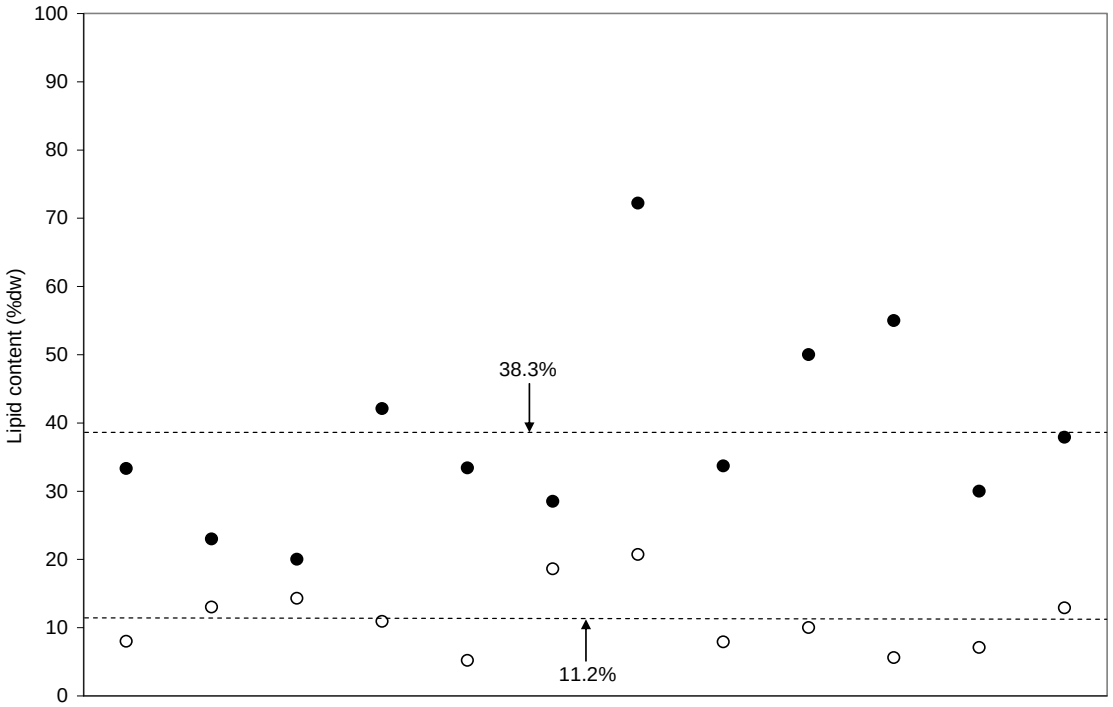


Fig. 25. Total cellular lipid content in various other oleaginous species under normal and stressed growth conditions. Closed circles: data obtained under nitrogen-depleted or other stress conditions. Dashed lines: maxima under stress and minima under normal growth conditions.

Table 12. List of references from which data for stressed/unstressed lipid plots were obtained.

Class (ITIS Scheme)	Species	Strain	Lipid (%dw)	Condition	Reference
Diatoms					
Coscinodiscophyceae	*Biddulphia aurita*	B-1	32.6	stressed	Shifrin & Chisholm 1981
Coscinodiscophyceae	*Biddulphia* sp.	Uknown	17.4	stressed	Uriarte et al. 2006
Coscinodiscophyceae	*Chaetoceros cal.citrans*	Uknown	26.9	stressed	Harrison et al. 90
Coscinodiscophyceae	*Chaetoceros muellerii*	CHAET57	45	stressed	Johansen et al. 90
Coscinodiscophyceae	*Chaetoceros* sp.	CH-X-1	25.4	stressed	Sanchez-Saavedra & Voltolina 1996
Coscinodiscophyceae	*Cyclotella cryptica*	7C	36.8	stressed	Shifrin & Chisholm 1981
Bacillariophyceae	*Navicula cf tenelloides*	Uknown	13.4	stressed	al.-hasan et al. 90
Bacillariophyceae	*Navicula incerta*	UTEX2044	77.8	stressed	Tan & Johns 1996
Bacillariophyceae	*Navicula pelliculosa*	IndianaCC668	38.8	stressed	Orcutt & Patterson 1975
Bacillariophyceae	*Navicula* sp.	NT4	24.2	stressed	Renaud et al. 1994
Bacillariophyceae	*Nitzchia laevis*	UTEX2047	35.7	stressed	Tan & Johns 1996
Bacillariophyceae	*Nitzschia frustulum*	Uknown	33.5	stressed	Renaud & Parry 1994
Bacillariophyceae	*Nitzschia inconspicua*	Uknown	20.5	stressed	Chu et al. 1996
Bacillariophyceae	*Nitzschia longissima*	Uknown	21	stressed	Orcutt & Patterson 1975
Bacillariophyceae	*Nitzschia oval.is*	IndianaCC680	11.8	stressed	Orcutt & Patterson 75
Bacillariophyceae	*Nitzschia* sp.	Uknown	18	stressed	Ben-Amotz et al. 1985
Bacillariophyceae	*Phaeodactylum tricornutum*	UTEX640	39.2	stressed	Yongmanitchai & Ward 1992
Bacillariophyceae	*Skeletonema costatum*	SKEL	30.3	stressed	Shifrin & Chisholm 1981
Coscinodiscophyceae	*Skeletonema* sp.	CS252	20	stressed	Brown & Jeffrey 1995
Coscinodiscophyceae	*Thal.asiossira weissflogii*	Actin	24	stressed	Shifrin & Chisholm 1981
Coscinodiscophyceae	*Thal.assiosira pseudonana*	CS-173	31	stressed	Brown et al. 1996
Coscinodiscophyceae	*Amphora coffeaformis*	NT13	19.7	unstressed	Renaud et al. 1999
Bacillariophyceae	*Amphora exigua*	IndianaCC685	18.1	unstressed	Orcutt & Patterson 1975
Bacillariophyceae	*Amphora luciae*	Uknown	6.4	unstressed	Gordon et al. 06
Bacillariophyceae	*Amphora* sp.	Uknown	19.3	unstressed	Orcutt & Patterson 1975
Bacillariophyceae	*Biddulphia sinensis*	MBAUK	9.6	unstressed	Yongmanitchai & Ward 91a
Coscinodiscophyceae	*Biddulphia sp.*	Uknown	15.2	unstressed	Uriarte et al. 2006
Coscinodiscophyceae	*Chaetoceros calcitrans*	Uknown	11.4	unstressed	Rivero-Rodriguez et al. 07
Coscinodiscophyceae	*Chaetoceros gracilis*	CS176	7.2	unstressed	Brown 91

Coscinodiscophyceae	*Chaetoceros muellerii*	CHAET39	11	unstressed	Johansen et al. 90
Coscinodiscophyceae	*Chaetoceros* sp.	CH-X-1	10.1	unstressed	Lopez-Elias et al. 93
Coscinodiscophyceae	*Cylindrotheca fusiformis*	MACC/B200	7.3	unstressed	Liang et al. 2005
Bacillariophyceae	*Fragilaria pinnata*	GOC1	14.9	unstressed	Renaud et al. 99
Fragilariophyceae	*Fragilaria* sp.	Uknown	10.2	unstressed	Orcutt & Patterson 75
Fragilariophyceae	*Lauderia annulata*	CS30	19	unstressed	Brown & Jeffrey 1995
Coscinodiscophyceae	*Melosira* sp.	NT3	18.5	unstressed	Renaud et al. 1994
Coscinodiscophyceae	*Navicula cf tenelloides*	Uknown	4.3	unstressed	al.-hasan et al. 90
Bacillariophyceae	*Navicula* cf. *lenzii*	Uknown	14.6	unstressed	Gordon et al. 06
Bacillariophyceae	*Navicula incerta*	Uknown	23.5	unstressed	Uriarte et al.. 2006
Bacillariophyceae	*Navicula jeffreyi*	CS-46	6.6	unstressed	Mansour et al. 05
Bacillariophyceae	*Navicula* sp.	CS-786	3.3	unstressed	Mansour et al. 05
Bacillariophyceae	*Nitzschia* cf. *frustulum*	CS258-1	13.9	unstressed	Renaud et al. 99
Bacillariophyceae	*Nitzschia conspicua*	111	2.7	unstressed	Chu et al. 94
Chlorophytes					
Prasinophyceae	*Tetraselmis suecica*	Uknown	23.4	stressed	Thomas et al. 1984a
Prasinophyceae	*Tetraselmis* sp.	CS-362	16	stressed	Brown et al. 98
Prasinophyceae	*Tetraselmis maculata*	Uknown	15.3	stressed	Wikfors et al. 84
Prasinophyceae	*Tetraselmis gracilis*	CN1	12.9	stressed	Lourenco et al. 02
Prasinophyceae	*Tetraselmis chui*	CS26	17	stressed	Brown 91
Prasinophyceae	*Tetraselmis* sp.	TEQL01	13.8	stressed	Renaud etal. 99
Prasinophyceae	*Teraselmis tetrathele*	Uknown	19.2	stressed	de La Pena & Villegas 05
Eustigmatophyceae	*Nannochloris sp.*	UTEX LB1999	42.4	stressed	Yamaberi et al. 98
Prasinophyceae	*Micromonas pusilla*	CS-170	29.9	stressed	Martinez-Fernandez & Southgate 07
Chlorophyceae	*Dunal.iella sal.ina*	NT1	28.1	stressed	Renaud et al. 1994
Chlorophyceae	*Dunal.iella primolecta*	Uknown	26.2	stressed	Uriarte et al. 1993
Trebouxiophyceae	*Chlorella* sp.	MFD-1	25.5	stressed	James et al. 1989
Trebouxiophyceae	*Chlorella minutissima*	Uknown	23.2	stressed	Seto et al. 1984
Chlorophyceae	*Botryococcus braunii*	Uknown	54.2	stressed	Thomas et al. 1984
Prasinophyceae	*Tetraselmis suecica*	Uknown	2.5	unstressed	Servel et al. 94
Prasinophyceae	*Tetraselmis sp.*	Uknown	6.2	unstressed	Molina Grima et al. 91
Prasinophyceae	*Tetraselmis maculata*	Uknown	8.3	unstressed	Wikfors et al. 84
Prasinophyceae	*Tetraselmis gracilis*	CN1	9.1	unstressed	Lourenco et al. 02
Prasinophyceae	*Tetraselmis chui*	CS26	13.9	unstressed	Dunstan et al. 92
Prasinophyceae	*Tetraselmis* sp.	NT18	12.6	unstressed	Renaud et al. 99
Prasinophyceae	*Tetraselmis tetrathele*	Uknown	12.8	unstressed	de La pena & Villegas 05
Eustigmatophyceae	*Nannochloris* sp.	Uknown	18	unstressed	Ben-Amotz et al. 1985
Prasinophyceae	*Micromonas pusilla*	CS170	10.9	unstressed	Dunstan et al. 92
Chlorophyceae	*Dunal.iella sal.ina*	UTEX200	8.5	unstressed	Ben-amotz et al. 85
Chlorophyceae	*Dunal.iella primolecta*	PlymouthCC81	16.6	unstressed	Thomas et al. 84A
Trebouxiophyceae	*Chlorella* sp.	CS247	11.1	unstressed	Dunstan et al. 92

Trebouxiophyceae	*Chlorella minutissima*	CN1	8.1	unstressed	Lourenco et al. 02
Chlorophyceae	*Botryococcus braunii*	UTEX572	18.7	unstressed	Ben-Amotz et al. 1985
Other Oleaginous Species					
Xanthophyceae	*Ellipsoidion* sp.	70-01	33.3	stressed	Xu et al. 2000
Prymnesiophyceae	*Emiliania huxleyi*	Bt-6	23	stressed	Pillsbury 1985
Prymnesiophyceae	*Hymenomonas carterae*	Cocco2	20	stressed	Shifrin & Chisholm 1981
Prymnesiophyceae	*Isochrysis gal.bana*	ISO	42.1	stressed	Fidal.go et al. 98
Prymnesiophyceae	*Isochrysis* sp.	T-ISO	33.4	stressed	Thomas et al. 1984b
Prymnesiophyceae	*Isochrysis* T-ISO	CS177	28.5	stressed	Renaud et al. 1995
Xanthophyceae	*Monal.lantus sal.ina*	GSB Sticho	72.2	stressed	Shifrin & Chisholm 1981
Eustigmatophyceae	*Nannochloropsis oculata*	CS170	33.7	stressed	Renaud & Parry 1994
Eustigmatophyceae	*Nannochloropsis sal.ina*	Uknown	50	stressed	Emdadi & Berland 1989
Eustigmatophyceae	*Nannochloropsis* QII	Uknown	55	stressed	Suen et al. 87
Prymnesiophyceae	*Pavlova lutheri*	Uknown	30	stressed	Emdadi & Berland 1989
Prymnesiophyceae	*Prymnesium parvum*	Uknown	37.9	stressed	Ricketts 1966
Xanthophyceae	*Ellipsoidion* sp.	70-01	8	unstressed	Xu et al. 00
Prymnesiophyceae	*Emiliania huxleyi*	UTEXLB1016	13	unstressed	Yongmanitchai & Ward 91a
Prymnesiophyceae	*Hymenomonas carterae*	Cocco2	14.3	unstressed	Shifrin & Chisholm 81
Prymnesiophyceae	*Isochrysis gal.bana*	II-4	10.9	unstressed	Molina Grima et al. 94B
Prymnesiophyceae	*Isochrysis* sp.	UTEX2307	5.2	unstressed	Ben-Amotz etal. 85
Prymnesiophyceae	*Isochrysis* T-ISO	CS177	18.6	unstressed	Renaud et al. 1995
Xanthophyceae	*Monal.lantus sal.ina*	GSB Sticho	20.7	unstressed	Thomas et al. 1984b
Eustigmatophyceae	*Nannochloropsis oculata*	CS216	7.9	unstressed	Volkman et al. 93
Eustigmatophyceae	*Nannochloropsis sal.ina*	GSB STICHO	10	unstressed	Boussiba et al. 87
Eustigmatophyceae	*Nannochloropsis* sp.	Uknown	5.6	unstressed	Servel et al. 94
Prymnesiophyceae	*Pavlova lutheri*	MONO	7.1	unstressed	Brown et al. 93A
Prymnesiophyceae	prymnesiophyte	CS260	12.9	unstressed	Thinh et al. 99

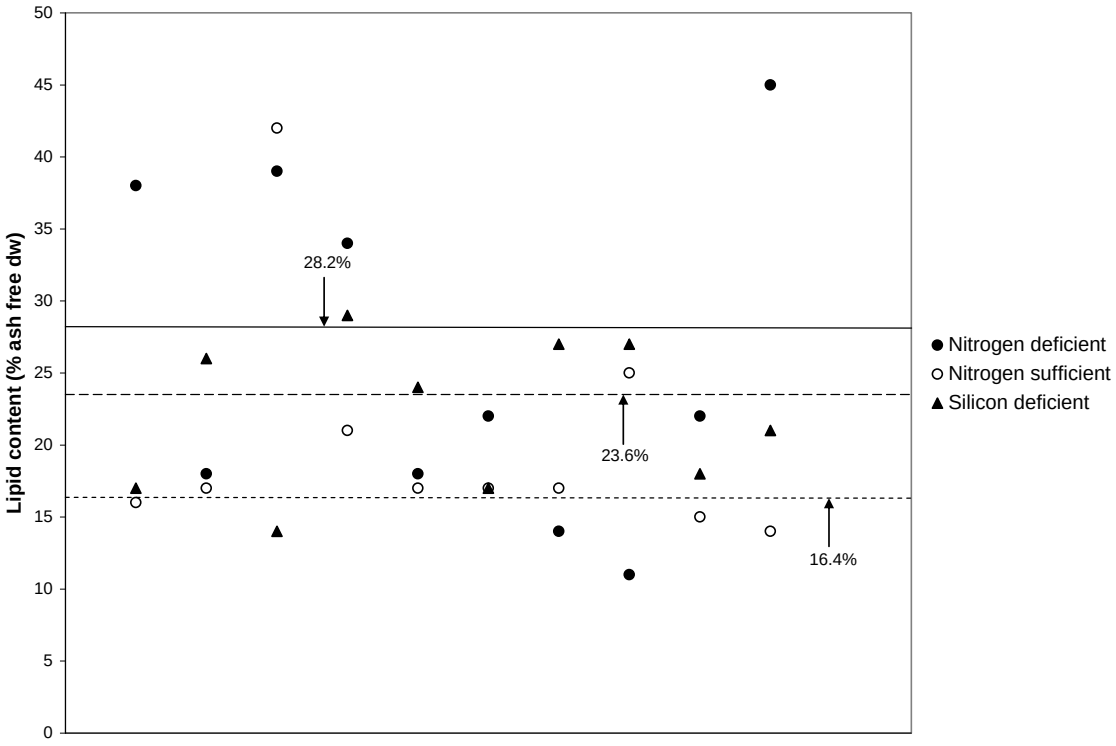


Fig. 26. Variation in total cellular lipid content (% ash free dry weight) in ten strains of *Chaetoceros muellerii*. Lines represent average content under nitrogen (solid) and silicon deficiency (long dash) and nitrogen sufficiency (short dash) respectively. Adapted from Johansen et al. 1990.

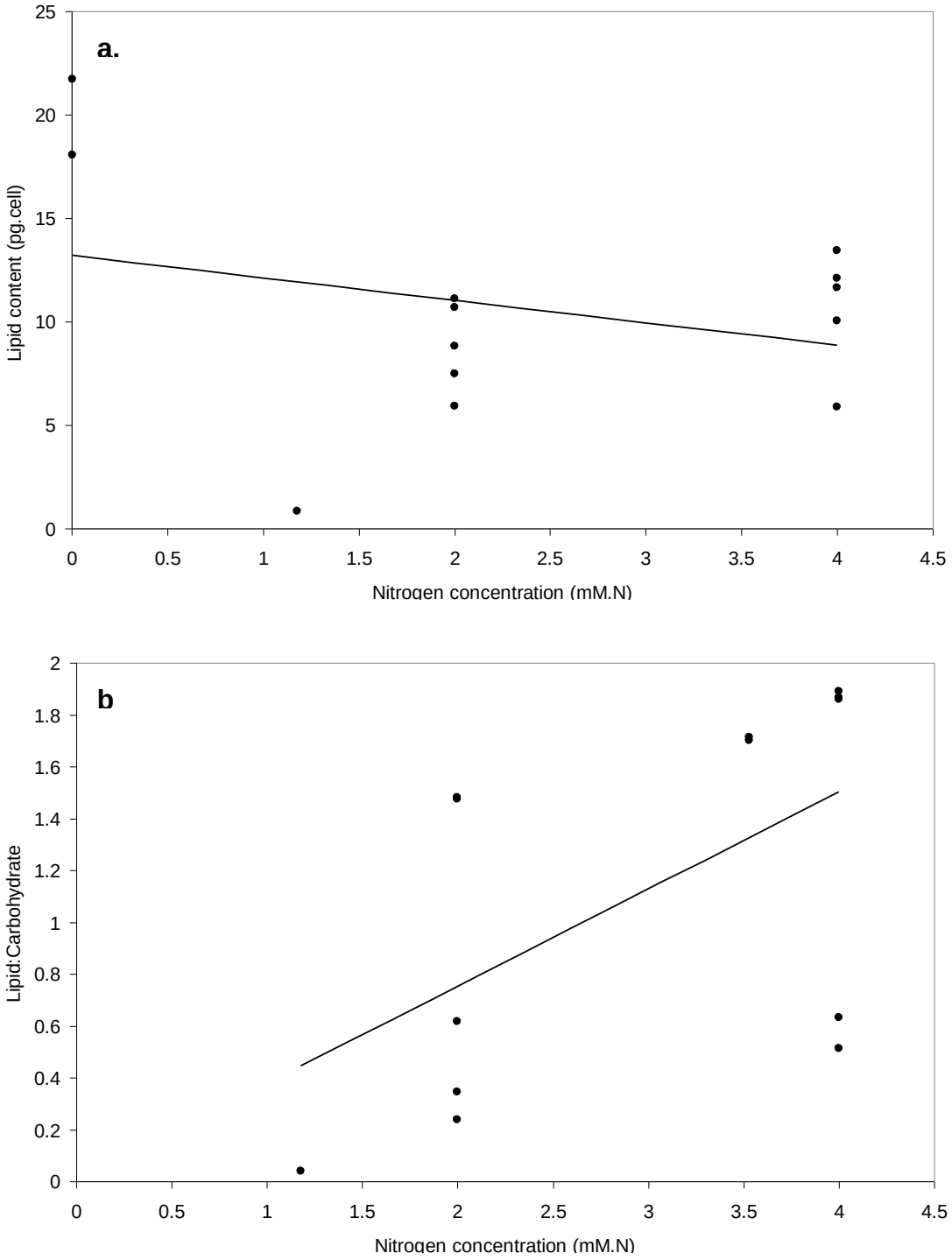


Fig. 27. . Ordinary LSR plots showing a) total intracellular lipid concentrations (r^2 = 0.09) and b) intracellular ratio of lipid to carbohydrate (r^2 = 0.3) as a function of nitrogen concentration in batch cultures of *Dunaliella primolecta* and *Dunaliella tertiolecta.* P<0.05.

Table 13. List of references from which data for nitrogen v's both total lipid and lipid:carbohydrate plots were obtained.

Class (ITIS Scheme)	Species	Strain	Nitrogen (µ.M NO_3)	Lipid:Carb	Reference
Chlorophyceae	*Dunaliella tertiolecta*	Unknown	2	0.23	Fabregas et al 95a
		Unknown	2	0.34	Fabregas et al 95a
		Unknown	2	0.61	Fabregas et al 95a
		Unknown	2	1.47	Fabregas et al 95a
		Unknown	2	1.48	Fabregas et al 95a
		Unknown	4	0.51	Fabregas et al 95a
		Unknown	4	0.63	Fabregas et al 95a
		Unknown	4	1.86	Fabregas et al 95a
		Unknown	4	1.86	Fabregas et al 95a
		Unknown	4	1.89	Fabregas et al 95a
		Unknown	3.53	1.70	Uriarte et al 93
	Dunaliella primolecta	Unknown	3.53	1.711	Uriarte et al 93
		Unknown	1.18	0.03	Uriarte et al 93

Reasons for the high degree of inter and intra specific variability observed here are as yet poorly understood (Hu 2004). For example, within a single genus of *Chlorella,* the accumulation of large amounts of carbohydrates (as starch) was observed in some strains and not in others maintained under similar conditions of N-starvation (Richmond 1986). Using the CMD, such variability was assessed at the class level for the diatom, chlorophyte, eustigmatophyte, prasinophyte and pyrmnesiophyte taxa (Fig 28). Results indicate a generally decreasing trend in the ratios of intracellular lipid to carbohydrate as irradiance increases above 400 $\mu.E.m^{-2}.s^{-1}$, with a maximum regression coefficient (r^2 = 0.6) (Table 9) associated with the centric diatom class, *Coscinodiscophyceae*. At the species level, a similarly decreasing trend was observed for both the centric diatom species, *Thalassiosira pseudonanna* (r^2 = 0.5) (Fig. 29.b.) and to a lesser degree in the unidentified prymnesiophyte strain *Isochrysis* sp (r^2 = 0.05) (Fig. 30.b.).

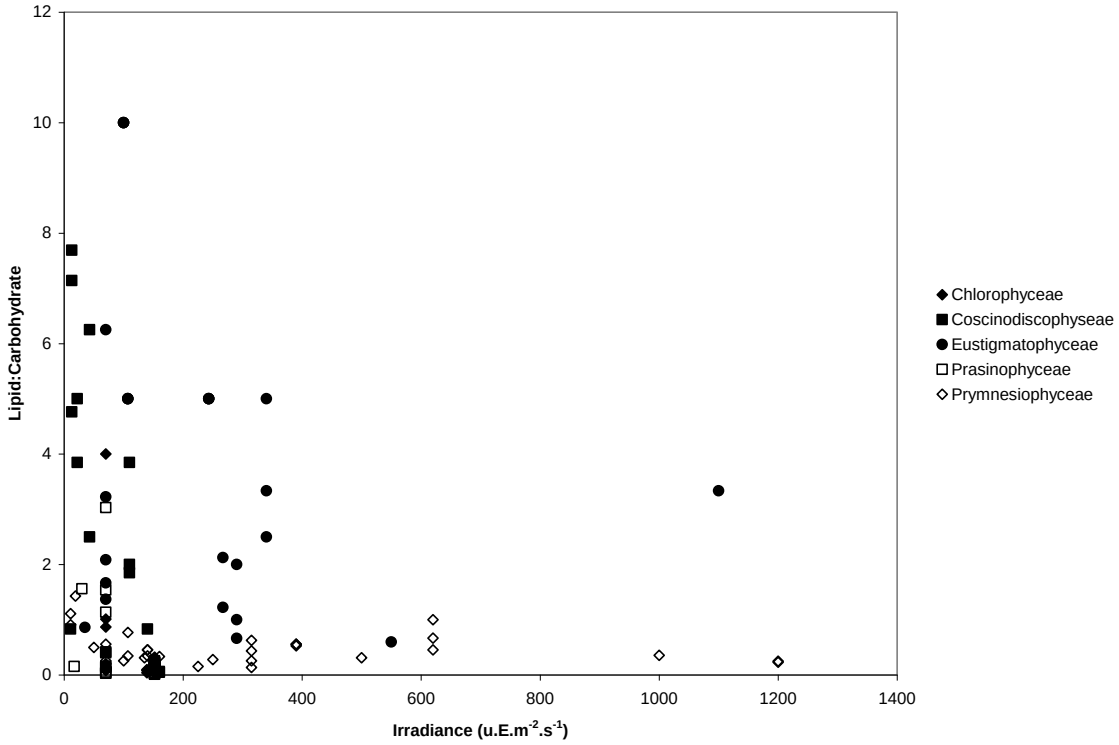


Fig. 28. Scatter plot showing decreasing ratio of lipid to carbohydrate under increasing culture irradiance in five microalgal classes.

In addition, a correlation between irradiance levels within the range of 6 to 225 µ.E.m^{-2}.s^{-1} and the relative percentages of both saturated fatty acids (max at 44 µ.E.m^{-2}.s^{-1}) and those containing more than 3 carbon double bonds was apparent in different cultures of the same strain of the diatom, *Chaetoceros simplex* (Fig. 31). To ensure compliance with international quality standards for commercial biodiesel these properties need to be maintained below 5% and 1% of total TAG fatty acids respectively. Noteworthy here was a high degree of both between species and between strain variability in fatty acid profiles in response to increases in both irradiance and temperature for several other CMD/pre-screen species. For example, increasing irradiance had the dual effect of increasing the overall chain length of unsaturated fatty acids while also producing the required decrease in the double bondedness of these chains.

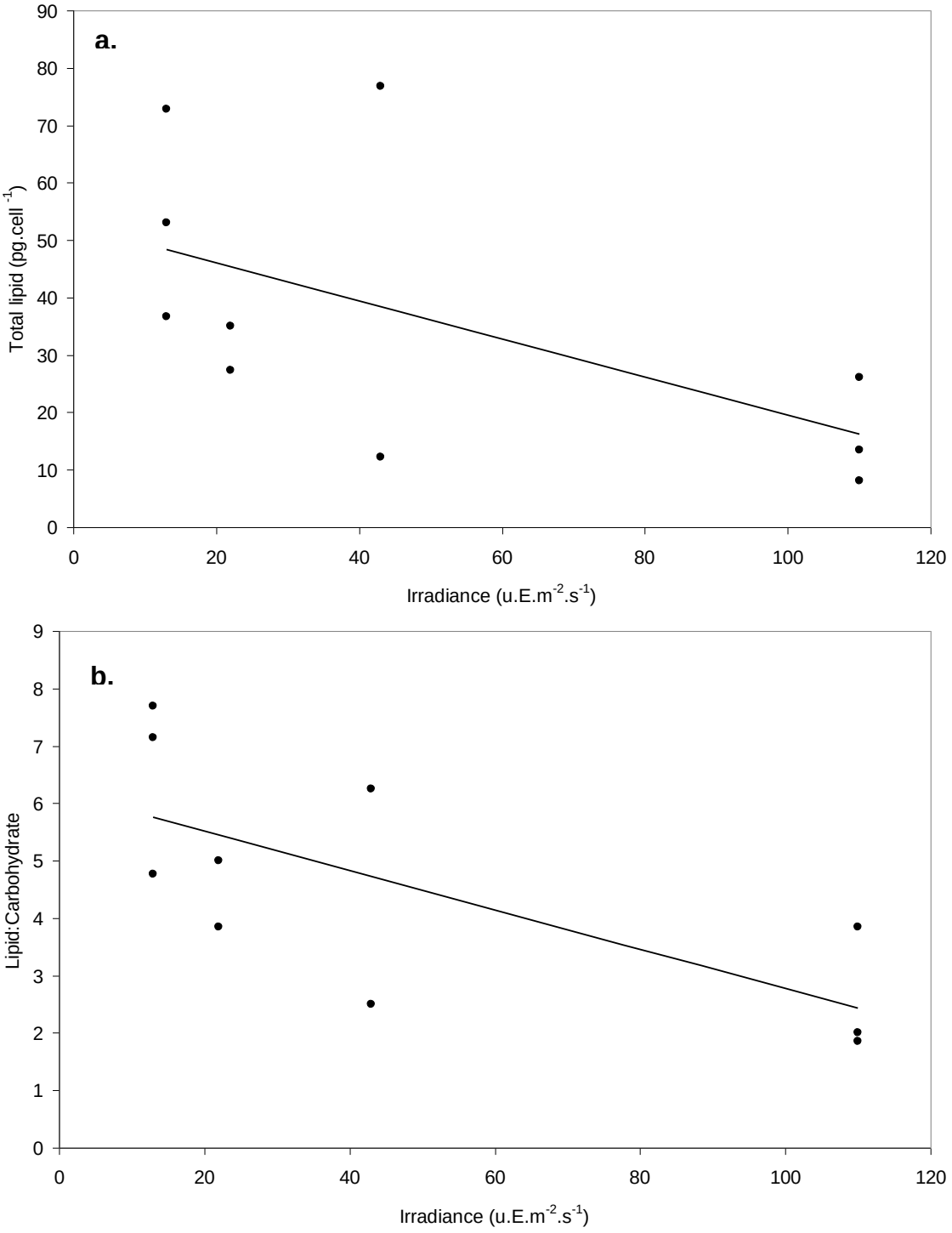
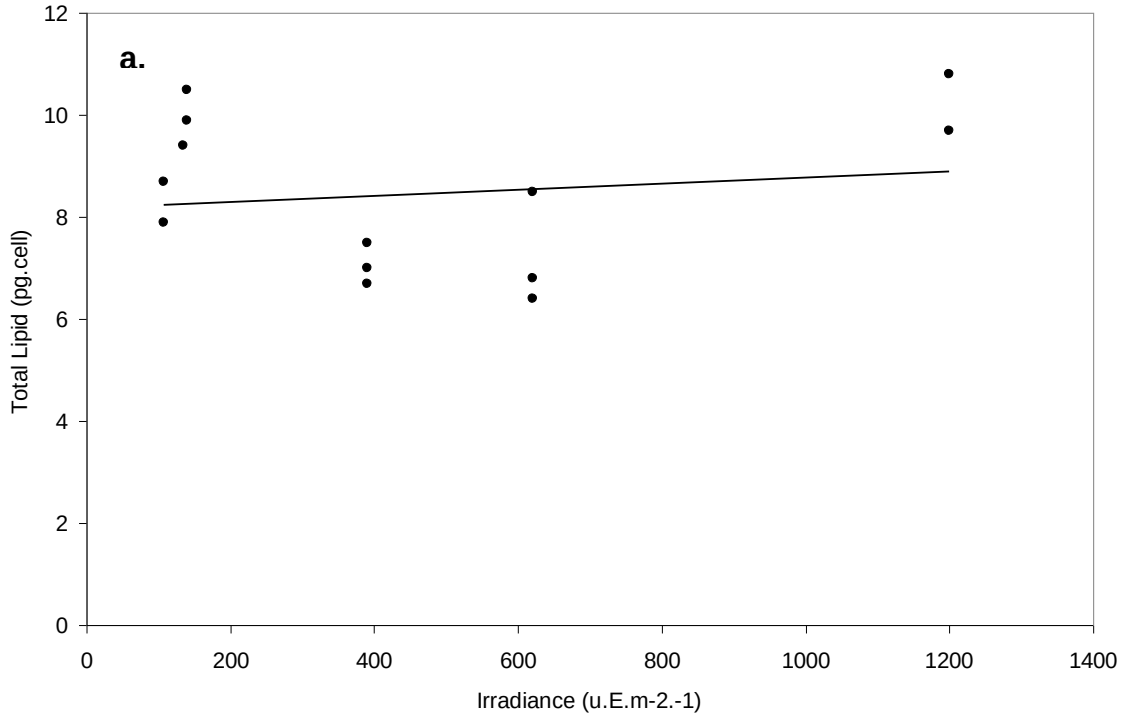


Fig. 29. Ordinary LSR plots showing a) total intracellular lipid concentrations (r^2 = 0.3) and b) intracellular ratio of lipid to carbohydrate (r^2 = 0.5) as a function of irradiance in batch cultures of *Thalassiosira pseudonanna.* P<0.05.



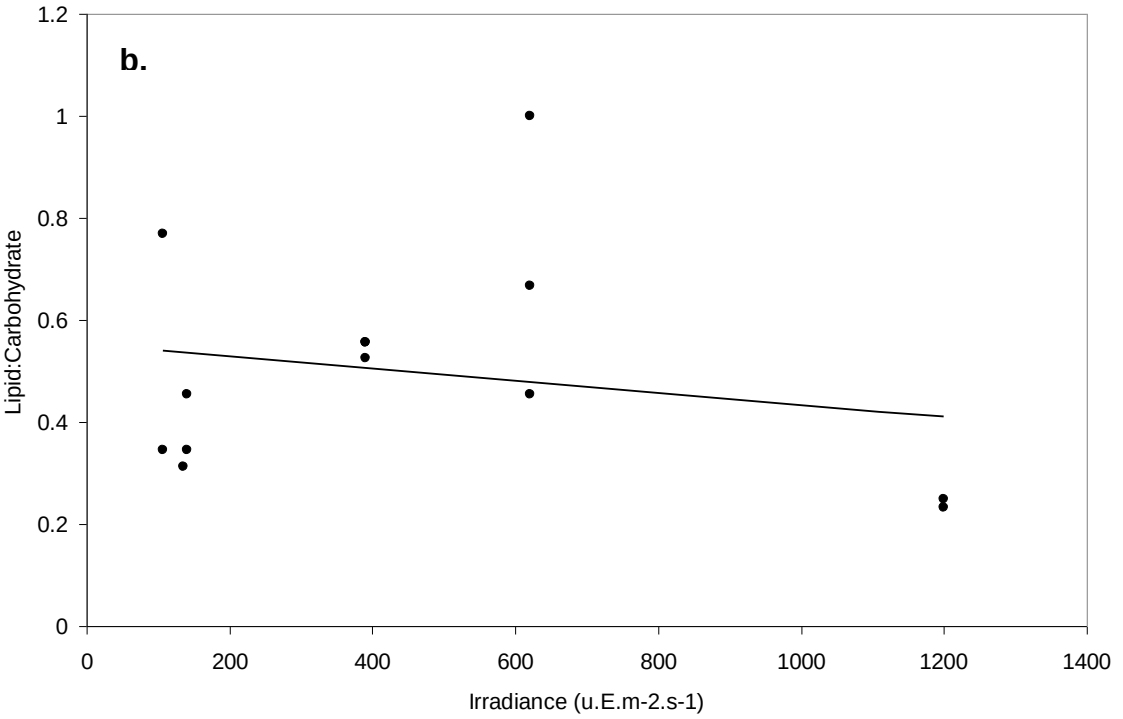


Fig. 30. Ordinary LSR plots showing a) total intracellular lipid concentrations (r^2 = 0.02) and b) intracellular ratio of lipid to carbohydrate (r^2 = 0.05) as a function of irradiance in batch cultures of *Isochrysis* sp.. P<0.05.

Table 14. Regression coefficients and statistical parameters associated with ordinary LSR of lipid:carbohydrate v's irradiance for pooled species data from five microalgal classes (*n* = number of sample points).

Class	*n*	r^2	St. Dev	Slope	y-intercept
Chlorophyceae	18	0.3	0.9	0.014	2.2
Coscinodiscophyceae	32	0.6	2.3	0.031	4.9
Eustigmatophyceae	25	0.06	2.8	0.003	4.6
Prasinophyceae	14	0.2	0.9	0.008	1.3
Prymnesiophyceae	34	0.02	0.3	0.017	0.5

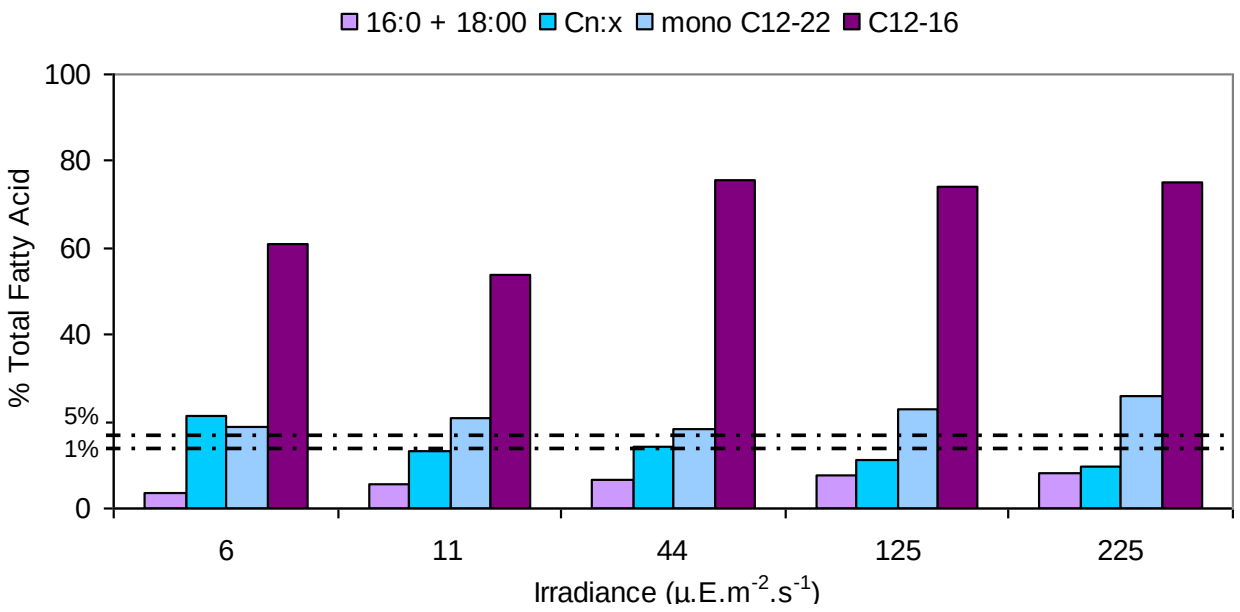


Fig. 31. Inter and intra-strain variation in relative proportions of fatty acids important in meeting international quality standards for commercial biodiesel as a function of irradiance in various culture collection strains of *C. simplex*. Dashed lines: critical limits of key chemical criteria i.e. Cn:x, where x> 3 < 1% TFA and maximum degree of saturation where C16:0 + C18:0 < 5% TFA.

Table 15. List of references from which data for irradiance v both total lipid and lipid:carbohydrate plots were obtained.

Class (IT IS Scheme)	Species	Strain	Irradiance $\mu.E.m^{2}.s^{-1}$	Lipid $pg.cell^{-1}$	Carb $pg.cell^{-1}$	Lipid:Carb	Reference
Coscinodiscophyceae	*Thalassiosira pseudonana*	NEPCC58	43	76.88	12.30	6.25	Thompson & Harrison 92
		NEPCC58	13	72.86	10.20	7.14	Thompson & Harrison 92
		NEPCC58	110	8.15	4.40	1.85	Thompson & Harrison 92
		NEPCC58	22	35.00	7.00	5.00	Thompson & Harrison 92
		NEPCC58	43	12.25	4.90	2.50	Thompson & Harrison 92
		NEPCC58	13	53.08	6.90	7.69	Thompson & Harrison 92
		NEPCC58	110	13.40	6.70	2.00	Thompson & Harrison 92
		NEPCC58	22	27.31	7.10	3.85	Thompson & Harrison 92
		NEPCC58	13	36.67	7.70	4.76	Thompson & Harrison 92
		NEPCC58	110	26.15	6.80	3.85	Thompson & Harrison 92
Prymnesiophyceae	*Isochrysis* sp.	T-ISO	107	2.72	7.90	0.34	Renaud et al 91
		T-ISO	107	6.69	8.70	0.77	Renaud et al 91
		T-ISO	135	2.94	9.40	0.31	Renaud et al 91
		T-ISO	140	4.50	9.90	0.45	Renaud et al 91
		T-ISO	140	3.62	10.50	0.34	Renaud et al 91
		T-ISO	390	3.72	6.70	0.56	Renaud et al 91
		T-ISO	390	3.89	7.00	0.56	Renaud et al 91
		T-ISO	390	3.95	7.50	0.53	Renaud et al 91
		T-ISO	620	4.27	6.40	0.67	Renaud et al 91
		T-ISO	620	6.80	6.80	1.00	Renaud et al 91
		T-ISO	620	3.86	8.50	0.45	Renaud et al 91
		T-ISO	1200	2.26	9.70	0.23	Renaud et al 91
		T-ISO	1200	2.70	10.80	0.25	Renaud et al 91

Table 16. List of references from which data for irradiance v fatty acid constituent (% Total Fatty Acids) charts were obtained for *Chaetoceros simplex*.

Class (IT IS Scheme)	Species	Strain	Irradiance ($\mu.E.m^2.s^{-1}$)	16:0+18:0	Cn:x	C12-22	C12-16	Reference
	Chaetoceros simplex	NEPCC595	6	3.8	21.3	18.7	61.1	Thompson et al 90
		NEPCC591	11	5.7	13.1	21.0	53.9	Thompson et al 93
		NEPCC594	44	6.8	14.4	18.5	75.75	Thompson et al 90
		NEPCC593	125	7.8	11.4	22.7	74.32	Thompson et al 90
		Average	225	8.2	9.6	25.8	75.095	Thompson et al 90
		NEPCC591 + 592						

Table 17. List of references from which data for temperature v fatty acid constituent (% Total Fatty Acids) charts (Fig. 36) were obtained for *Chaetoceros simplex.*

Class (IT IS Scheme)	Species	Strain	Temperature (°C)	16:0+18:0	Cn:x	C12-22	C12-16	Reference
	Chaetoceros simplex	NEPCC591	10	29.2	23.2	29.5	75.6	Thompson et al 92b
		NEPCC591	15	10.2	8.4	24.2	75.5	Thompson et al 92b
		NEPCC591	17.5	9.5	11.3	28.0	85.5	Thompson et al 92b
		NEPCC591	20	10.6	3.8	28.3	86.9	Thompson et al 92b
		NEPCC591	25	8.4	10.6	23.5	78.1	Thompson et al 92b

An examination of the effects of increasing temperature on both total lipid, the ratio of lipid to carbohydrate and key fatty acid constituents was also conducted at the class, species and strain levels using data from the CMD. These revealed a largely opposite linear trend in both total lipid concentration and lipid:carbohydrate as temperatures increased to a maximum of 30° C. At the class level, a comparatively strong positive relationship was returned for both the centric diatom (r^2 = 0.5) and chlorophyte (r^2 = 0.6) taxa (Fig. 32). This was supported in the former case by LSR analysis of data pertaining to the diatom, *Thalassiosira pseudonanna*, although a stronger temperature dependency was apparent in terms of the relative proportions of lipid to carbohydrate (r^2 0.4) (Fig. 34.b.) when compared to the overall effect of increasing temperature on total cellular lipid (r^2 = 0.03) (Fig. 34.a.). Interestingly, a weak negative relationship was found in terms of both total and proportional lipid concentrations in the chlorophyte species, *Pavlova lutheri* (Figs. 35) and is thought to reflect the reportedly greater tendency for carbohydrate accumulation in chlorophyte versus diatom species.

In contrast to the effects of increasing irradiance, increasing temperature was found to enhance the percentage of desirable short carbon chain (C12 -16) length fatty acids and these were found to attain abundances of > 80% total TAG in *Chaetoceros simplex* strain NEPCC591 (Fig. 36). Conversely, decreasing temperatures were found to increase the relative proportions of saturated fatty acids and those containing more than 1% carbon double bonds both of which are unfavorable properties for the production of high quality biodiesel fuel. Illustration of the high degree of intra-strain variability to these properties is also provided for in Fig. 37 for the diatoms, *Chaetoceros calcitrans* and *C. muellerii* both of which were of interest to the pre-screening process. Further illustration of variation in fatty acid profile is provided at the species level in Fig. 38. These analyses underline the need for species-specific cultivation strategies that regulate nutrient starvation according to seasonal patterns of irradiance and temperature conducive to the preferential and maximal synthesis of fatty acids suitable for biodiesel production.

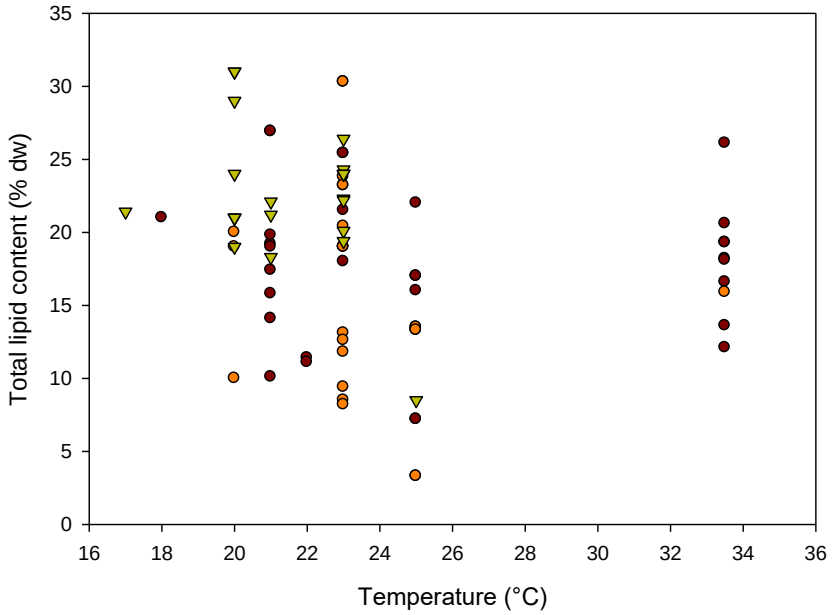


Fig. 32. Scatter plot showing decreasing ratio of lipid to carbohydrate under increasing culture temperature in five microalgal classes.

Table 18. Regression coefficients and statistical parameters associated with ordinary LSR of lipid to carbohydrate ratio v's temperature for pooled species data from five microalgal classes (*n* = number of sample points).

Class	***n***	**r^2**	**St. Dev**	**Slope**	**y-intercept**
Chlorophyceae	18	0.5	0.9	0.25	4.5
Coscinodiscophyceae	34	0.6	2.2	0.6	11.535
Eustigmatophyceae	25	0.02	2.8	0.55	0.18
Prasinophyceae	15	0.1	0.9	0.11	1.81
Prymnesiophyceae	34	0.02	0.3	0.005	0.5

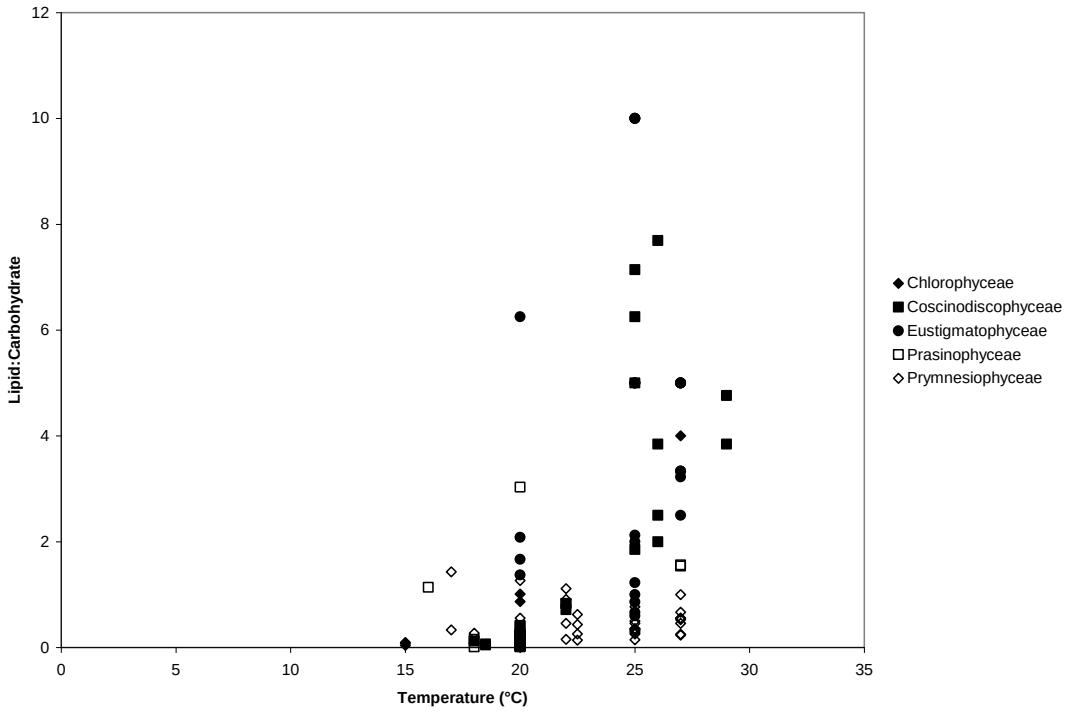


Fig. 33. Scatter plot showing variation in total cellular lipid content (% dry weight) as a function of growth temperature for pooled species data. Red circles: *Chaetoceros* species. Orange circles: *Skeletonema* species. Green Triangles: *Thalassiosira* species.

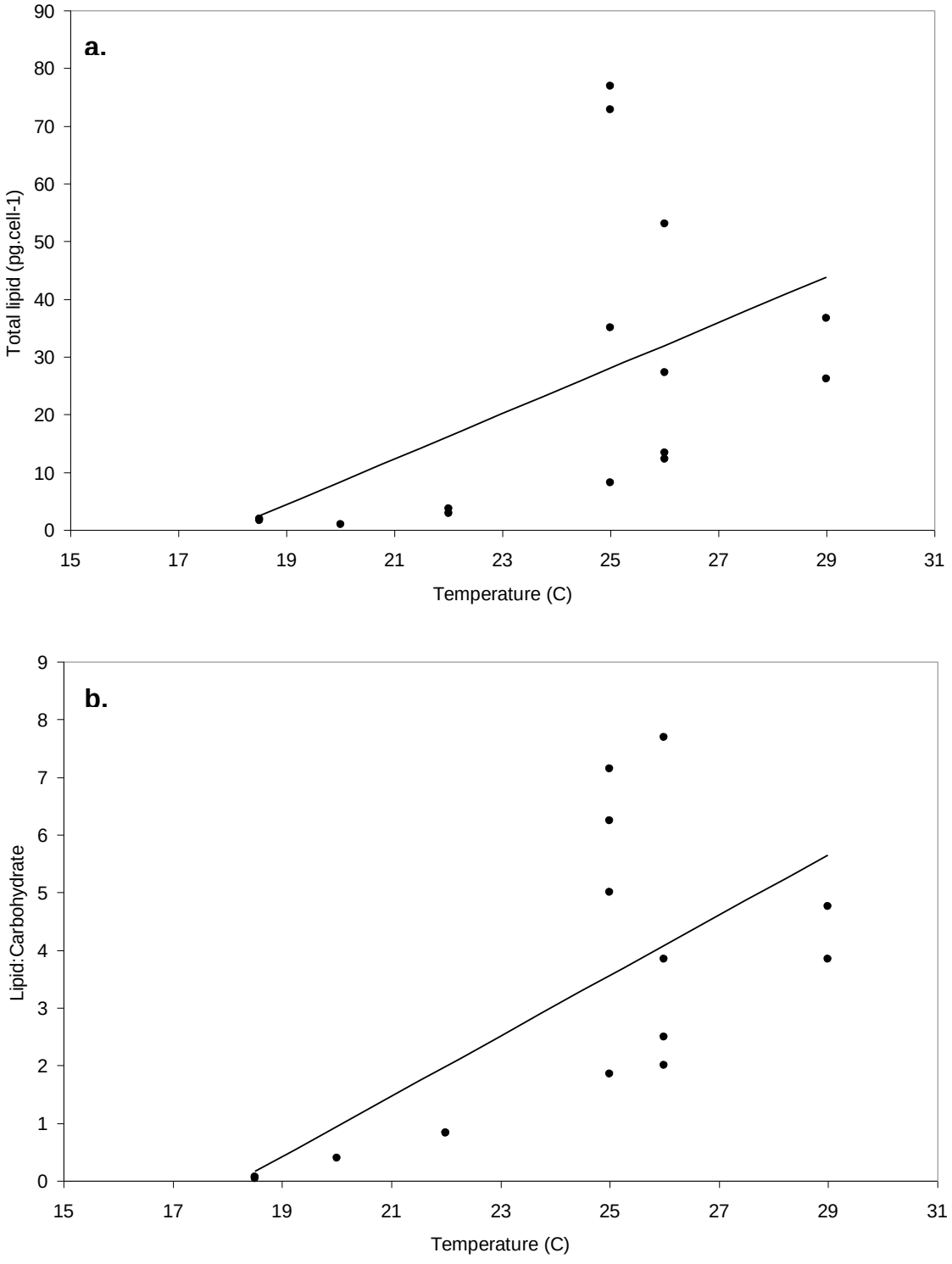


Fig. 34. Ordinary LSR plots showing a) total intracellular lipid concentrations (r^2 = 0.03) and b) intracellular ratio of lipid to carbohydrate (r^2 = 0.4) as a function temperature in batch cultures of *Thalassiosira pseudonanna.* P<0.05.

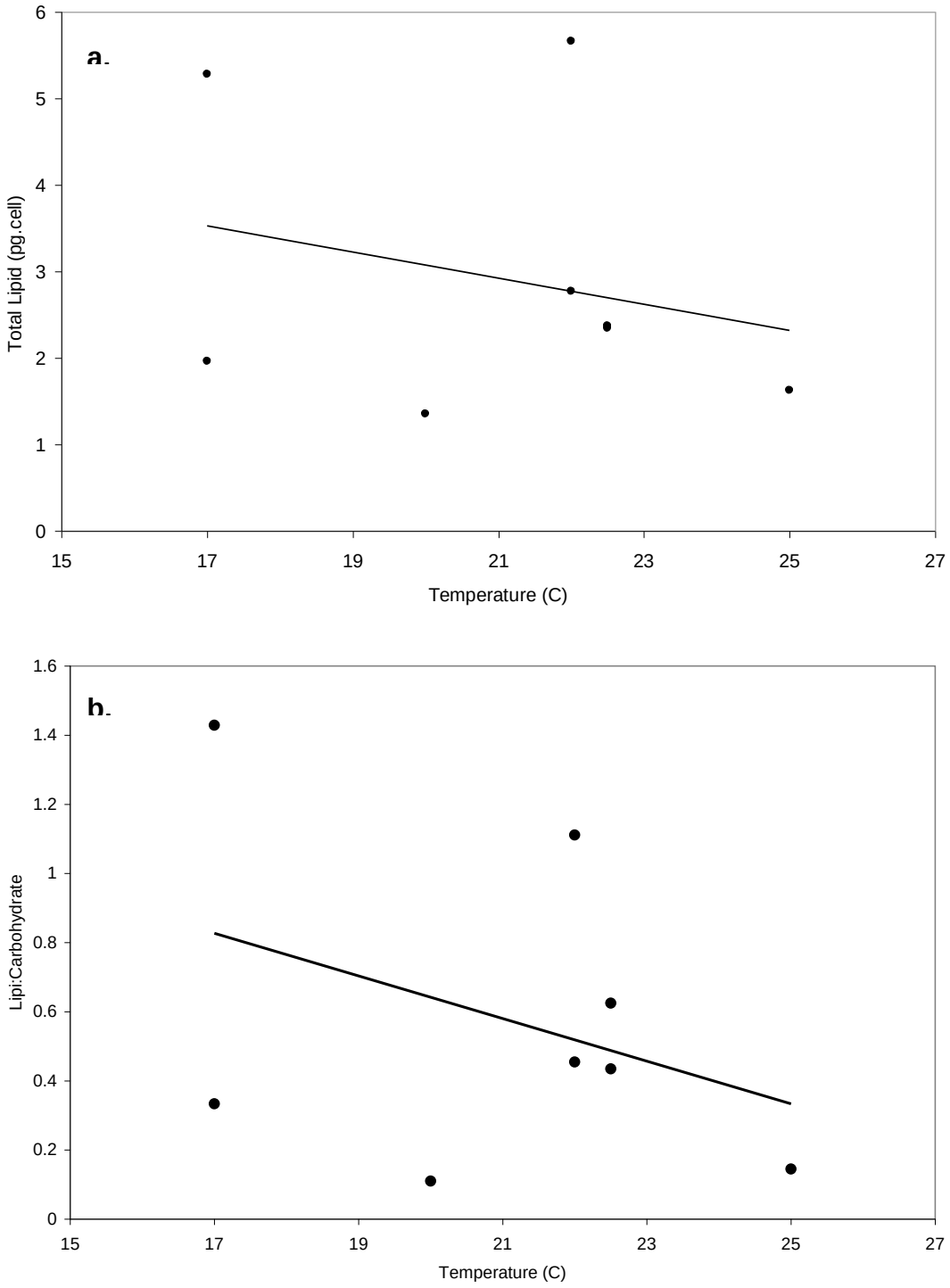


Fig. 35. Ordinary LSR plots showing a) total intracellular lipid concentrations (r^2 = 0.06) and b) intracellular ratio of lipid to carbohydrate (r^2 = 0.1) as a function temperature in batch cultures of *Pavlova lutheri*. P<0.05.

Table 19. List of references from which data for temperature v both total lipid and lipid:carbohydrate plots were obtained.

Class (IT IS Scheme)	Species	Strain	Reference
Coscinodiscophyceae	*Thalassiosira pseudonana*	CS173	Brown 91
		NEPCC58	Thompson et al 96
		NEPCC58	Thompson et al 96
		NEPCC58	Thompson & Harrison 92
		NEPCC58	Thompson & Harrison 92
		NEPCC58	Thompson & Harrison 92
		NEPCC58	Thompson & Harrison 92
		NEPCC58	Thompson & Harrison 92
		NEPCC58	Thompson & Harrison 92
		NEPCC58	Thompson & Harrison 92
		NEPCC58	Thompson & Harrison 92
		NEPCC58	Thompson & Harrison 92
		NEPCC58	Thompson & Harrison 92
		NEPCC58	Thompson & Harrison 92
	Thalassiosira weissflogii	Actin	Milligan & Harrison 00
		Actin	Milligan & Harrison 00
Prymnesiophyceae	*Pavlova lutheri*	NEPCC5	Thompson et al 94
		NEPCC5	Thompson et al 94
		CS182	Brown 91
		NEPCC5	Thompson et al 96
		NEPCC5	Thompson et al 96
		Unknown	Ponis et al 03
		Unknown	Ponis et al 03
	Pavlova salina	CS49	Brown 91

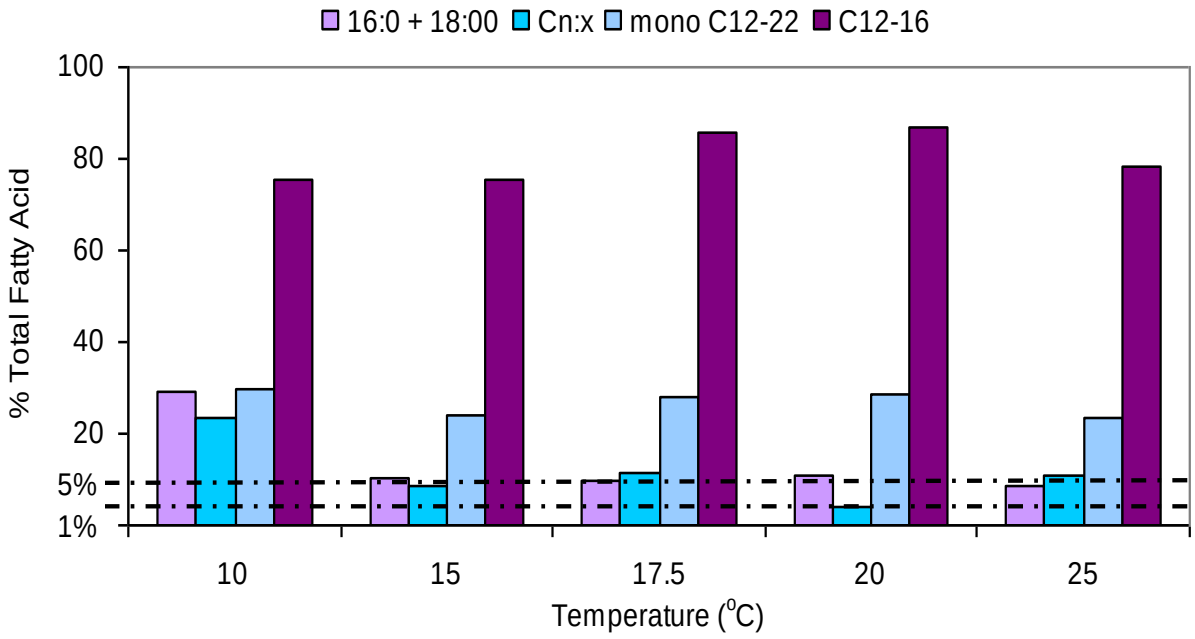


Fig. 36. Inter and intra-strain variation in relative proportions of fatty acids important in meeting international quality standards for commercial biodiesel as a function of growth temperature in various culture collection strains of *C. simplex*. Dashed lines: critical limits of key chemical criteria i.e. Cn:x, where x> 3 < 1% TFA and maximum degree of saturation where C16:0 + C18:0 < 5% TFA.

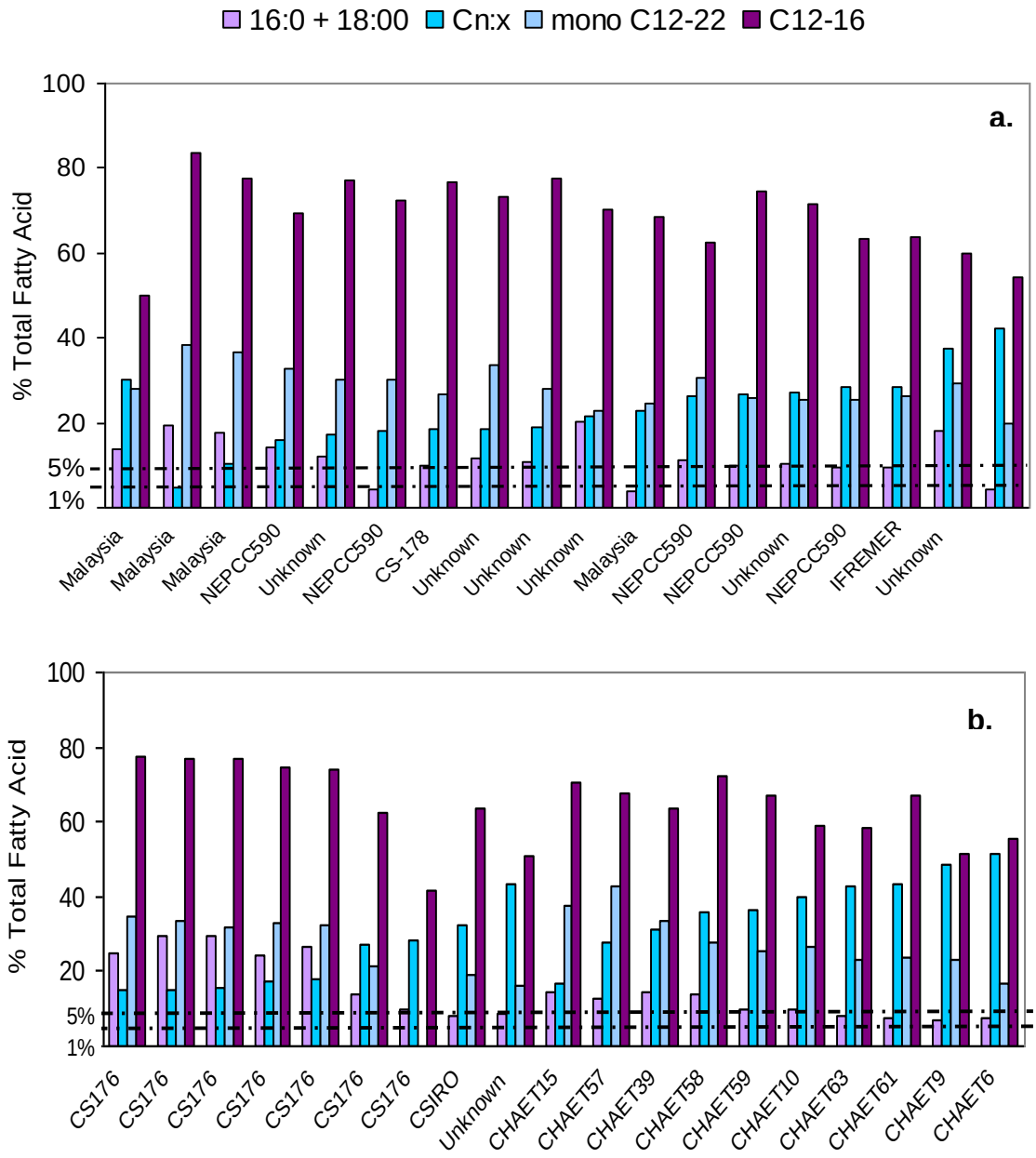


Fig. 37. Inter and intra-strain variation in relative proportions of fatty acids important in meeting international quality standards for commercial biodiesel in a) *C. calcitrans* and b) *C. muellerii* under different physiochemical regimes. Dashed lines: critical limits of key chemical criteria i.e. Cn:x, where x> 3 < 1% TFA and maximum degree of saturation where C16:0 + C18:0 < 5% TFA.

Table 20. List of references from which data for detailed lipid profile charts for *Chaetoceros* species *C. calcitrans* and *C. muellerii,* were obtained.

Species	Strain	16:0 + 18:0	Cn:x	12:1 - 22:1	C12-16	Reference
C. calcitrans	Unknown	20.1	21.4	22.8	70.2	Delaunay et al 93
	CCAP1010/05	13.6	30.4	28.2	50.1	Rico-Villa et al 06
	Unknown	4.3	42.3	19.8	54.3	Rivero-Rodriguez et al 07
	Malaysia	19.4	4.7	38.3	83.7	Shamsudin 92
	Malaysia	17.8	10.2	36.8	77.5	Shamsudin 92
	Malaysia	14.4	15.9	32.6	69.2	Shamsudin 92
	Malaysia	11.0	26.1	30.6	62.3	Shamsudin 92
	IFREMER	18.0	37.7	29.1	59.8	Servel et al 94
	NEPCC590	12.0	17.3	30.0	77.2	Thompson etal 92b
	Unknown	4.3	17.9	30.2	72.5	Thompson et al 90
	NEPCC590	9.9	18.5	26.6	76.7	Thompson et al 92b
	Unknown	11.0	18.9	28.1	77.8	Thompson et al 90
	Unknown	4.1	22.8	24.6	68.4	Thompson et al 90
	NEPCC590	10.1	26.6	26.0	74.7	Thompson et al 92b
	NEPCC590	10.4	27.2	25.5	71.6	Thompson et al 92b
	Unknown	9.6	28.4	25.5	63.2	Thompson et al 90
	NEPCC590	9.6	28.4	26.1	63.9	Thompson et al 92b
	CS178	11.5	18.6	33.8	73.2	Volkman et al 89
C. muellerii	CS176	24.6	15.2	34.9	77.5	Liang et al 06
	CS176	29.5	15.3	33.3	77	Liang et al 06
	CS176	29.4	15.8	31.9	76.7	Liang et al 06
	CS176	24.5	17.6	32.9	74.5	Liang et al 06
	CS176	26.7	17.8	32.1	73.8	Liang et al 06
	CS176	13.8	27.1	21.1	62.5	d'Souza & Loneragan 99
	CS176	10.1	28.4	0	41.4	d'Souza & Loneragan 99
	CSIRO	8.36	32.15	19.26	63.53	Payne & Rippingale 00
	Unknown	8.6	43.4	16.3	51.1	Rivero-Rodriguez et al 07
	CHAET15	14.5	16.5	37.3	70.8	Johansen et al 90
	CHAET57	12.7	27.6	43	67.5	Johansen et al 90
	CHAET39	14.5	31.3	33.6	63.5	Johansen et al 90
	CHAET58	13.9	36	28	72	Johansen et al 90
	CHAET59	10	36.6	25.7	67.2	Johansen et al 90
	CHAET10	10	40.1	26.5	58.7	Johansen et al 90
	CHAET63	8	42.9	23.4	58.3	Johansen et al 90
	CHAET61	7.4	43.2	23.9	67	Johansen et al 90
	CHAET9	6.7	48.4	23.2	51.3	Johansen et al 90
	CHAET6	7.7	51.2	16.9	55.3	Johansen et al 90

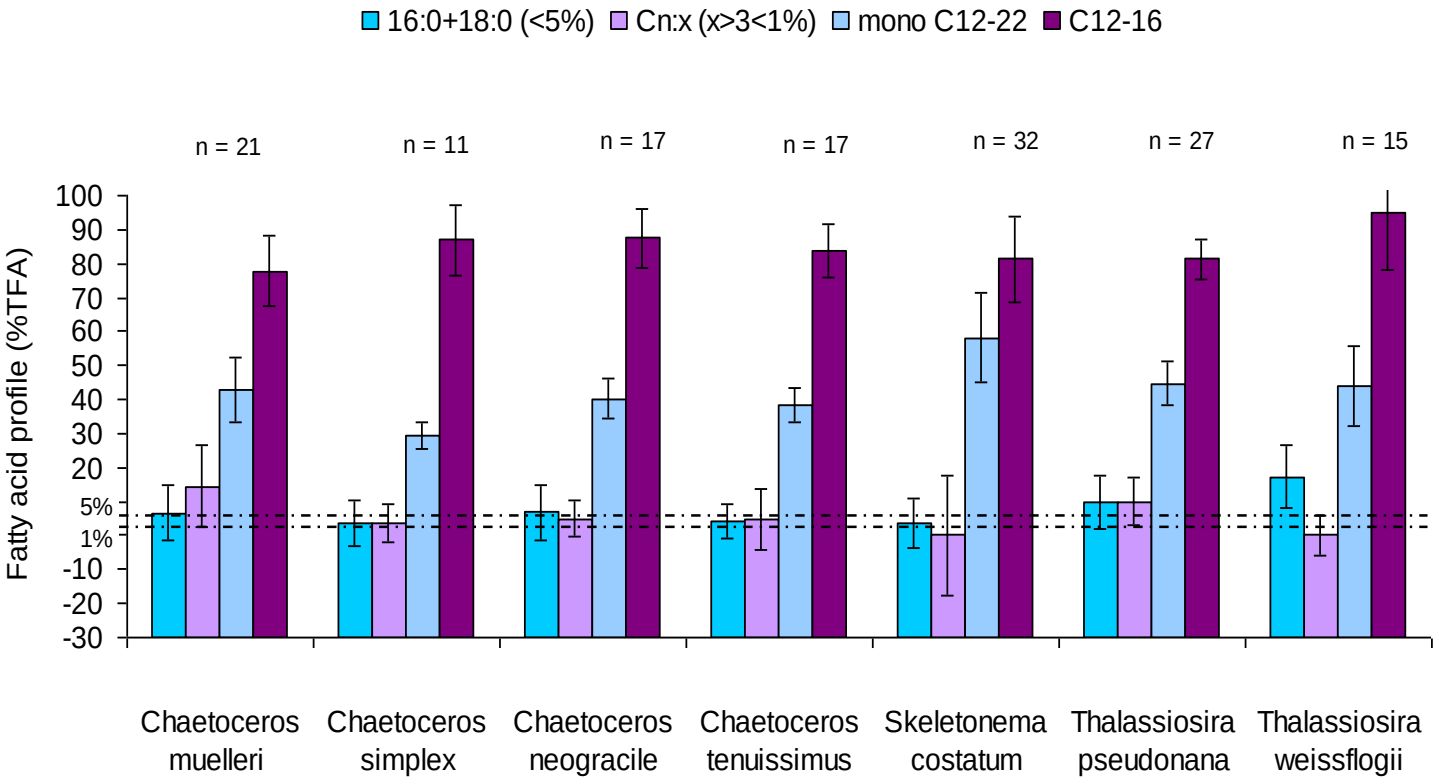


Fig. 38. Inter-specific variation in the relative proportions of important fatty acids in seven centric diatom species of interest to the pre-screening process. Dashed lines: critical limits of key chemical criteria i.e. Cn:x, where x> 3 < 1% TFA and maximum degree of saturation where C16:0 + C18:0 < 5% TFA. Error bars showing standard deviation between *n* data points.

Table 21. List of references from which HTS species fatty acid profile charts were obtained.

Species	Strain	Lipid	Temperature	Reference
Chaetoceros calcitrans	Unknown	26.9	21	Harrison et al 1990
	Unknown	19.2	21	Harrison et al 1990
	Unknown	11.4	22	Rivero-Rodriguez et al 2007
	CS178	19	23	McCausland et al. 1999
	CS178/CCMP1315	19	23	McCausland et al. 1999
	NSW Fisheries	18	23	McCausland et al. 1999
	CS178	16	25	Brown 1991
	Unknown	22	25	De Castro Araujo & Garcia 2005
Chaetoceros gracilis	CS176	7.2	25	Brown 1991
	CS176	7.2	25	Brown 1991
Chaetoceros muelleri	CS176	21	18	D'Souza & Loneragan 1999
	Unknown	11.1	22	Rivero-Rodriguez et al 2007
	CCMP1316	26.1	33.5	Lopez-Elias et al 2005
	CCMP1316	20.6	33.5	Lopez-Elias et al 2005
	CCMP1316	18.2	33.5	Lopez-Elias et al 2005
	CCMP1316	18.1	33.5	Lopez-Elias et al 2005
	CCMP1316	16.6	33.5	Lopez-Elias et al 2005
	CCMP1316	13.6	33.5	Lopez-Elias et al 2005
	CS-176	12.1	33.5	Martinez-Fernandez & Southgate 2007
Chaetoceros sp.	CH-X-1	15.8	21	Lopez-Elias et al. 1993
	CH-X-1	14.1	21	Lopez-Elias et al. 1993
	CH-X-1	10.1	21	Lopez-Elias et al. 1993
	CH-X-1	19.8	21	Lopez-Elias et al. 1993
	CH-X-1	19	21	Lopez-Elias et al. 1993
	CH-X-1	17.4	21	Lopez-Elias et al. 1993
	CH-X-1	25.4	23	Sanchez-Saavedra & Voltolina 1996
	CH-X-1	23.2	23	Sanchez-Saavedra & Voltolina 1996
	CH-X-1	21.5	23	Sanchez-Saavedra & Voltolina 1996
	CH-X-1	25.4	23	Sanchez-Saavedra & Voltolina 1996
	CS256	17	25	Renaud et al. 1999
	CS256	17	25	Thinh et al. 1999
	CS-256	19.3	33.5	Martinez-Fernandez & Southgate 2007
Skeletonema costatum	CS181	19	20	Brown & Jeffrey 1995
	CS181	10	20	Brown 1991
	CF1	13.1	23	Lourenco et al 2002
	CF1	12.6	23	Lourenco et al 2002
	CF1	11.8	23	Lourenco et al 2002
	NSW Fisheries	19	23	McCausland et al. 1999
	SK-C-2	9.4	23	Sanchez-Saavedra & Voltolina 1996
	SK-C-2	8.5	23	Sanchez-Saavedra & Voltolina 1996
	SK-C-2	8.2	23	Sanchez-Saavedra & Voltolina 1996
	SKEL	30.3	23	Shifrin & Chisholm 1981
	SKEL	23.8	23	Shifrin & Chisholm 1981
	FH-1	23.2	23	Shifrin & Chisholm 1981
	FH-1	20.4	23	Shifrin & Chisholm 1981

	SKEL	30.3	23	Shifrin & Chisholm 1981
	GOC36	13.5	25	Renaud et al. 1999
	GOC36	13.5	25	Thinh et al. 1999
Skeletonema sp.	CS252	20	20	Brown & Jeffrey 1995
	GOC27	13.3	25	Renaud et al. 1999
	CS-252	3.3	25	Mansour et al. 2005
	CS252	3.3	25	Mansour et al. 2005
	GOC27	13.3	25	Renaud et al. 1999
	GOC27	13.3	25	Thinh et al. 1999
	CS252	15.9	33.5	Martinez-Fernandez & Southgate 2007
Thalassiosira pseudonana	Unknown	21.4	17	Orcutt & Patterson 1975
	CS173	19	20	Brown 1991
	CS-173	31	20	Brown et al. 1996
	CS-173	29	20	Brown et al. 1996
	CS-173	24	20	Brown et al. 1996
	CS-173	21	20	Brown et al. 1996
	CS-173	21	20	Brown et al. 1996
	CS-173	21	20	Brown et al. 1996
	CS173	31	20	Brown et al. 1996
	3H	22.1	21	Harrison et al 1990
	3H	21.2	21	Harrison et al 1990
	3H	18.3	21	Harrison et al 1990
	3H	26.4	23	Shifrin & Chisholm 1981
	3H	24.3	23	Shifrin & Chisholm 1981
	TH-P-1	22.3	23	Sanchez-Saavedra & Voltolina 1996
	TH-P-1	20.1	23	Sanchez-Saavedra & Voltolina 1996
	TH-P-1	19.4	23	Sanchez-Saavedra & Voltolina 1996
	CS-20	8.5	25	Mansour et al. 2005
Thalassiosira weissflogii	Actin	24	23	Shifrin & Chisholm 1981
	Actin	24	23	Shifrin & Chisholm 1981
	Actin	22.2	23	Shifrin & Chisholm 1981

Table 22. Minimum relative proportions of saturated and multiple double bond (>3) fatty acids (% total fatty acid) important in meeting international biodiesel quality standards. Data obtained from strains grown in resource saturated conditions only.

Species	*n*	16:0 + 18:0	St. dev	Cn:x, x>3	St dev
C. muellerii	21	6.7	8.4	14.5	12.1
C. neogracile	17	6.8	8.1	5.0	5.5
C. simplex	11	3.8	6.7	3.8	5.6
C. tenuissimus	17	4.1	5.0	4.7	9.2
S. costatum	32	3.5	7.2	0.0	17.7
T. pseudonanna	27	9.7	7.8	9.9	7.0
T. weissflogii	15	17.3	9.0	0.1	5.8

Table 23. Maximum relative proportions of mono-unsaturated and short carbon chain fatty acids (% total fatty acid) important in meeting international biodiesel quality standards. Data obtained from strains grown in resource saturated conditions only.

Species	*n*	12:1 + 22:1	St. dev	C12 - 16	St dev
C. muellerii	21	43.0	9.4	77.8	10.2
C. neogracile	17	40.2	5.9	87.4	8.5
C. simplex	11	29.5	3.8	86.9	10.4
C. tenuissimus	17	38.3	4.9	83.7	7.7
S. costatum	32	58.2	13.3	81.4	12.5
T. pseudonanna	27	44.8	6.2	81.4	5.9
T. weissflogii	15	44.0	11.7	95.2	17.2

Concluding remarks

Reductions in growth rate due to photoinhibition at high irradiance can prematurely force cells into the stationary phase of growth. This presents problems in a mass cultivation scenario as lipid accumulation increases as an inverse function of growth rate (Hu 2008). Through the resulting decrease in both biomass and lipid yields, production costs can be increased beyond critical economic levels and for this reason, pre-screen selections were, for the most part, confined to species with a relatively high capacity to photoacclimate and maintain photosynthetic efficiency at levels (i.e. ~3% efficiency) conducive to the maintenance of specific growth rates >1.4$\mu.d^{-1}$ (Huntley and Redalje 2006) Assessment of these parameters however was constrained by a paucity of relevant studies for species of interest and to overcome this, extrapolation of results from a small number of studies relating indirectly to these effects in species of interest allowed qualitative inferences to be made on the performance potential of genetically related, morphologically similar species.

On this basis, pre-screen selections of predominantly smaller and thus more photosynthetically and metabolically efficient centric diatom species (class *Coscinodiscophyceae*) originating from high temperature tropical and sub-tropical latitudes were selected for further biochemical evaluation and optimization of lipid yields under High Throughput Screening. Due to their comparatively higher affinities for nutrient uptake and growth under high temperature and irradiance levels, strains of species from the genera *Chaetoceros* and *Thalassiosira* were considered here to rank among the most promising candidates for meeting and sustaining the average target production rate of 15 $g.m^{-2}.d^{-1}$ total lipid. Of particular interest here are strains of *C. muellerii*, *S. costatum*, *T. weissfloggii* and *T. pseudonanna*. The enormous morphological and genetic diversity reported for these classes however, also maximizes the potential for both discovery of new oleaginous species/strains and the optimization of fatty acid profiles under HTS to better meet the chemical requirements for high quality biodiesel production. For these reasons, the work conducted as part of the HTS process promises to yield valuable information in both environmental and economic

terms, for the amelioration and reduction of transport sector greenhouse gas emissions (Huntley and Redalje 2006).

Table 24. List of references used to determine ammonia preferences for each ranked species.

Species	Reference
Amphora sp.	Admiraal 1987
Ankistrodesmus sp.	Syrett 1973
Asterionella glacialis	Collos and Lewin 1974
Bacteriastrum hyalina	Gotschalk and Alldredge 1989
Biddulphia aurita	Lui 1972
Biddulphia sinensis	Lui 1972
Botryococcus braunii	Ohmori 1984
Chaetoceros affinis	Dortch & Conway 1984
Chaetoceros calcitrans	Yusoff et al 2001
Chaetoceros cf. *wighami*	Lagos et al 2004
Chaetoceros gracilis	Bressler & Ahmed 1984
Chaetoceros muellerii	Giordano et al 2002
Chaetoceros neogracile	López-Ruiz et al 1994
Chaetoceros simplex	Goldman & Gilbert 1982
Chaetoceros sp.	Cloern 1977
Chaetoceros sp. *'tenuissimus-like'*	
Chlorella minutissima	Kanazawa et al 1972; Lourenco et al 2002
Chlorella sp.	Kanazawa et al 1972; Syrett 1953
Cryptomonas sp	Cloern 1977
Cyclotella cryptica	Wheeler 1979
Cyclotella sp	Wheeler 1979
Cylindrotheca fusiformis	Hildebrand 2004
Ditylium brightwellii	Bressler & Ahmed 1984
Dunaliella bardawil	Tan et al 1993
Dunaliella primolecta	Uriarte et al 1993
Dunaliella salina	Pick 1991
Dunaliella tertiolecta	Lourenco et al 2002
Ellipsoidion sp.	Flynn & Flynn 1992
Emiliania huxleyi	Varella & Harrison 1999
Extobucellus spinifera	
Hymenomonas carterae	Wheeler et al 1982
Isochrysis aff *galbana*	Guillard 1958

Isochrysis galbana	Guillard 1958
Isochrysis sp.	Guillard 1958
Isochrysis T-ISO	Guillard 1958
Lauderia annulata	Trigueras & Orive 2001
Leptocylindrus danicus	Trigueras & Orive 2001
Melosira cf nummuloides	Rodrigo et al 2001
Minutocellus polymorphus	Vaulot 1994
Nannochloris atomus	Melack et al 1982
Nannochloris sp.	Lourenco et al 2002
Nannochloropsis oculata	Lourenco et al 2002
Nannochloropsis salina	Lourenco et al 2002
Nannochloropsis sp.	Lourenco et al 2002
Navicula jeffreyi	Knuckey et al 2002
Nitzchia laevis	Wen & Chen 2001
Nitzschia closterium	Zobell 1935
Nitzschia frustulum	Rushforth & Brock 1991
Nitzschia inconspicua	Fawzi et al 2002
Nitzschia longissima	Carlson et al 1993
Nitzschia paleacea	Hürlimann & Schanz 1992
Odontella mobilensis	Horabun 1997
Odontella sp.	Horabun 1997
Pavlova lutheri	Bressler & Ahmed 1984
Pavlova salina	Bressler & Ahmed 1984
Pavlova sp.	Bressler & Ahmed 1984
Pedinomonas minor	Smith 1999
Prymnesium parvum	Flynn et al 1993; McLaughlin 1958
Rhizosolenia setigera	Villareal 1990
Rhodomonas lens	Guillard 1958
Rhodomonas salina	Guillard 1958
Skeletonema costatum	Bressler & Ahmed 1984; Lourenco et al 2002
Skeletonema tropicum	Haernstroem 2007
Stephanopyxis palmeriana	Alonso-Rodriguez et al 2000
Synechococcus subsalsa	Lourenco et al 2002
Tetraselmis sp	Lourenco et al 2002
Tetraselmis suecica	Lourenco et al 2002

Tetraselmis tetrathele	Lourenco et al 2002
Thalasiossira weissflogii	Goldman and McCarthy 1978
Thalassiosira floridiana	Goldman and McCarthy 1978
Thalassiosira fluviatilis	Goldman and McCarthy 1978
Thalassiosira oceanica	Goldman and McCarthy 1978
Thalassiosira pseudonanna	Bressler & Ahmed 1984
Thalassiosira rotula	Goldman and McCarthy 1978

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