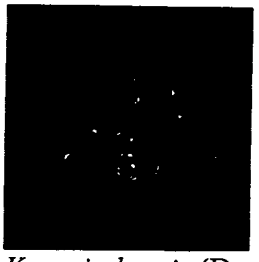
Final Report

The effect of environmental factors on the growth rate of *Karenia brevis* (Davis) G. Hansen and Moestrup



Karenia brevis (Davis)

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SUMMARY

Mass mortalities of marine organisms in the Gulf of Mexico have been associated with blooms of the unarmored dinoflagellate, *Karenia brevis* (Davis) G. Hansen & Moestrup (*K. brevis*). This investigation focuses on the environmental factors affecting the growth rate of *K. brevis* under laboratory conditions. Field observations and laboratory experiments have established light, salinity, and temperature tolerances of a clone from Texas waters.

The effects of environmental factors on growth rates were determined by culturing *K. brevis* under a combination of various light (19, 31, 52, 67, and 123 μmol m^{-2} s^{-1}), salinity (25, 30, 35, 40 and 45), and temperatures (15, 20, 25, and 30°C). Exponential growth rates increased with increasing irradiance. Maximum growth rates ranged from 0.17 to 0.36 div day^{-1}. No growth was supported at 19 μmol m^{-2} s^{-1} for any experiment. Maximum growth rates 15°C were much lower than at other temperatures.

The results indicate only minor differences between the Texas and Florida strains of *K. brevis*. Maximum growth rates of the Texas clone (SP3) fell within the range of Florida clones reported in the literature (0.17 - 0.36 div day^{-1} vs. 0.2 – 1.0 div day^{-1}). Light saturation for the Texas clone (SP3) was very similar to that for a Florida clone (67 vs. 65 μmol m^{-2} s-1) reported by Shanley and Vargo (1993). Light compensation (I_c) was similar to previous studies (26.25 – 30 μmol m^{-2} s^{-1}) with the exception of Shanley and Vargo (1993) who reported I_c at 6 μmol m^{-2} s^{-1}.

Our results combined with literature data and the concurrent genetic study by Campbell indicate that while *K. brevis* from Texas and Florida are genetically identical and growth rates responses are similar, toxin profiles differ. This area of intra-specific variability clearly requires more study both for understanding temporal evolution of blooms as well as basic autecology of the species. The Texas strain of *K. brevis* is similar to the Florida strain for many criteria, but differs substantially from Louisiana *K. brevis*, which salinities < 10.

In summary, the Texas clone (SP3) varied in growth rate when cultured under different light intensity, salinity, and temperature criteria. The Texas clone (SP3) had a very similar light saturation point compared to that of the Florida isolate (67 vs. 65 μmol m^{-2} s^{-1}), and light compensation (20-30 μmol m^{-2} s^{-1}) with the exception of Shanley and Vargo who reported a light compensation of 6 μmol m^{-2} s^{-1}. The upper salinity tolerance of the Texas clone was lower (40

vs. 46) than that of some Florida clones; however, lower salinity tolerance was similar. The Texas clone had the same temperature tolerance of Florida clones (15-30°C).

BACKGROUND

Marine phytoplankton are diverse photosynthetic organisms that occur worldwide in temperate to tropical waters. When environmental conditions become favorable, certain species can produce enormous blooms through either growth or aggregation. These noxious blooms, termed Harmful Algal Blooms (HABs), are a natural phenomenon and are considered "harmful" either because of their potential threat to human health through consumption of contaminated shellfish or respiratory irritation (Buskey et al.1996). Blooms of dinoflagellates are generally referred to as red tides. Red tides occur globally and their increasing frequency has caused increased losses in aquaculture, fisheries, and tourism (Hallengraeff 1993). Over the past 20 years red tide events have increased in frequency, intensity, and geographic distribution (Hallegraeff 1993). The term "red tide" describes the sudden proliferation or accumulation of microorganisms that give rise to discolored water (Martin and Martin 1976).

The causative agents of this phenomenon are microscopic single-cell protists that occur in fresh and seawater. Species of dinoflagellates are most commonly responsible, though other flagellates, or ciliates, can cause red tide blooms (Iwasaki 1979). The name dinoflagellate comes from the Greek word *dinos* meaning whirling and describes the mode of locomotion, spiraling forward (Spector 1984). They are found in the subtropics, tropics, or temperate water during the warmer, and usually the warmest time of the year (Ryther 1955, Steidinger 1975).

Hallegraeff (1993) offered five possible explanations for the apparent increased frequency of red tides; 1) increased scientific awareness, 2) increased utilization of coastal waters for aquaculture 3) increase of algal blooms caused by cultural eutrophication, 4) stimulation of algal blooms by unusual climatological conditions, or 5) transport of dinoflagellate cysts in ships' ballast water or associated with transfer of shellfish stocks. No single explanation has been shown to definitively predict red tide blooms since many hydrological and meteorological conditions must coincide for a bloom to occur.

Red tides may be classified as toxic or nontoxic (Steidinger and Haddad 1981). Non-toxic red tides do not produce a toxin, although rapid increases in the population of red tide organisms can deplete ambient dissolved oxygen in a body of water ultimately causing mortalities of vertebrates and invertebrates (Martin and Martin 1976). Less than 20 species of red tide dinoflagellates produce toxins. These toxic species may also have more than one active fraction including neurotoxins, ichthyotoxins, hemolytic agents, or human respiratory irritants (Steidinger and Haddad 1981). The toxins produced by red tides are small (approximately 30 Angstroms) non-peptidic materials known to be some of the most toxic natural substances (Fleming and Baden 1998). These toxins are tasteless, odorless, lipid soluble, and heat and acid stable (Hemmert 1975, Trebatoski 1988, Fleming and Baden 1998, Fleming et al. 2001).

Karenia brevis (*K. brevis*) is an unarmored dinoflagellate that produces potent chemical toxins (brevetoxins) that are harmful to both fish and mammals. A recent change in nomenclature has renamed *Gymnodinium breve* Davis to *Karenia brevis* (Davis) G. Hansen and Moestrup (Daugberg et al. 2000). *Karenia brevis* is found throughout the Gulf of Mexico, and at times in parts of the southeast Atlantic coast of the U.S. and the Caribbean. The continental shelf off the west coast of Florida has experienced *K. brevis* blooms in 24 of the last 26 years (Tester and Steidinger 1997), yet in Texas red tide blooms are relatively rare. Buskey et al. (1996) suggests that this is due in part to limited documentation of blooms in Texas prior to WWII,

before establishment of the cause and effect relationship between HABs and fish kills. Villareal et al. (2000) suggested that the sparse coastal human population, itself, has limited observations of *K. brevis* blooms in Texas.

The maximum growth rates for Florida isolates of *K. brevis* have been reported by Steidinger (1983) (0.2-0.5 div day^{-1}), Wilson (1966), Shanley and Vargo (1993) (0.2-1.0 div day^{-1}), and by Doig (1973) (0.16-0.19 div day^{-1}). Temperature and salinity requirements for the Florida have been reported as 15-30°C and salinity of 22.5 to 46. However, Dortch et al. (1998) reported *K. brevis* in low salinity waters off the Louisiana coast. Counts exceeded the regulatory limit between salinities of 14 and 30 and *K. brevis* was observed at salinities from 5 to 35 (Dortch et al. 1998). This is evidence of perhaps several populations (genotypes) responding to different environmental conditions. This study was designed to determine how the Texas strain of *K. brevis* responds to environmental factors.

Environmental Effects Study

Few multifactorial experiments have attempted to identify synergistic effects involving different levels of environmental parameters and cell densities in *K. brevis* (Steidinger and Haddad 1981). This study will define the range of environmental factors that provide optimal growth for a Texas clone of *K. brevis* under various environmental conditions (e.g. light, salinity, and temperature) and to compare the Texas clone to data on Florida and Louisiana clones being processed by Drs. K.A. Steidinger and Q. Dortch's labs. Since this study was completed prior to these other labs, the data from this study was compared to literature data.

METHODS and MATERIALS

A 10 mL sample of *K. brevis*, designated SP3, was obtained from the University of Texas Marine Science Institute (UTMSI), Port Aransas, Texas as xenic, unialgal cultures and cultured in L1-Si media (Guillard and Hargraves 1993). The *K. brevis* clone (SP3) was isolated by Suzanne Pargee at UTMSI from an October, 1999 red tide bloom in Brownsville, Texas. The seawater ($\approx$ salinity of 36) used in the preparation of media was collected 200 miles offshore and aged in the dark for several months prior to use. Various salinities were obtained by diluting with 18 meg Ohm deionized water (Millipore® Corp. Milli-Q Reagent water system) to reduce the salinity to 25, 30, and 35. Evaporating seawater with an aquarium heater and aeration increased the salinity to achieve the desired treatment levels of 40 and 45. Salinity was measured ($\pm$ 1 PSU) with a Fisher Scientific refractometer (Cat. # 13-946-27) and calibrated with seawater standard of salinity 35. L1-Si medium (Guillard and Hargraves 1993) was prepared using sterile filtered seawater. The medium was sterile-filtered at through a 0.2 μm surfactant free cellulose acetate filter (SFCA) (Nalgene® Co.) at 250 mm Hg vacuum (Brinkman vacuum aspirator model B-169). For this study, the L1-Si media was modified by replacing $NaH_2PO_4 \cdot H_2O$ with equimolar K_2HPO_4. Stock cultures were acclimated to five salinity levels (25, 30, 35, 40, 45) over a two-week period. Stock cultures were maintained in 1L borosilicate bottles (Kimax Corp.) at 25°C in a separate incubator (Hotpack Corp. model 352622) under 20w "daylight" and 40w "cool white" fluorescent lights at 12/12 L: D photoperiod.

All glassware, culture vessels, and utensils were washed in hot water and detergent (Liquinox™) and then rinsed three times with house-deionized water. All items were placed in a 5% HCl water bath and allowed to soak overnight to remove trace metals. Items were rinsed three times with house-deionized water and then rinsed three times with 18 meg Ohm deionized water and left to air dry inverted in a dish rack to reduce contamination. Borosilicate culture

tubes (25mm x 150 mm Kimble Co.) were filled with 18 meg Ohm deionized water, capped with a friction-fit cap (Corning closure model 26795-25) and autoclaved at 121° C at 15 psi for 30 minutes with a 30 minute cool down period in an Amsco Scientific Eagle Century Isothermal Sterilizer (model SI-120). Culture tubes were allowed to cool prior to inoculation.

Aliquots (150 mL) from stock cultures were transferred into 250 mL polycarbonate square bottles (Nalgene Corp.) in a laminar flow hood (Forma Scientific Inc. model 1128) to minimize secondary contamination. These cultures were acclimated in a walk-in incubator (EJS Limited Inc. model GC-1-DL3C-6139) to the designated light intensity, salinity, and temperature for two weeks prior to inoculation.

Culture test tubes were filled with 30 mL enriched seawater and inoculated several days later. Triplicate culture tubes (25 x 150 mm) were each aseptically inoculated in a laminar flow hood with 3 mL of inoculum from parent cultures. Culture tubes were placed along the perimeter of 4x10 test tube racks (Fisher*brand* blue epoxy-coated racks cat. # 14-793-6) and transferred to the walk-in incubator.

There is no standard lighting system in *K. brevis* research; researchers have typically used "cool white" 40 watts (40w) fluorescent lighting for culture studies, while others have used fluorescent lamps that simulating the light spectrum of natural sunlight. This study employed fluorescent "warm white" 40w lights for all experiments. A 12/12 L:D photoperiod was maintained for all experiments.

Light irradiance was measured with a Li-Cor 4 π quantum sensor (model Li-1000) at the surface of the culture table prior to beginning each experiment. The level of the table was adjusted to obtain an equivalent irradiance level for all experiments. Five light attenuation levels were achieved by placing successive layers of neutral density screening (Phifer wire products, Inc. charcoal fiberglass) over the culture tube racks. Irradiance was measured at the top, middle, and bottom level of culture tubes with a calibrated 4 π quantum sensor (Biospherical Instruments model QSL-170) and values were averaged to yield mean irradiance values of 19, 31, 52, 67, and 123 μmol $m^{-2} s^{-1}$. Inoculated tubes were placed in the walk-in incubator and maintained at the appropriate temperature (15, 20, 25, 30, or 35° C $\pm$ 1°C). Infrequently, a small (< 5μm) colorless flagellate was observed co-existing in *K. brevis* cultures (25 and 30 PSU) during 15 and 30°C experiments. This flagellate did not seem to affect the growth rate of *K. brevis*. Since it never achieved significant abundance it was concluded that it was unimportant.

Growth Determinations

Culture tubes were sampled at the time of inoculation and subsampled at subsequent 3-day intervals. On sampling days, culture tubes were removed from their rack and placed in a darkened water bath to eliminate the effects of sudden temperature change and light shock. Sampling was performed in a laminar flow hood to reduce secondary contamination. Each culture tube was vortexed (VWR Vanlab model K550-G) at the lowest setting for five seconds and a 1 mL sample extracted. Each sample was placed in a labeled 1-dram screw-top glass vial (Cabisco model 71-5121) and preserved with Lugol's solution (Thorsden 1978). Culture tubes were subsampled for a period of 14 days (Wilson et al. (1975) reported that maximum growth occurs during the first 15 days). Cell counts were enumerated using a stereomicroscope (Olympus Corp. model SZH-ILLD) at 64x on a grided Sedgewick-Rafter (Gradicules Ltd. model S52) counting chamber (Guillard 1979). Raw data (Appendix A-D) was transcribed into an Excel spreadsheet (Microsoft Corp.). Growth rates (div day^{-1}) were calculated using the slope of the line (Appendix E-H) for a better determination of exponential growth phase (Guillard 1973).

Growth rates were calculated using the growth constant (K_e) where $K_e = \ln (N_1/N_o)/(t_1 - t_o)$ and dividing by 0.6931 to yield divisions per day (k) (Guillard 1973). Growth rates were plotted on a Deltagraph® 4.0 graphing program and curve fit to the Jassby-Platt (1976) hyperbolic tangent function model where the data was represented mathematically.

The Jassby-Platt (1976) hyperbolic tangent function equation is as follows;

$$\text{rate} = a*\tanh(b*I/a)+r;$$

where a = maximum growth , tanh = hyperbolic tangent, b = alpha slope, I = irradiance, and r = "y" intercept.

Raw data was transformed to the natural log for growth rate calculations using SPSS® statistical software version 10.0 (SPSS Inc.), and descriptive statistics (mean and standard error) were calculated.

RESULTS

Growth Rates, Light Intensity and Salinity/Temperature

As was noted in previous studies of *K. brevis*, there was substantial variability in growth rates. Growth rate curves in general followed the Jassby-Platt (J-P) formulation (Figs. 1-4). Goodness of fit became increasingly poor at physical criteria extremes, especially at the higher salinities and lower temperatures. Exponential growth rates of *K. brevis* at 20 and 25°C increased with increasing irradiance. The exceptions to this pattern were 15 and 30°C cultures maintained at salinity of 45, which had no growth. Maximum growth rates for all temperature combinations were 0.36 div day^{-1} at salinity of 30 at 20°C, and salinity of 35 at 25°C (Fig. 3). No growth was supported at light intensity of 19 $\mu mol\ m^{-2}\ s^{-1}$ at any temperature/salinity combinations. Growth at salinity of 45 only occurred at 20 °C (0.15 div day^{-1}) (Fig. 2) and 25°C (0.2 div day^{-1}) (Fig. 3) at the highest light intensity of 123 $\mu mol\ m^{-2}\ s^{-1}$.

Maximum growth rates at 15°C were 50% lower than those at the higher temperatures. There was little variability in the J-P formulations for salinities of 25, 30, 35, and 40 at light intensities above 52 $\mu mol\ m^{-2}\ s^{-1}$ (Fig. 1). At 15°C, no growth was observed at salinity of 45 at any light intensity. At 20°C, J-P fits were similar for cultures at salinities of 25, 30, and 35 at light intensities from 31 $\mu mol\ m^{-2}\ s^{-1}$ to 123 $\mu mol\ m^{-2}\ s^{-1}$ (Fig. 2). No growth was observed for salinities of 40 and 45 below light intensity of 123 $\mu mol\ m^{-2}\ s^{-1}$. At 25°C, growth was only observed at light intensities above 52 $\mu mol\ m^{-2}\ s^{-1}$ (Fig. 3). The exception to the pattern was at salinity of 45, which only had growth at 123 $\mu mol\ m^{-2}\ s^{-1}$. At 30°C, no growth was observed at salinity of 45 at any light intensity (Fig. 4).

Growth Rates and Salinity

The *K. brevis* clone (SP3) could not be acclimated to salinity levels above 45 or below 24. The salinities that yielded the greatest growth were 30 and 35 at 20 and 25°C. In general, the pattern for maximum growth rate was a gradual increase as salinity increased then a decrease at the higher salinities. At 15°C, maximum growth rate increased from salinity of 25 to 40 (Fig. 5a). At 20°C, the pattern for maximum growth rate was an increase as the salinity increased from 25 to 30 then steadily decreased as salinity increased (Fig. 5b). No growth was observed for salinity of 40 and 45 below light intensity of 123 $\mu mol\ m^{-2}\ s^{-1}$. The pattern at 25°C was similar to that of 20°C in that maximum growth rate increased with increasing salinity up to 35 then decreased as salinity increased (Fig. 6a). The pattern for maximum growth rates at 30°C was an increase from salinity 25 to 30 and remained steady through to salinity of 40 (Fig. 6b). For most experiments, no growth occurred at salinity of 45 except at 20 and 25°C.

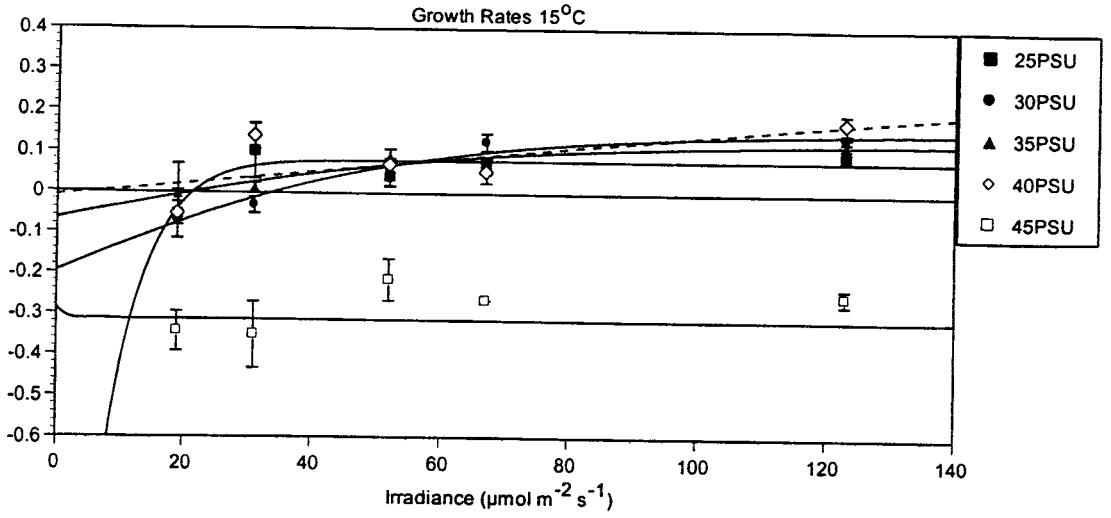
Growth Rates and Temperature

Karenia brevis clone SP3 could not be acclimated to temperatures above 30°C. Attempts to increase temperature above 31°C in the incubator resulted in a rapid death of cells. Growth rates for cultures at 15°C indicated that this temperature was also suboptimal for *K. brevis* since the maximum growth rates at 15°C were 50% lower than those at subsequently higher temperatures. The minimum and maximum growth rates at 15°C were 0.09 div day^{-1} at salinity of 25 and 0.17 div day^{-1} at salinity of 40 (Fig. 2). Maximum growth rates for 20 to 25 and 30°C were very similar. The minimum and maximum growth rates at 20°C were 0.15 div day^{-1} at salinity of 45 and 0.36 div day^{-1} at salinity of 30 (Fig. 3). The minimum and maximum growth rates at 25°C were 0.20 div day^{-1} at salinity of 45 and 0.36 div day^{-1} at salinity of 35 (Fig. 4). The minimum and maximum growth rates at 30°C were 0.20 div day^{-1} at salinity of 25 and 0.34 div day^{-1} at salinity of 40 (Fig.4).

Light compensation (I_c) and Salinity/Temperature

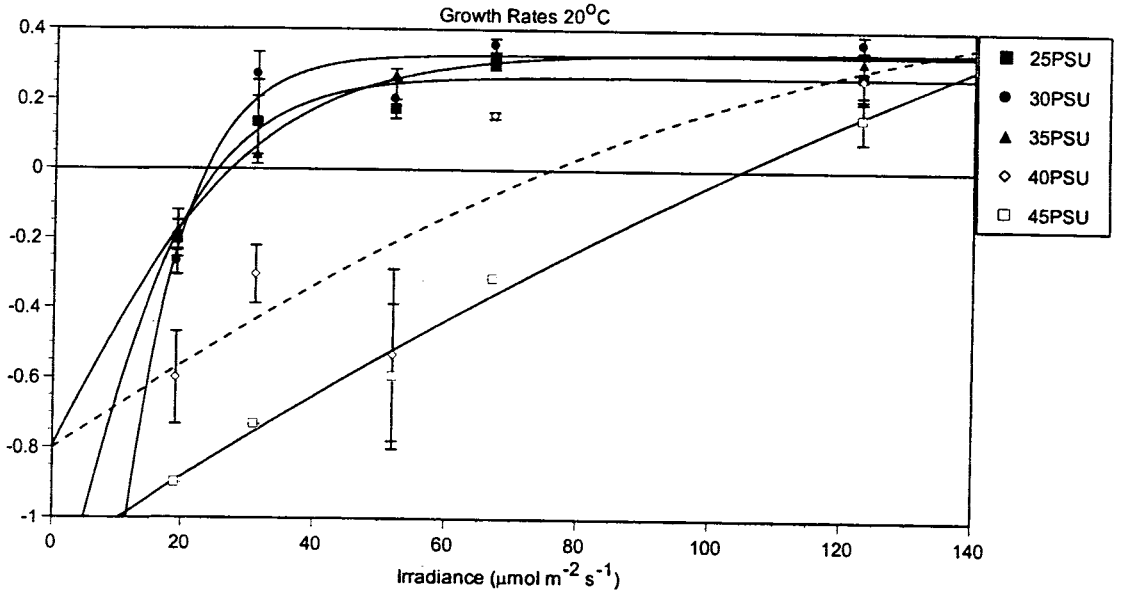
At 15°C, I_c increased from salinity of 25 to 30 (21 to 35 μmol m^{-2} s^{-1}) and then decreased as salinity increased to 35 and 40 (21 to 7.9 μmol m^{-2} s^{-1}) (Fig. 5a). Light compensation for cultures at 15°C ranged from 7.9 to 35 μmol m^{-2} s^{-1}. The lowest value recorded for I_c for all experiments was 7.9 μmol m^{-2} s^{-1} at 15°C at salinity of 40 (Fig. 5a). At 20°C, I_c remained constant from a salinity of 25 to 35 (25 to 28 μmol m^{-2} s^{-1}) and then rapidly increased at salinity of 40 and 45 (77 and 105 μmol m^{-2} s^{-1} respectively Fig. 5b). Light compensation ranged from 23.0 μmol m^{-2} s^{-1} at salinity of 30 to 105.0 μmol m^{-2} s^{-1} at salinity of 45. At 25°C, I_c gradually decreased from salinity of 25 to 40 (42, 35, 32.5, and 30 μmol m^{-2} s^{-1}), then increased rapidly at salinity of 45 (85 μmol m^{-2} s^{-1}, Fig. 6a). Light compensation ranged from 30.0 μmol m^{-2} s^{-1} at salinity of 40 to 85.0 μmol m^{-2} s^{-1} at salinity 45. At 30°C, I_c remained constant between salinities of 25 to 40 (22 to 25 μmol m^{-2} s^{-1} Fig. 6b). Light compensation ranged between 22.0 μmol m^{-2} s^{-1} at salinity of 25 to 27.0 μm m^{-2} s^{-1} at salinity of 35 (Fig. 6b). Light compensation could not be calculated for cultures at salinity of 45 at the temperature extremes of 15 and 30°C. Results can be found in Table 1.

Fig. 1. Growth rates at 15°C. Each point represents average of triplicate cultures.



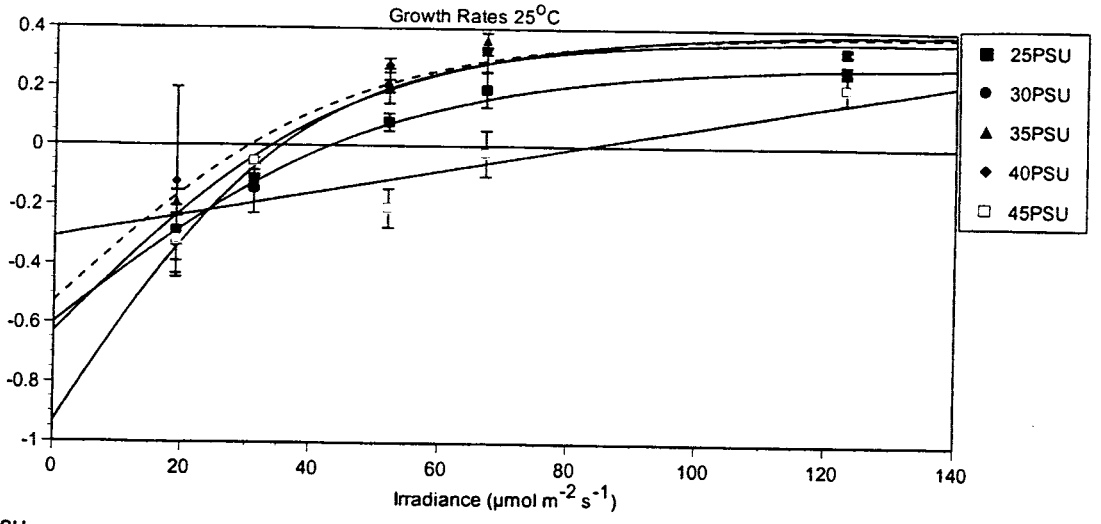
25 PSU	30 PSU	35 PSU	40 PSU	45 PSU
µmax=0.09	µmax=0.13	µmax=0.12	µmax=0.17	µmax=no growth
I_c=21.0 µmol m^{-2} s^{-1}	I_c=35.0 µmol m^{-2} s^{-1}	I_c=21.0 µmol m^{-2} s^{-1}	I_c=7.9 µmol m^{-2} s^{-1}	I_c= N/A
r^2=0.8	r^2=0.96	r^2=0.92	r^2=0.45	r^2=0.22

Fig. 2. Growth rates at 20°C. Each point represents average of triplicate cultures.



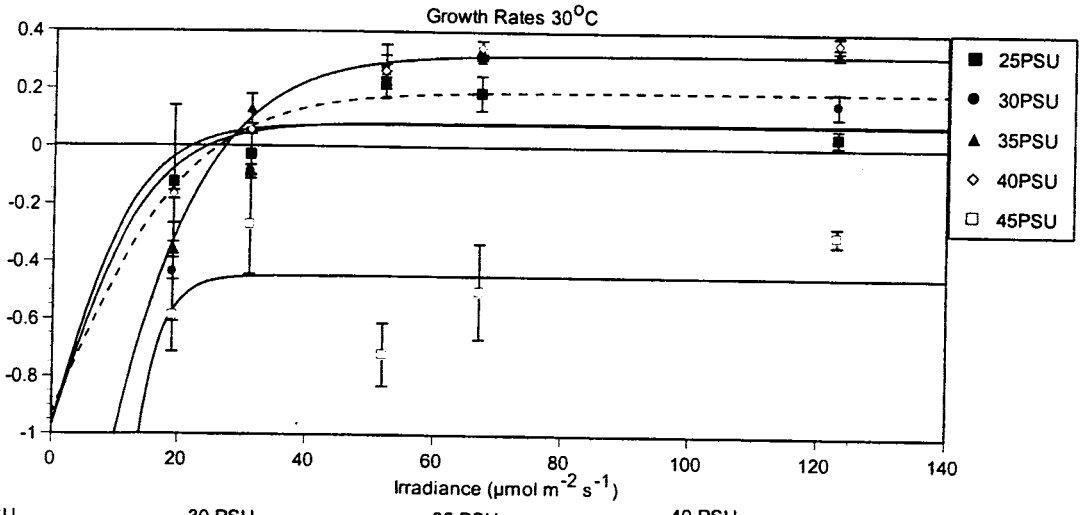
25 PSU	30 PSU	35 PSU	40 PSU	45 PSU
µmax=0.30	µmax=0.36	µmax=0.32	µmax=0.26	µmax=0.15
I_c=25.0 µmol m^{-2} s^{-1}	I_c=23.0 µmol m^{-2} s^{-1}	I_c=28.0 µmol m^{-2} s^{-1}	I_c=77.0 µmol m^{-2} s^{-1}	I_c=N/A
r^2=0.95	r^2=0.92	r^2=0.99	r^2=0.72	r^2=0.98

Fig. 3. Growth rates at 25°C. Each point represents average of triplicate cultures.



25 PSU	30 PSU	35 PSU	40 PSU	45 PSU
µmax=0.26	µmax=0.32	µmax=0.36	µmax=0.33	µmax=0.2
I_c=42.0 µmol m^{-2} s^{-1}	I_c=35.0 µmol m^{-2} s^{-1}	I_c=32.5 µmol m^{-2} s^{-1}	I_c=30.0 µmol m^{-2} s^{-1}	I_c=85.0 µmol m^{-2} s^{-1}
r^2=0.99	r^2=0.98	r^2=0.92	r^2=0.90	r^2=0.86

Fig. 4. Growth rates at 30°C. Each point represents average of triplicate cultures.



25 PSU	30 PSU	35 PSU	40 PSU	45 PSU
µmax=0.2	µmax=0.32	µmax=0.33	µmax=0.34	µmax=no growth
I_c=22.0 µmol m^{-2} s^{-1}	I_c=25.0 µmol m^{-2} s^{-1}	I_c=27.0 µmol m^{-2} s^{-1}	I_c=25.0 µmol m^{-2} s^{-1}	I_c= N/A
r^2=0.86	r^2=0.88	r^2=0.99	r^2=0.83	r^2=0.86

Fig. 5a

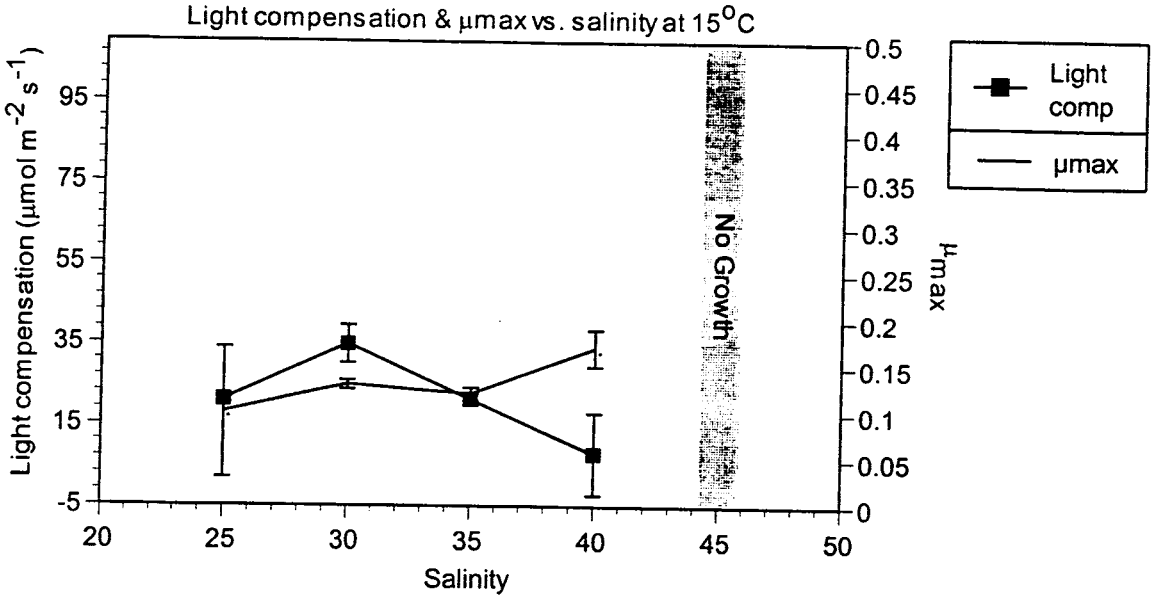

Light compensation & µmax vs. salinity at 15°C
Light compensation (µmol m-2 s-1)
95
75
55
35
15
-5
20
25
30
35
40
45
50
Salinity
No Growth
0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
µmax
Light comp
µmax

Fig. 5b

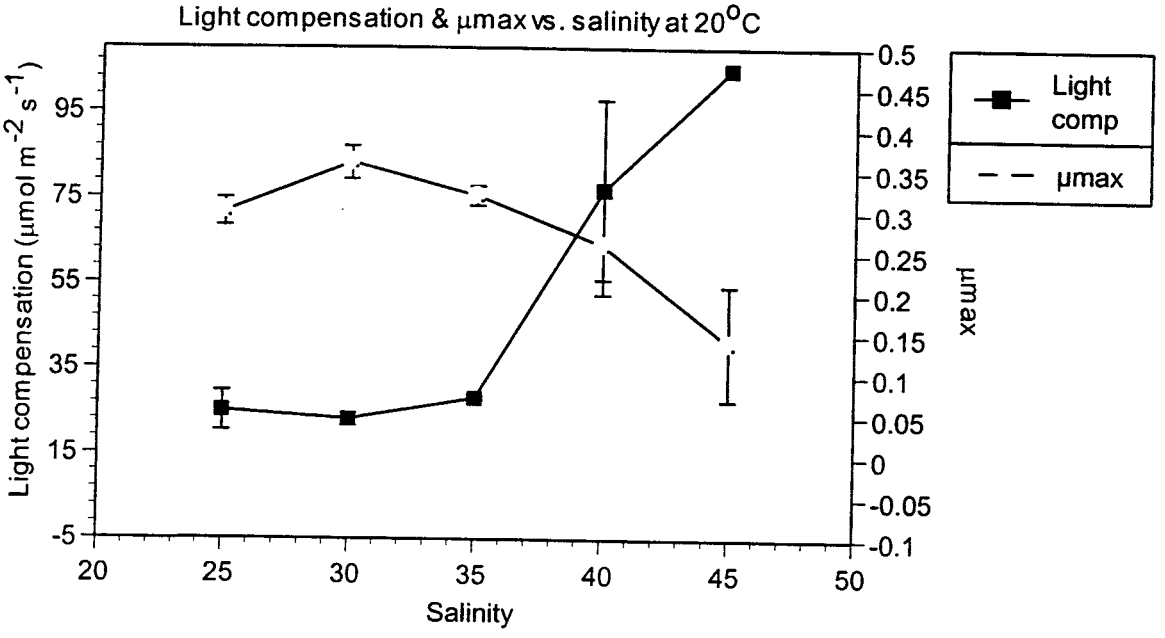

Light compensation & µmax vs. salinity at 20°C
Light compensation (µmol m-2 s-1)
95
75
55
35
15
-5
20
25
30
35
40
45
50
Salinity
0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
-0.05
-0.1
µmax
Light comp
µmax

Fig. 6a

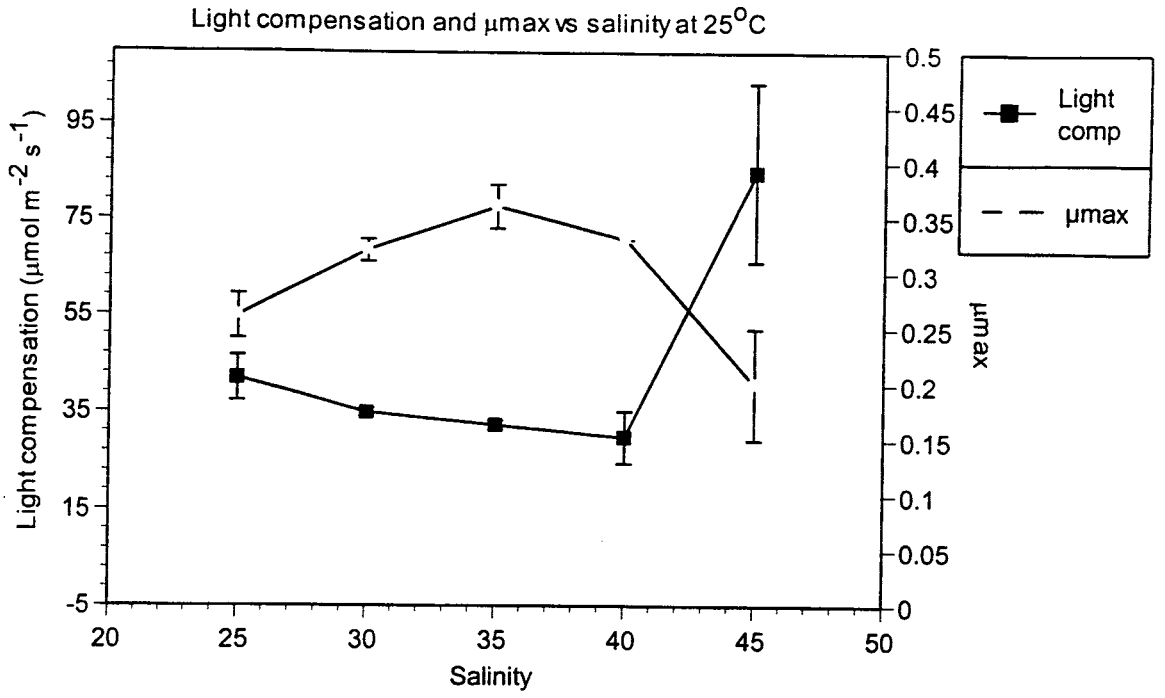

Light compensation and μmax vs salinity at 25°C
Light compensation (μmol m-2 s-1)
95
75
55
35
15
-5
20
25
30
35
40
45
50
Salinity
0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
μmax
Light comp
μmax

Fig. 6b

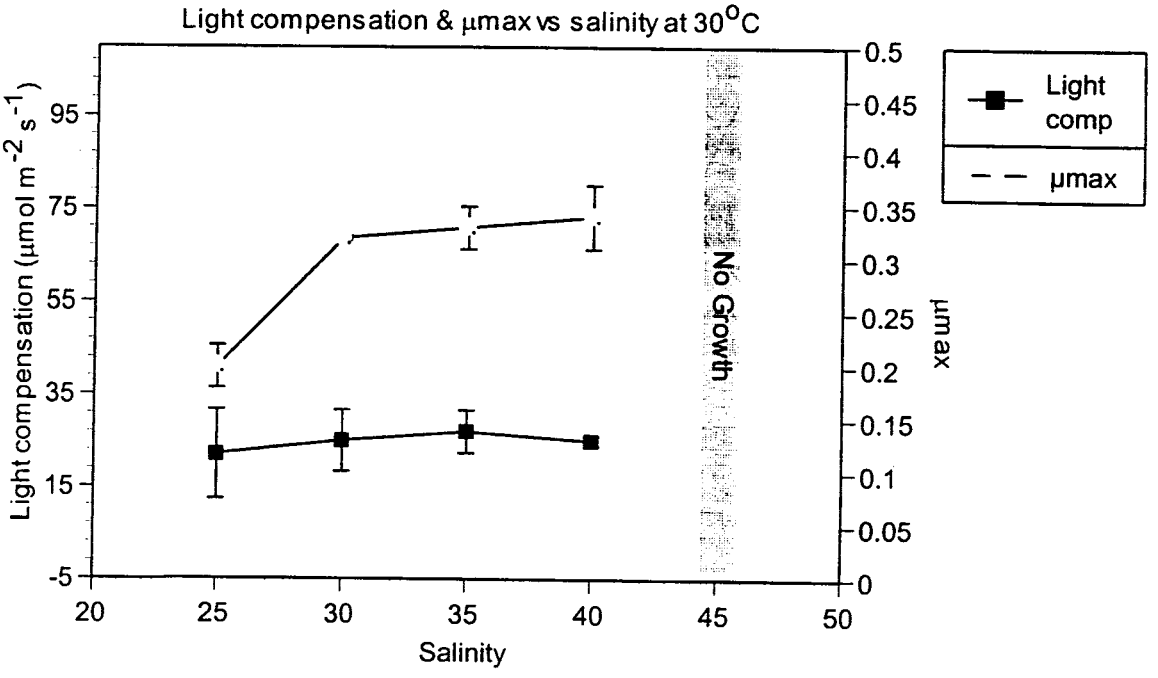

Light compensation & μmax vs salinity at 30°C
Light compensation (μmol m-2 s-1)
95
75
55
35
15
-5
20
25
30
35
40
45
50
Salinity
No Growth
0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
μmax
Light comp
μmax

Table 1. Results of μ_{max}, I_c, and R^2 for all experiments. μmax = div day^{-1}, I = μmol m^{-2} s^{-1} (lowest irradiance that supported saturated growth rates), I_c = μmol m^{-2} s^{-1} (compensation light intensity). R^2 is the fit to the Jassby-Platt hyperbolic tangent function for light dependent growth.

	25 psu	30 psu	35 psu	40 psu	45 psu
15°C	μ_{max} = 0.09 I = 31 I_c = 21.0 R^2 = 0.80	μ_{max} = 0.13 I = 67 I_c = 35.0 R^2 = 0.96	μ_{max} = 0.12 I = 123 I_c = 21.0 R^2 = 0.92	μ_{max} = 0.17 I = 123 I_c = 7.9 R^2 = 0.45	μ_{max} = no growth I_c = no growth R^2 = 0.80
20°C	μ_{max} = 0.30 I = 67 I_c = 25.0 R^2 = 0.95	μ_{max} = 0.36 I = 123 I_c = 23.0 R^2 = 0.92	μ_{max} = 0.32 I = 67 I_c = 28.0 R^2 = 0.99	μ_{max} = 0.26 I = 123 I_c = 77.0 R^2 = 0.72	μ_{max} = 0.15 I = 123 I_c = 105.0 R^2 = 0.98
25°C	μ_{max} = 0.26 I = 123 I_c = 42.0 R^2 = 0.99	μ_{max} = 0.32 I = 67 I_c = 35.0 R^2 = 0.98	μ_{max} = 0.36 I = 67 I_c = 32.5 R^2 = 0.92	μ_{max} = 0.33 I = 67 I_c = 30.0 R^2 = 0.90	μ_{max} = 0.20 I = 123 I_c = 85.0 R^2 = 0.86
30°C	μ_{max} = 0.20 I = 52 I_c = 22.0 R^2 = 0.86	μ_{max} = 0.32 I = 67 I_c = 25.0 R^2 = 0.88	μ_{max} = 0.33 I = 123 I_c = 27.0 R^2 = 0.99	μ_{max} = 0.34 I = 123 I_c = 25.0 R^2 = 0.83	μ_{max} = no growth I_c = no growth R^2 = 0.86

Discussion

Light and growth rates

The maximum growth rates (0.17-0.36 div day^{-1}) shown by *K. brevis* in this study fell within the range reported by Steidinger (0.2-0.5 div day^{-}1, 1983), Wilson (1966), and Shanley and Vargo (0.2-1.0 div day^{-}1, 1993), but higher than those reported by Doig (0.16-0.19 div day^{-1}, 1973). In the present study, growth rates increased with increasing irradiance from 31 to 123 μmol m^{-2} s^{-1}. Results for all temperature and salinity combinations indicated that a light intensity of approximately 67 μmol m^{-2} s^{-1} could be the saturation point for the Texas clone under a common range of environmental conditions. Light saturation for the Texas clone (salinity of 30 to 40) closely approximated that of a Florida clone (67 vs. 65 μmol m^{-2} s^{-1}, respectively) reported by Shanley and Vargo (1993) at 24° C. Eng-Wilmont et al. (1977) illuminated cultures from below with light intensity limited to 50 $\pm$ 13 ft-c (7.5 μmol m^{-2} s^{-1}) at the top and 207 $\pm$ 6 ft-c (31 μmol m^{-2} s^{-1}) at the bottom of the cultures. They reported intensity of light (200 ft-c $\approx$ 30 μmol m^{-2} s^{-1}) incident to the cultures was low, but within the light limiting region of growth.

One difference between previous studies and the present study was the light source ("cool white" vs. "warm white" fluorescent). Though "cool white" and "warm white" lamps have similar output in the spectral range of 410-710 nm (Figs. 8 and 9), "cool white" lights have seven peaks within this range while the "warm white" only have four peaks. The "cool white" lamps have a much higher spectral photon irradiance ranging from 0.1 to 0.7 μmol m^{-2} s^{-1} nm^{-1} compared to the "warm white" which ranges from 0.01 to 0.22 μmol m^{-2} s^{-1} nm^{-1}. The "warm white" fluorescent lamps also has less photosynthetically available radiation (PAR) than "cool white" 40.7 μmol s^{-1} vs. 42.4 μmol s^{-1}, and less efficient photosynthetically utilizable radiation efficiency (PUReff); 0.51μmol s^{-1} watt^{-1} vs. 0.45 μmol s^{-1} watt^{-1}; (Busko 2000). Further studies are needed to establish a standard lighting system and determine spectral quality effects for *K. brevis*. Few experiments have been conducted to compare fluorescent light sources and growth. Saturated growth rates suggest more light would have not increased maximum growth. However, light quality may have affected the compensation light intensity.

Light Compensation (I_c) and Growth Rates

Despite the substantial variability in growth rates of *K. brevis*, growth rate clearly varied as a function of salinity and temperature. The results from this study indicate that the *K. brevis* clone (SP3) requires light levels at or above approximately 20-30 μmol m^{-2} s^{-1}, similar to values reported in previous studies (26.25 to 30 μmol m^{-2} s^{-1}; Wilson 1955, Aldrich 1959, Aldrich 1962, and Eng-Wilmot et al. 1977). The values for I_c reported in the present study were higher than those reported by Shanley and Vargo (1993) who reported an I_c for *K. brevis* at 6 μmol m^{-2} s^{-1}. Texas clone has a higher I_c requirement than that reported for this one Florida clone; however one experimental combination (15°C salinity 40) in the present study had an I_c of 7.9 μmol m^{-2} s^{-1}. The variation of compensation light intensity with growth conditions suggests that in the field local conditions may substantially alter the light needs of *K. brevis*. High salinities lead to a significant increase in the compensation light intensity. This may explain why few red tides enter the upper Laguna Madre since that negative estuary is typically hypersaline and turbid during the time frame red tides occur along the south Texas coast.

In general, these results are reasonably consistent with the results from previous studies; however, further investigations of other clones and different temperature/salinity combinations are warranted to identify additional discrepancies associated with the Shanley and Vargo (1993) study.

Salinity and Growth Rates

Results from this study indicate only a slightly narrower range of salinity tolerance (25-46) than those reported from previous studies. The *Karenia brevis* clone (SP3) could not be acclimated to salinity levels above 45 or below 24. This is a slightly narrower range than Aldrich and Wilson (1960) who reported a salinity minimum of 22.5 and a maximum of 46 for *K. brevis*. Brydon et al. (1971) and Hitchcock (1976) reported that *K. brevis* grew well within a salinity range of 27-37 and 30-34, respectively. The higher salinity minimum of 25 shown by the present study agreed with that of Finucane (1960). Rounsefell and Nelson (1966) reported that the upper salinity limit for abundance of *K. brevis* (37) appeared to vary with temperature. Growth was poor above salinity of 36, in combination with temperature in excess 23°C. Our results show a similar effect in that no growth occurred at a salinity of 45 at the temperature extremes of 15 and 30° C. We noted that it did not grow at salinity of 45 except at the highest light intensity of 123 $\mu mol\ m^{-2}\ s^{-1}$ and at 20 and 25°C. It is clear from our results that characterizing the salinity tolerance of *K. brevis* also requires knowledge of the light and temperature field.

The salinity tolerance of clone SP3 sharply contrasted with Dortch et al.'s (1998) results. They reported *K. brevis* found in the northern Gulf of Mexico off Louisiana at salinity as low as 5. There is no evidence that clone SP3 is able to tolerate this low salinity.

Temperature and Growth Rates

A temperature tolerance for Florida *K. brevis* has been established in the field and the laboratory at 15-30°C (Kusek et al. 1999). The temperature tolerance of the Texas clone (SP3) appeared to generally resemble that for the Florida clones, with only minor differences shown at the temperature extremes. Results compare favorably with those of Eng-Wilmont et al. (1977) who reported rapid decline in viability of cells above 31°C. Growth rates in this study were significantly lower at 15 °C than other temperatures and are consistent with the general decline of Texas red tide blooms in cooler water. The upper temperature for growth (33°C) reported by Hitchcock (1976) is somewhat higher than the results noted in the present study since all attempts to culture *K. brevis* (SP3) above 31°C failed. As noted below, water quality variations may play a significant role in growth rates and survival.

Karenia brevis and Inter-Clonal Variation

Intra-specific variability in an organism's response to changes in environmental conditions is a significant problem for interpreting laboratory results, especially with marine microalgae. Gallagher (1980) examined the allozyme banding patterns for five enzyme loci in the diatom *Skeletonema costatum* and found genetic differences between winter and summer blooms are as great as those found between species of terrestrial organisms.

There is evidence that such variability occurs in *K. brevis*. Baden and Tomas (1988) analyzed major toxin composition for six clones of *K. brevis*, two from Corpus Christi, TX (CC5 and CC6) and four from Florida, Sarasota and Gasparilla Pass (CT5D2 and CT5D3) and two clones from Tampa Bay (78P5 and W53DB). Clones were subjected to identical culture conditions in NH-15 medium (Aldrich and Wilson 1960) at 24°C and light intensity of 50-70 $\mu mol\ m^{-2}\ s^{-1}$. An evaluation of the three major toxins showed variations among all clones. Baden and Tomas (1988) proposed reasons for variation among clones derived from different geographic areas as well as within geographic areas: and/or variation in metabolic capability,

ecological and/or physiological factors which could affect the metabolic machinery for toxin production, serving to intensify or reduce the potency of clones or blooms.

Loret et al. (submitted) analyzed the molecular characterization of internal transcribed spacers (ITS) and the 18S DNA region of five clonal cultures of *K. brevis*. Three strains were from an Oct. 1999 red tide event in Brownsville, Texas and referred to as SP1, SP2, and SP3, and two Florida strains were referred to as CCMP718 and "Piney Island" B4. Cultures were maintained under identical conditions in L1 medium (Guillard and Hargraves 1993) at 25°C and a light intensity of 70 μmol m^{-2} s^{-1}. Analysis and alignment of ITS and 18S regions of *K. brevis* from Texas and Florida revealed a striking conservation of the sequence--all five isolates were identical at the loci examined.

An unrelated study by Singler and Villareal (unpublished data) using the same Texas clones (SP1, SP2, and SP3) found variability in growth rates. These clones were cultured in triplicate in water collected at the Flower Gardens and enriched with L1 medium (Galliard and Hargraves 1993) at a salinity of 36 and at 25°C. Cultures were maintained in a 12:12, (L: D) photoperiod under 40 watt "cool white" fluorescent lights at 85 μmol m^{-2} s^{-1}. Growth rates for SP1, SP2, and SP3 averaged 0.94±0.04, 0.58±-0.05, 0.73±0.04, respectively. Clone SP1 grew significantly faster ($p<0.03$) than the other two clones. Apparently, further studies are needed to locate specific DNA sequences for identification of genetic differences among *K. brevis* clones.

These growth rates are much higher than those of the present study and are likely due to differences in water quality and possibly light source (water in this study was collected several hundred kilometers offshore in a dense *Trichodesmium* bloom whereas Singler used Flower Garden water). While there cannot be a direct comparison between these two studies, results indicate variation among clones isolated from the same bloom.

In conclusion, *K. brevis* from Texas and Florida are apparently genetically identical, but toxin profiles differ between the different areas of the Gulf of Mexico, and growth rates vary significantly within a single region. Intra-specific variability among clones clearly requires more study, both for understanding the temporal evolution of blooms as well as the basic autecology of the species. The Texas *K. brevis* is similar to the Florida *K. brevis* in its salinity and temperature tolerance, but differs substantially from the Louisiana *K. brevis* in the reported salinity tolerance.

Summary

The Texas clone (SP3) varied in growth rate when cultured under different light intensity, salinity, and temperature criteria. SP3 had a very similar light saturation point compared to that of the Florida isolate (67 vs. 65 μmol m^{-2} s^{-1}), and a similar light compensation point (20-30 μmol m^{-2} s^{-1}) to most of the published data. The exception was a reported light compensation of 6 μmol m^{-2} s^{-1} (Shanley and Vargo 1993). Texas clone had the same temperature tolerance of Florida clones (15-30°C) and was very similar in its salinity tolerance (25-45). However, the two factors interact and growth did not occur at all temperature-salinity combinations in the growth range. The light requirements for growth and compensation increased with salinity.

Fig. 8 "Cool white" fluorescent spectrum (Courtesy of Pedro J. Aphalo. University of Joensuu. Joensuu, Finland).

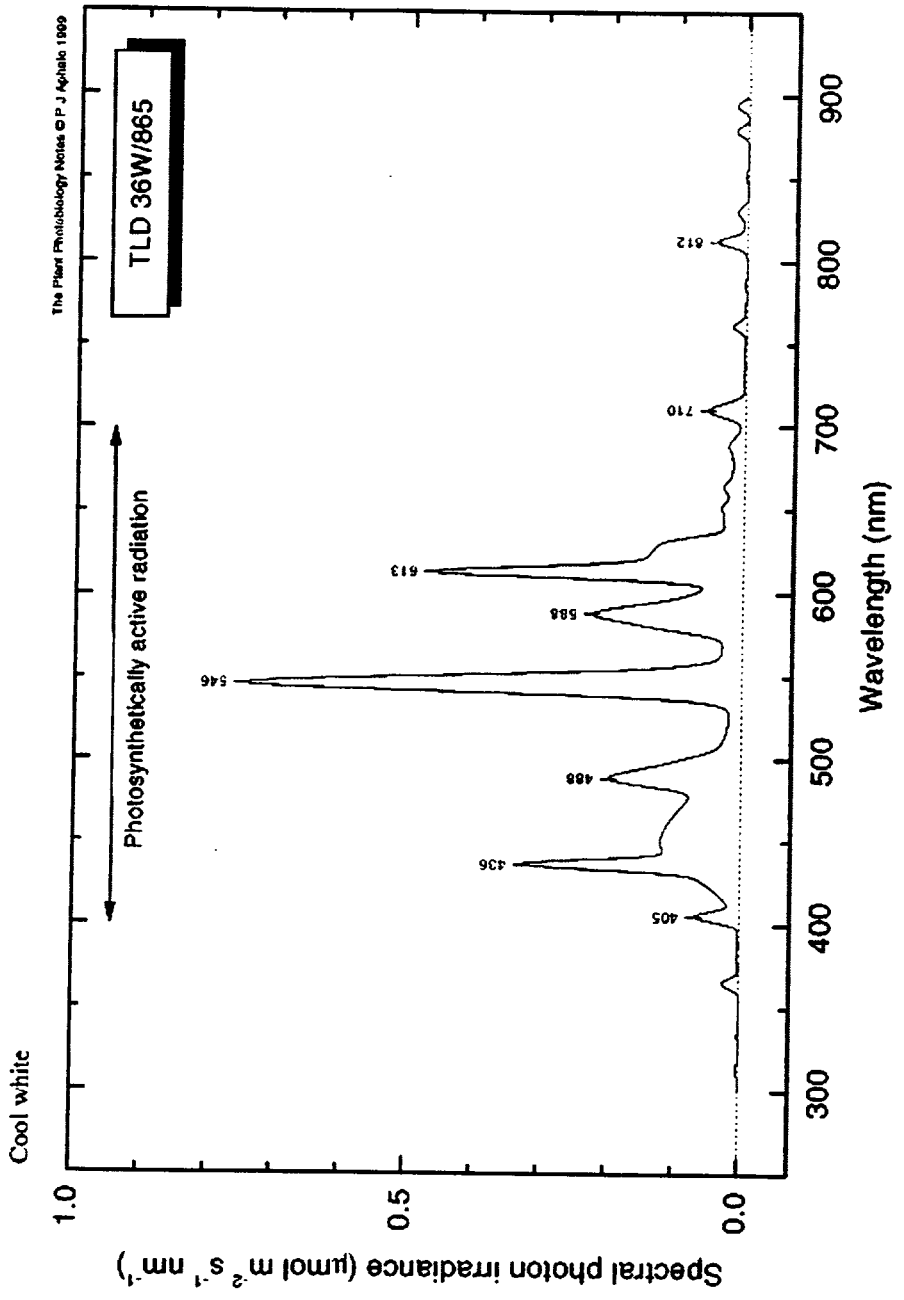


Fig. 9 "Warm white" fluorescent spectrum (Courtesy of Pedro J. Aphalo. University of Joensuu. Joensuu, Finland).

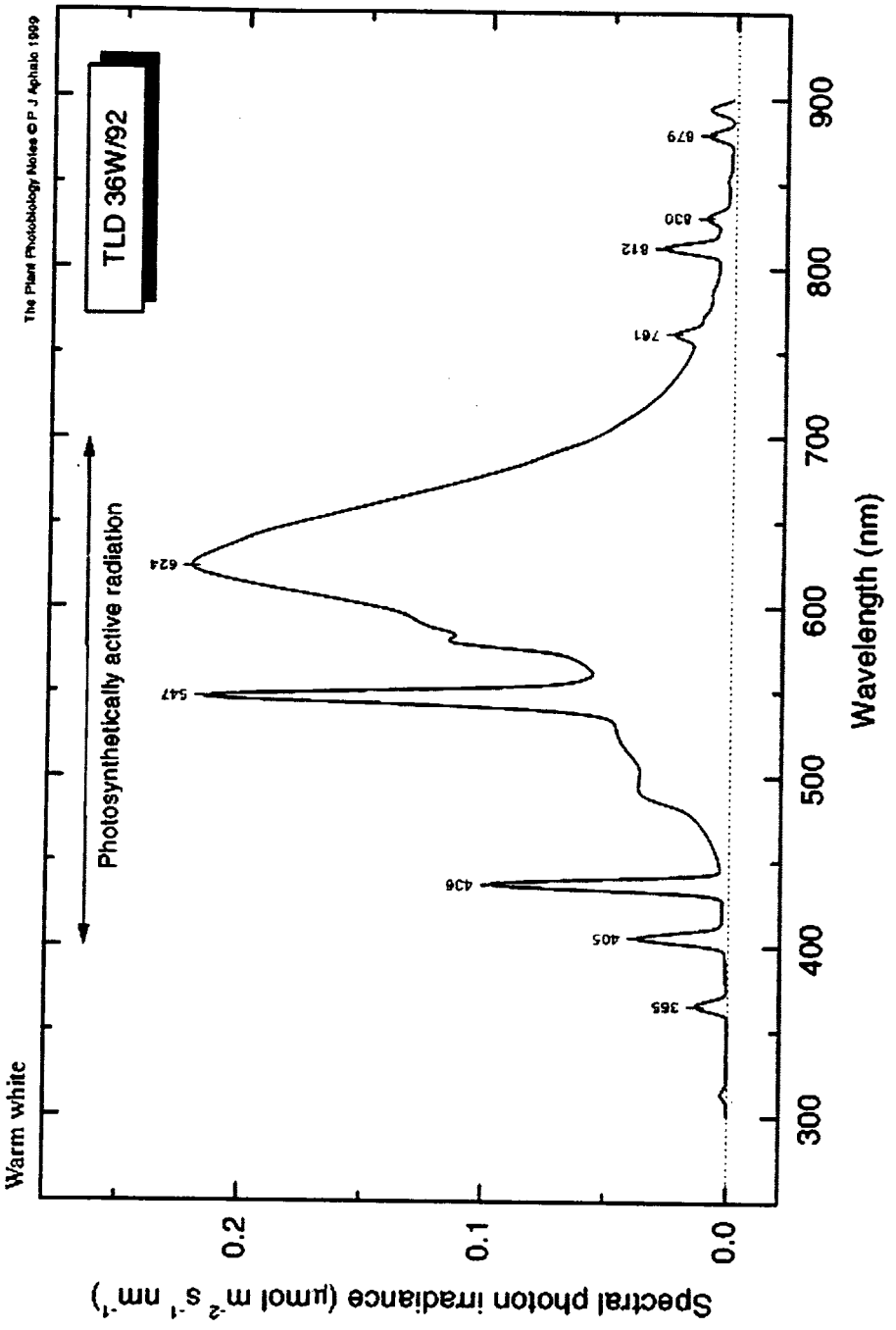
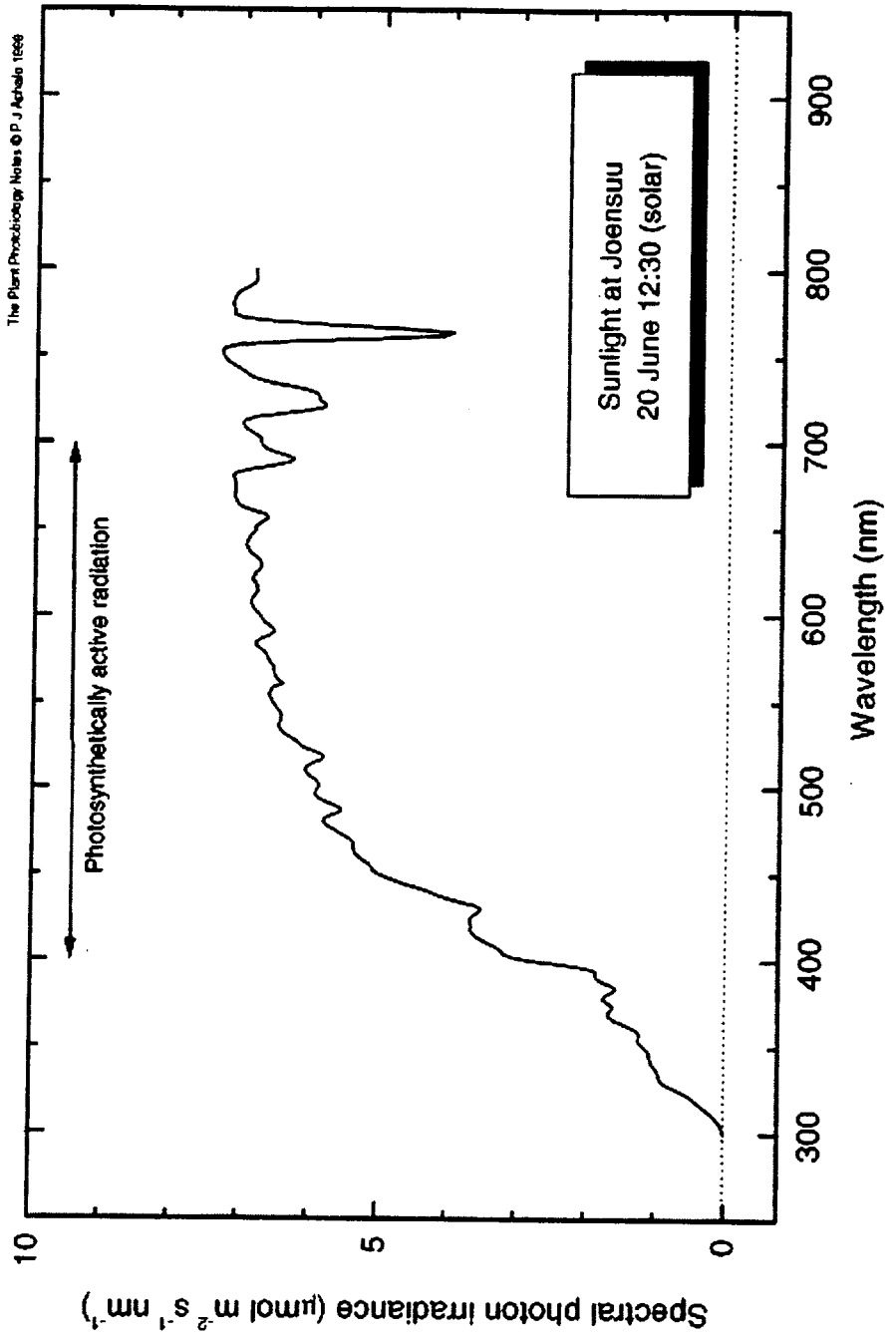


Fig. 10 Natural sunlight spectrum (Courtesy of Pedro J. Aphalo. University of Joensuu. Joensuu, Finland).



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Appendices

Appendix A. Raw data for growth experiments at 15°C
Each column represents a replicate culture (e.g. 25a = Salinity 25 replicate tube "a")
Heading in first cell indicates light irradiance in $\mu mol\ m^{-2}\ s^{-1}$
Second group of data under "Day" heading are raw data in exponential phase

123µ	15°C														
Date	25a	25b	25c	30a	30b	30c	35a	35b	35c	40a	40b	40c	45a	45b	45c
14-Mar	826	889	882	514	306	410	604	667	632	639	604	993	76	42	42
17-Mar	979	882	708	354	389	354	493	514	396	938	861	729	56	28	21
20-Mar	1243	1069	1326	382	507	465	757	576	479	896	931	1028	21	21	28
23-Mar	1472	1285	1479	590	653	722	771	806	785	1674	1424	1625	21	7	62
26-Mar	1549	1535	1510	722	917	826	1035	1028	854	2160	2090	2811	0	0	7

Day	25a	25b	25c	30a	30b	30c	35a	35b	35c	40a	40b	40c	45a	45b
4	979	882	708	354	389	354	493	514	396	938	861	729	76	42
7	1243	1069	1326	382	507	465	757	576	479	896	931	1028	56	28
10	1472	1285	1479	590	653	722	771	806	785	1674	1424	1625	21	21
13	1549	1535	1510	722	917	826	1035	1028	854	2160	2090	2811	21	7

67µ	15°C														
Date	25a	25b	25c	30a	30b	30c	35a	35b	35c	40a	40b	40c	45a	45b	45c
14-Mar	701	583	660	417	271	326	722	604	576	361	417	510	35	35	28
17-Mar	792	736	771	361	361	264	660	493	444	410	479	500	21	42	42
20-Mar	1375	826	806	500	410	333	493	576	660	396	646	576	14	56	35
23-Mar	944	826	1063	597	528	521	826	708	563	792	847	1056	7	42	14
26-Mar	1097	951	1083	826	778	653	2677	1264	799	833	1132	2153	0	21	0

Day	25b	25c	30a	30b	30c	35b	35c	40a	40b	40c	45a	45c
1	583	660	361	361	264	493	444	361	417	510	21	42
4	736	771	500	410	333	576	660	410	479	500	14	35
7	826	806	597	528	521	708	563	396	646	576	7	14
10	826	1063	826	778	653	1264	799	792	847	1056	0	0
13	951	1083						833	1132	2153		

52µ	15°C														
Date	25a	25b	25c	30a	30b	30c	35a	35b	35c	40a	40b	40c	45a	45b	45c
14-Mar	583	361	417	236	181	236	611	510	396	486	285	417	63	56	49
17-Mar	472	299	389	236	174	215	472	424	333	500	410	382	35	42	14
20-Mar	479	403	410	313	187	257	375	438	486	715	410	493	21	42	7
23-Mar	479	379	493	479	181	285	632	597	549	694	466	618	21	21	7
26-Mar	569	597	403	500	410	396	604	660	764	979	660	660	14	7	0

Day	25a	25b	25c	30a	30b	30c	35a	35b	35c	40a	40b	40c	45a	45b	45c
1	472	299	389	236	181	236	472	424	333	486	285	417	63	56	49
4	479	403	410	236	174	215	375	438	486	500	410	382	35	42	14
7	479	379	493	313	187	257	632	597	549	715	410	493	21	42	7
10	569	597	403	479	181	285	604	660	764	694	466	618	21	21	7
13				500	410	396				979	660	660	14	7	0

31µ	15°C														
Date	25a	25b	25c	30a	30b	30c	35a	35b	35c	40a	40b	40c	45a	45b	45c
14-Mar	69	201	174	158	118	149	389	479	340	83	90	167	35	56	56
17-Mar	153	257	189	139	132	181	313	458	438	215	167	167	42	21	28
20-Mar	139	340	201	118	111	146	438	486	514	111	69	132	14	14	7
23-Mar	208	306	118	194	118	208	347	431	479	104	104	181	7	35	21
26-Mar	250	181	208	319	208	229	618	597	493	222	139	181	7	7	7

31µ	15°C													
Day	25a	25c	30a	30b	30c	35a	35b	35c	40a	40b	40c	45a	45b	45c
1	69	174	158	118	149	313	458	438	111	69	132	35	56	56
4	153	189	139	132	181	438	486	514	104	104	181	42	21	28
7	139	201	118	111	146	347	431	479	222	139	181	14	14	7
10	208	118	194	118	208	618	597	493				7	35	21
13	250	208	319	208	229							7	7	7

19µ	15°C														
Date	25a	25b	25c	30a	30b	30c	35a	35b	35c	40a	40b	40c	45a	45b	45c
14-Mar	146	181	153	146	146	111	271	125	194	35	139	146	21	21	7
17-Mar	125	181	104	90	83	90	208	208	201	28	104	83	28	21	42
20-Mar	132	90	174	118	132	69	118	167	139	35	125	69	7	7	7
23-Mar	132	160	174	76	146	167	271	264	174	28	83	83	7	7	7
26-Mar	104	160	167	49	181	104	201	410	319	14	111	56	0	0	0

Day	25a	25b	25c	30b	30c	35b	35c	40a	40b	40c	45a	45b	45c
1	146	181	153	146	111	125	194	28	104	83	28	21	42
4	125	181	104	83	90	208	201	35	125	69	7	7	7
7	132	90	174	132	69	167	139	28	83	83	7	7	7
10	132	160	174	146	167	264	174	14	111	56	0	0	0
13	104	160	167	181		410	319						

Appendix B. Raw data for growth experiments at 20°C
Each column represents a replicate culture (e.g. 25a = Salinity 25 replicate tube "a")
Heading in first cell indicates light irradiance in μmol m^{-2} s^{-1}.
Second group of data under "Day" heading are raw data in exponential phase

123μ 20°C

Day	25a	25b	25c	30a	30b	30c	35a	35b	35c	40a	40b	40c	45a	45b	45c
16-Feb	1111	590	1181	958	840	868	632	660	549	472	431	486	49	56	28
19-Feb	2177	2258	2678	1875	1812	1826	889	993	1042	785	944	688	56	35	28
22-Feb	3303	3056	2313	4929	4088	3222	2367	2376	2050	1583	1917	924	35	63	69
25-Feb	2493	1667	2278	9125	9074	8300	4275	4711	3667	2347	1764	2045	14	56	14
1-Mar	1535	1938	1979	13800	13800	12167	9762	11167	6933	1729	1826	2034	14	21	7

Day	25a	25b	25c	30a	30b	30c	35a	35b	35c	40a	40b	40c	45a	45b	45c
1	1111	590	1181	958	840	868	632	660	549	785	944	688	49	56	28
4	2177	2258	2678	1875	1812	1826	889	993	1042	1583	1917	924	56	35	28
7	3303	3056	2313	4929	4088	3222	2367	2376	2050	2347	1764	2045	35	63	69
10				9125	9074	8300	4275	4711	3667				14	56	14
14				13800	13800	12167	9762	11167	6933				14	21	7

67μ 20°C

Day	25a	25b	25c	30a	30b	30c	35a	35b	35c	40a	40b	40c	45a	45b	45c
16-Feb	583	243	604	777	722	479	1042	1201	1083	326	271	250	7	77	7
19-Feb	1153	840	1431	1194	1424	979	1965	1889	1458	319	403	521	7	7	7
22-Feb	2564	1514	2625	4275	2733	2166	3396	4000	4976	632	569	521	14	14	14
25-Feb	2967	1993	4078	6848	1373	3789	7444	8000	7916	715	660	833	28	7	7
1-Mar	3449	3182	4444	6394	4762	5119	13933	17333	13333	1299	1243	1465	1	1	1

Day	25a	25b	25c	30a	30c	35a	35b	35c	40a	40b	40c	45b	45c
1	583	243	604	777	479	1042	1201	1083	326	271	250	7	7
4	1153	840	1431	1194	979	1965	1889	1458	319	403	521	14	14
7	2564	1514	2625	4275	2166	3396	4000	4976	632	569	521	7	7
10	2967	1993	4078	6848	3789	7444	8000	7916	715	660	833	1	1
14	3449	3182	4444	6394	5119	13933	17333	13333	1299	1243	1465		

52μ 20°C

Day	25a	25b	25c	30a	30b	30c	35a	35b	35c	40a	40b	40c	45a	45b	45c
16-Feb	479	299	333	333	215	208	319	368	382	49	63	49	28	35	7
19-Feb	750	458	583	361	326	514	639	472	451	42	14	14	7	21	7
22-Feb	979	507	840	493	556	674	1056	1063	778	28	7	1	1	7	7
25-Feb	1569	1049	1076	534	660	819	1208	1229	1229	21	7	7	0	7	1
1-Mar	2313	1194	1889	1486	1257	1549	2403	2361	1757	1	1	1	0	1	0

Day	25a	25b	25c	30a	30b	30c	35a	35b	35c	40a	40b	40c	45a	45b
1	479	299	333	333	215	208	319	368	382	49	63	49	28	35
4	750	458	583	361	326	514	639	472	451	42	14	14	7	21
7	979	507	840	493	556	674	1056	1063	778	28	7	1	1	7
10	1569	1049	1076	534	660	819	1208	1229	1229	21	7	7	0	7
14	2313	1194	1889	1486	1257	1549	2403	2361	1757	1	1	1	0	1

31µ 20°C

Day	25a	25b	25c	30a	30b	30c	35a	35b	35c	40a	40b	40c	45a	45b	45c
16-Feb	139	90	63	118	104	69	49	83	83	35	42	35	21	14	7
19-Feb	83	49	28	35	28	49	76	76	132	14	7	14	7	1	1
22-Feb	83	83	63	76	63	69	76	146	76	7	21	14	1	0	0
25-Feb	56	104	104	111	132	97	90	90	104	1	1	1	0	0	0
1-Mar	140	188	215	284	153	125	125	181	139	0	0	0	0	0	0

Day	25a	25b	25c	30a	30b	30c	35a	35c	40a	40c	45a	45b	45c
1	83	49	28	35	28	49	76	132	35	35	21	14	7
4	83	83	63	76	63	69	76	76	14	14	7	1	1
7	56	104	104	111	132	97	90	104	7	14	1	0	0
10	140	188	215	284	153	125	125	139	1	1	0	0	0
14									0	0			

19µ 20°C

Day	25a	25b	25c	30a	30b	30c	35a	35b	35c	40a	40b	40c	45a	45b	45c
16-Feb	63	42	97	56	63	83	35	69	56	7	21	7	7	7	42
19-Feb	28	35	14	28	28	49	56	42	28	1	7	7	1	1	7
22-Feb	42	14	35	14	21	35	14	42	21	0	1	1	0	0	1
25-Feb	14	42	21	63	35	28	7	21	21	0	0	0	0	0	0
1-Mar	7	21	7	28	28	56	28	7	14	0	0	0	0	0	0

Day	25a	25b	25c	30a	30b	30c	35b	35c	40a	40b	40c	45a	45b	45c
1	63	42	97	56	63	83	69	56	7	21	7	7	7	42
4	28	35	14	28	28	49	42	28	1	7	7	1	1	7
7	42	14	35	14	21	35	42	21	0	1	1	0	0	1
10	14	42	21				21	21	0	0	0	0	0	0
14	7	21	7				7	14						

Appendix C. Raw data for growth experiments at 25oC
Each column represents a replicate culture (e.g. 25a = Salinity 25 replicate tube "a")
Heading in first cell indicates light irradiance in □mol m-2 s-1
Second group of data under "Day" heading are raw data in exponential phase

123µm 25°C

Day	25a	25b	25c	30a	30b	30c	35a	35b	35c	40a	40b	40c	45a	45b	45c
18-Dec	1014	694	882	1021	1069	910	1181	1243	1271	1340	965	1264	646	556	889
21-Dec	2826	1981	2667	3533	2701	2942	3759	2222	2431	2280	1772	1964	424	951	1725
24-Dec	8731	1611	2436	5467	3206	4646	5765	3167	2564	4463	3056	2973	1305	667	1292
27-Dec	5359	3981	6417	9208	8267	7444	11429	11292	10333	10833	4804	11037	2973	2986	2049
3-Jan	10476	4786	6182	10810	4556	4333	12238	8148	7250	15933	6636	9583	965	1660	1743

Day	25b	25c	30a	30b	30c	35a	35b	35c	40a	40c	45a	45b	45c
1	694	882	1021	1069	910	1181	1243	1271	1340	1264	646	556	889
4	1981	2667	3533	2701	2942	3759	2222	2431	2280	1964	424	951	1725
7	1611	2436	5467	3206	4646	5765	3167	2564	4463	2973	1305	667	1292
10	3981	6417	9208	8267	7444	11429	11292	10333	10833	11037	2973	2986	2049
16	4786	6182											

67µm 25°C

Day	25a	25b	25c	30a	30b	30c	35a	35b	35c	40a	40b	40c	45a	45b	45c
14-Mar	1389	597	1229	958	417	514	750	813	486	410	1160	910	326	403	521
17-Mar	6848	1083	1833	1576	542	368	1646	1146	639	604	1319	1732	417	708	1104
20-Mar	2422	1403	3524	4216	2315	1152	3693	3000	2433	1583	4098	3722	208	271	896
23-Mar	4700	1563	7861	2469	1313	1694	6194	674	2877	2718	2795	4063	632	944	1389
26-Mar	7593	1020	3639	2087	2465	1486	1146	1806	910	6205	1167	896	299	431	424

Day	25a	25c	30a	30b	30c	35a	35b	35c	40a	40b	40c	45a	45b	45c
1	1389	1229	958	417	514	750	813	486	410	1160	910	326	403	521
4	6848	1833	1576	542	368	1646	1146	639	604	1319	1732	417	708	1104
7	2422	3524	4216	2315	1152	3693	3000	2433	1583	4098	3722	208	271	896
10	4700	7861	2469	1313	1694				2718	2795	4063	632	944	1389

52µm 25°C

Day	25a	25b	25c	30a	30b	30c	35a	35b	35c	40a	40b	40c	45a	45b	45c
14-Mar	896	1042	694	576	410	333	472	354	417	181	243	361	271	465	368
17-Mar	1319	1153	542	1021	667	396	847	667	569	472	542	715	181	375	285
20-Mar	500	1278	1111	1528	806	813	1000	1319	674	493	840	1021	76	174	257
23-Mar	2737	1993	1042	1097	799	639	1951	1257	1313	1083	924	729	118	222	354
26-Mar	1868	1132	875	1222	556	389	819	722	292	326	347	840	63	76	139

Day	25a	25b	25c	30a	30b	30c	35a	35b	35c	40b	40c	45a	45b	45c
1	896	1042	694	576	410	333	472	354	417	243	361	271	465	368
4	1319	1153	542	1021	667	396	847	667	569	542	715	181	375	285
7	500	1278	1111	1528	806	813	1000	1319	674	840	1021	76	174	257
10	2737	1993	1042	1097	799	639	1951	1257	1313			118	222	354

31μm 25°C

Day	25a	25b	25c	30a	30b	30c	35a	35b	35c	40a	40b	40c	45a	45b	45c
14-Mar	201	340	167	146	173	153	313	257	347	111	215	125	160	174	188
17-Mar	174	174	361	160	201	167	215	174	326	243	208	299	306	361	382
20-Mar	188	194	382	111	132	139	243	111	382	132	208	186	215	333	229
23-Mar	194	118	194	90	104	76	139	28	250	69	125	160	174	243	339
26-Mar	64	90	201	69	90	35	76	139	104	90	49	70	97	146	118

Day	25a	25b	30a	30b	30c	35a	35b	35c	40a	40b	40c	45a	45b	45c
1	201	340	146	173	153	313	257	347	111	215	125	160	174	188
4	174	174	160	201	167	215	174	326	243	208	299	306	361	382
7	188	194	111	132	139	243	111	382	132	208	186	215	333	229
10	194	118	90	104	76	139	28	250	69	125	160	174	243	339
16	64	90	69	90	35	76	139	104	90	49	70	97	146	118

19μm 25°C

Day	25a	25b	25c	30a	30b	30c	35a	35b	35c	40a	40b	40c	45a	45b	45c
14-Mar	76	28	35	97	149	188	69	111	76	90	146	125	167	160	167
17-Mar	7	7	0	104	125	111	118	153	111	7	243	194	90	215	375
20-Mar	0	0	0	42	28	14	69	76	43	42	201	90	76	69	76
23-Mar	7	7	7	28	21	21	28	35	14	28	42	76	28	83	7
26-Mar	0	7	0	0	0	7	7	7	14	56	21	7	7	14	0

Day	25a	25b	25c	30a	30b	30c	35a	35b	35c	40a	40b	40c	45a	45b	45c
1	76	28	35	97	149	188	69	111	76	90	146	125	167	160	167
4	7	7	0	104	125	111	118	153	111	7	243	194	90	215	375
7	0	0	0	42	28	14	69	76	43	42	201	90	76	69	76
10	7	7	7	28	21	21	28	35	14	28	42	76	28	83	7
16	0	7	0	0	0	7	7	7	14	56	21	7	7	14	0

Appendix D. Raw data for growth experiments at 30°C
Each column represents a replicate culture (e.g. 25a = Salinity 25 replicate tube "a")
Heading in first cell indicates light irradiance in μmol m^{-2} s^{-1}
Second group of data under "Day" heading are raw data in exponential phase

123μ 30°C

Day	25a	25b	25c	30a	30b	30c	35a	35b	35c	40a	40b	40c	45a	45b	45c
15-Jan	910	667	861	1125	861	743	2745	2368	1785	1285	576	1430	52	35	17
18-Jan	819	1215	3433	2292	2181	1840	5074	4255	3400	3416	3000	2248	139	49	28
21-Jan	1215	701	1292	1549	1639	1903	12667	9000	6361	5214	6685	7231	63	28	14
24-Jan	0	0	0	1965	1403	2042	16444	13722	9814	8410	7100	8711	56	0	0
27-Jan	0	0	0	5385	1319	3103	17667	13833	15667	9292	8542	9958	0	0	0

Day	25a	25b	30a	30b	30c	35a	35b	35c	40a	40b	40c	45b	45c
1	910	667	1125	861	743	2745	2368	1785	1285	576	1430	35	17
4	819	1215	2292	2181	1840	5074	4255	3400	3416	3000	2248	49	28
7	1215	701	1549	1639	1903	12667	9000	6361	5214	6685	7231	28	14
10	0	0	1965	1403	2042	16444	13722	9814	8410	7100	8711	0	0
13	0	0	5385	1319	3103	17667	13833	15667	9292	8542	9958	0	0

67μ 30°C

Day	25a	25b	25c	30a	30b	30c	35a	35b	35c	40a	40b	40c	45a	45b	45c
15-Jan	396	292	326	56	160	153	1799	1486	1111	340	472	382	35	104	83
18-Jan	1340	785	1153	208	257	229	2433	2479	1514	722	757	743	56	56	70
21-Jan	896	410	1083	743	604	590	6533	5275	2792	1722	1653	1590	14	111	35
24-Jan	0	0	0	1805	1222	882	9815	6795	4317	1479	2305	2588	7	42	0
27-Jan	0	0	0	958	514	1708	10500	8417	6733	2693	3400	3551	0	0	0

Day	25a	25b	25c	30a	30b	30c	35a	35b	35c	40a	40b	40c	45a	45c
1	396	292	326	56	160	153	1799	1486	1111	340	472	382	35	83
4	1340	785	1153	208	257	229	2433	2479	1514	722	757	743	56	70
7	896	410	1083	743	604	590	6533	5275	2792	1722	1653	1590	14	35
10	0	0	0	1805	1222	882	9815	6795	4317	1479	2305	2588	7	0
13	0	0	0				10500	8417	6733	2693	3400	3551	0	0

52μ 30°C

Day	25a	25b	25c	30a	30b	30c	35a	35b	35c	40a	40b	40c	45a	45b	45c
15-Jan	424	222	201	438	208	319	604	299	257	396	250	90	14	28	14
18-Jan	486	563	410	764	431	777	729	514	563	715	764	215	14	35	14
21-Jan	938	674	493	1250	694	1049	1319	840	1174	806	1104	194	42	35	14
24-Jan	1333	806	799	1819	924	1431	1547	1018	1285	1125	1187	188	7	0	0
27-Jan	1819	1201	1229	2229	1632	1764	1833	1035	1479	1201	1097	222	0	0	0

Day	25a	25b	25c	30a	30b	30c	35a	35b	35c	40a	40b	45a	45b	45c
1	424	222	201	438	208	319	604	299	257	396	250	14	28	14
4	486	563	410	764	431	777	729	514	563	715	764	14	35	14
7	938	674	493	1250	694	1049	1319	840	1174	806	1104	42	35	14
10	1333	806	799	1819	924	1431	1547	1018	1285	1125	1187	7	0	0
13	1819	1201	1229	2229	1632	1764	1833	1035	1479	1201	1097	0	0	0

31µ 30°C

Day	25a	25b	25c	30a	30b	30c	35a	35b	35c	40a	40b	40c	45a	45b	45c
15-Jan	69	42	56	14	76	83	181	667	396	389	229	181	215	118	104
18-Jan	153	49	49	42	35	42	542	625	576	444	333	194	97	132	146
21-Jan	69	76	28	21	35	21	361	799	375	486	319	174	63	83	104
24-Jan	0	49	35	7	49	49	736	1076	451	653	438	257	42	21	97
27-Jan	0	0	0	7	63	28	764	1292	465	424	181	118	0	14	21

Day	25b	25c	30b	30c	35a	35b	35c	40a	40b	40c	45a	45b	45c
1	42	56	76	83	181	667	396	389	229	181	215	118	104
4	49	49	35	42	542	625	576	444	333	194	97	132	146
7	76	28	35	21	361	799	375	486	319	174	63	83	104
10	49	35	49	49	736	1076	451	653	438	257	42	21	97
13	0	0	63	28	764	1292	465				0	14	21

19µ 30°C

Day	25a	25b	25c	30a	30b	30c	35a	35b	35c	40a	40b	40c	45a	45b	45c
15-Jan	14	35	42	42	63	174	63	160	118	21	340	174	97	111	90
18-Jan	21	14	42	7	77	56	69	188	146	14	250	125	28	21	42
21-Jan	7	7	7	7	7	35	21	83	146	0	42	56	0	0	14
24-Jan	0	0	7	0	7	21	7	21	49	0	14	21	0	0	0
27-Jan	0	0	0	0	0	7	0	14	0	0	0	0	0	0	0

Day	25a	25b	25c	30a	30b	30c	35a	35b	35c	40a	40b	40c	45a	45b	45c
1	14	35	42	42	63	174	63	160	118	21	340	174	97	111	90
4	21	14	42	7	77	56	69	188	146	14	250	125	28	21	42
7	7	7	7	7	7	35	21	83	146	0	42	56	0	0	14
10	0	0	7	0	7	21	7	21	49	0	14	21	0	0	0
13	0	0	0	0	0	7	0	14	0	0	0	0	0	0	0

Appendix E. Results from linear regression 15°C
Lin = Linear regression, d.f. = degrees of freedom, Sigf = significance, b1 = slope of line

123ln15C	Method	Rsq.	d.f.	Sigf	b1	Div/Day	Avg.
a25	Lin	0.93	2	0.035	0.0515	0.074304	
b25	Lin	1	2	0	0.0615	0.088732	0.093
c25	Lin	0.737	2	0.142	0.0794	0.114558	
a30	Lin	0.939	2	0.031	0.0858	0.123792	
b30	Lin	0.995	2	0.002	0.0942	0.135911	0.134
c30	Lin	0.965	2	0.017	0.0994	0.143414	
a35	Lin	0.9	2	0.052	0.0748	0.107921	
b35	Lin	0.97	2	0.015	0.0805	0.116145	0.120
c35	Lin	0.933	2	0.034	0.0933	0.134613	
a40	Lin	0.864	2	0.07	0.1042	0.150339	
b40	Lin	0.939	2	0.031	0.1028	0.148319	0.172
c40	Lin	0.99	2	0.005	0.1502	0.216708	
a45	Lin	0.879	2	0.062	-0.1613	-0.23272	-0.253
b45	Lin	0.908	2	0.047	-0.1888	-0.2724	

67ln15C	Method	Rsq.	d.f.	Sigf	b1	Div/Day	Avg.
b25	Lin	0.963	1	0.123	0.0581	0.083826	0.065936
c25	Lin	0.907	1	0.198	0.0333	0.048045	
a30	Lin	0.972	1	0.107	0.0838	0.120906	
b30	Lin	0.965	1	0.12	0.0634	0.091473	0.125283
c30	Lin	0.968	1	0.115	0.1133	0.163468	
b35	Lin	0.993	1	0.051	0.0603	0.087	0.072068
c35	Lin	0.354	1	0.594	0.0396	0.057135	
a40	Lin	0.495	1	0.503	0.0154	0.022219	
b40	Lin	0.957	1	0.133	0.073	0.105324	0.052277
c40	Lin	0.631	1	0.416	0.0203	0.029289	
a45	Lin	0.978	1	0.096	-0.1831	-0.26418	-0.26418
c45	Lin	0.87	1	0.234	-0.1831	-0.26418	

52In15C	Method	Rsq.	d.f.	Sigf	b1	Div/Day	Avg.
a25	Lin	0.664	2	0.185	0.0187	0.02698	
b25	Lin	0.82	2	0.094	0.0671	0.096811	0.041761
c25	Lin	0.125	2	0.647	0.0097	0.013995	
a30	Lin	0.862	2	0.071	0.0802	0.115712	
b30	Lin	0.1	2	0.684	0.0024	0.003463	0.051652
c30	Lin	0.639	2	0.201	0.0248	0.035781	
a35	Lin	0.454	2	0.326	0.0421	0.060742	
b35	Lin	0.912	2	0.045	0.0546	0.078777	0.088395
c35	Lin	0.968	2	0.016	0.0871	0.125667	
a40	Lin	0.793	2	0.109	0.0475	0.068533	
b40	Lin	0.807	2	0.102	0.0492	0.070985	0.069495
c40	Lin	0.766	2	0.125	0.0478	0.068966	
a45	Lin	0.883	2	0.06	-0.1269	-0.18309	
b45	Lin	0.829	2	0.089	-0.0981	-0.14154	-0.21291
c45	Lin	0.844	2	0.081	-0.2177	-0.3141	

31In15C	Method	Rsq.	d.f.	Sigf	b1	Div/Day	Avg.
a25	Lin	0.649	1	0.404	0.1167	0.168374	0.101501
c25	Lin	0.993	1	0.054	0.024	0.034627	
a30	Lin	0.995	1	0.045	-0.0487	-0.07026	
b30	Lin	0.121	1	0.774	-0.012	-0.01731	-0.03083
c30	Lin	0.007	1	0.945	-0.0034	-0.00491	
a35	Lin	0.09	1	0.806	0.0172	0.024816	
b35	Lin	0.256	1	0.662	-0.0101	-0.01457	0.01058
c35	Lin	0.311	1	0.623	0.0149	0.021498	
a40	Lin	0.68	1	0.383	0.1155	0.166643	
b40	Lin	0.99	1	0.063	0.1167	0.168374	0.136969
c40	Lin	0.75	1	0.333	0.0526	0.075891	
a45	Lin	0.606	1	0.432	-0.1527	-0.22031	
b45	Lin	0.946	1	0.15	-0.231	-0.33329	-0.35122
c45	Lin	0.964	1	0.121	-0.3466	-0.50007	

19ln15C	Method	Rsq.	d.f.	Sigf	b1	Div/Day	Avg.
a25	Lin	0.409	1	0.558	-0.0168	-0.02424	
b25	Lin	0.75	1	0.333	-0.1164	-0.16794	-0.05377
c25	Lin	0.058	1	0.846	0.0214	0.030876	
b30	Lin	0.028	1	0.893	-0.0168	-0.02424	-0.06925
c30	Lin	0.995	1	0.043	-0.0792	-0.11427	
b35	Lin	0.322	1	0.616	0.0483	0.069687	-0.00527
c35	Lin	0.671	1	0.389	-0.0556	-0.08022	
b40	Lin	0.302	1	0.629	-0.0376	-0.05425	-0.05425
c40	Lin	0	1	1	7.4^-17		
a45	Lin	0.75	1	0.333	-0.231	-0.33329	
b45	Lin	0.75	1	0.333	-0.1831	-0.26418	-0.34276
c45	Lin	0.75	1	0.333	-0.2986	-0.43082	

Appendix F. Results from linear regression at 20°C

Lin = Linear regression, d.f. = degrees of freedom, Sigf = significance, b1 = slope of line

123ln20C	Method	Rsq.	d.f.	Sigf	b1	Div/Day	Avg.
a25	Lin	0.982	1	0.086	0.1818	0.2623	
b25	Lin	0.883	1	0.223	0.2741	0.39547	0.273121
c25	Lin	0.593	1	0.441	0.112	0.161593	
a30	Lin	0.989	1	0.066	0.273	0.393883	
b30	Lin	1	1	0.01	0.2637	0.380465	0.363247
c30	Lin	0.994	1	0.049	0.2186	0.315395	
a35	Lin	0.928	1	0.173	0.2201	0.317559	
b35	Lin	0.958	1	0.131	0.2135	0.308036	0.314144
c35	Lin	1	1	0.01	0.2196	0.316837	
a40	Lin	0.974	1	0.102	0.1825	0.26331	0.262661
c40	Lin	0.935	1	0.165	0.1816	0.262011	
a45	Lin	0.483	1	0.511	0.0561	0.080941	0.148896
c45	Lin	0.75	1	0.333	0.1503	0.216852	

67ln20C	Method	Rsq.	d.f.	Sigf	b1	Div/Day	Avg.
a25	Lin	0.941	2	0.03	0.1894	0.273265	
b25	Lin	0.909	2	0.047	0.2301	0.331987	0.303323
c25	Lin	0.978	2	0.011	0.2112	0.304718	
a30	Lin	0.957	2	0.022	0.2601	0.375271	0.355937
c30	Lin	0.996	2	0.002	0.2333	0.336604	
a35	Lin	0.995	2	0.003	0.2149	0.310056	
b35	Lin	0.99	2	0.005	0.2146	0.309623	0.321887
c35	Lin	0.945	2	0.028	0.2398	0.345982	
a40	Lin	0.844	2	0.081	0.1013	0.146155	
b40	Lin	0.965	2	0.018	0.1005	0.145001	0.154956
c40	Lin	0.879	2	0.063	0.1204	0.173712	
b45	Lin	0.55	2	0.258	-0.2177	-0.3141	-0.3141
c45	Lin	0.55	2	0.258	-0.2177	-0.3141	

52In20C	Method	Rsq.	d.f.	Sigf	b1	Div/Day	Avg.
a25	Lin	0.979	1	0.093	0.119	0.171692	
b25	Lin	0.888	1	0.217	0.088	0.126966	0.173712
c25	Lin	0.985	1	0.077	0.1542	0.222479	
a30	Lin	0.897	1	0.209	0.0654	0.094359	
b30	Lin	0.995	1	0.045	0.1584	0.228538	0.201847
c30	Lin	0.912	1	0.192	0.1959	0.282643	
a35	Lin	0.991	1	0.059	0.1995	0.287837	0.271462
b35	Lin	0.914	1	0.189	0.1768	0.255086	
a40	Lin	0.937	1	0.162	-0.0933	-0.13461	
b40	Lin	0.957	1	0.134	-0.3662	-0.52835	-0.53292
c40	Lin	0.959	1	0.129	-0.6486	-0.9358	
a45	Lin	0.991	1	0.062	-0.5554	-0.80133	-0.59414
b45	Lin	0.957	1	0.132	-0.2682	-0.38696	

31In20C	Method	Rsq.	d.f.	Sigf	b1	Div/Day	Avg.
a25	Lin	0.75	1	0.333	-0.0656	-0.09465	
b25	Lin	0.949	1	0.145	0.1254	0.180926	0.133939
c25	Lin	0.982	1	0.086	0.2187	0.315539	
a30	Lin	0.962	1	0.125	0.1924	0.277593	
b30	Lin	0.999	1	0.017	0.2584	0.372818	0.271534
c30	Lin	1	1	0.001	0.1138	0.16419	
a35	Lin	0.75	1	0.333	0.0282	0.040687	0.040687
a40	Lin	0.994	1	0.051	-0.2682	-0.38696	-0.30364
c40	Lin	0.75	1	0.333	-0.1527	-0.22031	
a45	Lin	0.975	1	0.101	-0.5074	-0.73207	-0.73207

19ln20c	Method	Rsq.	d.f.	Sigf	b1	Div/Day	Avg.
a25	Lin	0.25	1	0.667	-0.0676	-0.09753	
b25	Lin	0.87	1	0.234	-0.1831	-0.26418	-0.20228
c25	Lin	0.277	1	0.647	-0.1699	-0.24513	
a30	Lin	1	1		-0.231	-0.33329	
b30	Lin	0.93	1	0.171	-0.1831	-0.26418	-0.26836
c30	Lin	0.984	1	0.081	-0.1439	-0.20762	
b35	Lin	0.75	1	0.333	-0.0827	-0.11932	-0.17761
c35	Lin	0.946	1	0.149	-0.1635	-0.2359	
b40	Lin	0.975	1	0.101	-0.5074	-0.73207	-0.59999
c40	Lin	0.75	1	0.333	-0.3243	-0.4679	
c45	Lin	0.857	1	0.247	-6.8333	-9.85904	-9.85904

Appendix G. Results from linear regression at 25°C
Lin = Linear regression, d.f. = degrees of freedom, Sigf = significance, b1 = slope of line

123ln25C	Method	Rsq.	d.f.	Sigf	b1	Div/Day	Avg.
b25	Lin	0.816	2	0.097	0.1678	0.242101	0.262011
c25	Lin	0.869	2	0.068	0.1954	0.281922	
a30	Lin	0.936	2	0.033	0.2345	0.338335	
b30	Lin	0.944	2	0.028	0.2103	0.303419	0.32232
c30	Lin	0.938	2	0.031	0.2254	0.325206	
a35	Lin	0.962	2	0.019	0.2412	0.348002	
b35	Lin	0.929	2	0.036	0.2325	0.335449	0.329438
c35	Lin	0.86	2	0.072	0.2113	0.304862	
a40	Lin	0.987	2	0.007	0.2314	0.333862	0.333213
c40	Lin	0.911	2	0.045	0.2305	0.332564	
a45	Lin	0.745	2	0.137	0.1901	0.274275	
b45	Lin	0.644	2	0.198	0.1563	0.225509	0.202135
c45	Lin	0.613	2	0.217	0.0739	0.106622	

67ln25C	Method	Rsq.	d.f.	Sigf	b1	Div/Day	Avg.
a25	Lin	0.118	1	0.777	0.0927	0.133747	0.193551
c25	Lin	0.981	1	0.088	0.1756	0.253354	
a30	Lin	0.965	1	0.119	0.247	0.35637	
b30	Lin	0.862	1	0.243	0.2857	0.412206	0.320877
c30	Lin	0.473	1	0.517	0.1345	0.194056	
a35	Lin	1	1	0.005	0.2657	0.38335	
b35	Lin	0.93	1	0.17	0.2176	0.313952	0.361516
c35	Lin	0.873	1	0.232	0.2684	0.387246	
a40	Lin	0.943	1	0.154	0.2252	0.324917	
b40	Lin	0.825	1	0.274	0.2103	0.303419	0.322368
c40	Lin	0.998	1	0.032	0.2348	0.338768	
a45	Lin	0.406	1	0.56	-0.0749	-0.10807	
b45	Lin	0.169	1	0.73	-0.0661	-0.09537	-0.02434
c45	Lin	0.489	1	0.507	0.0904	0.130429	

52ln25C	Method	Rsq.	d.f.	Sigf	b1	Div/Day	Avg.
b25	Lin	1	1	0.003	0.034	0.049055	0.081085
c25	Lin	0.416	1	0.554	0.0784	0.113115	
a30	Lin	0.99	1	0.064	0.1626	0.234598	
b30	Lin	0.939	1	0.158	0.1127	0.162603	0.203963
c30	Lin	0.889	1	0.216	0.1488	0.214688	
a35	Lin	0.906	1	0.198	0.1251	0.180493	
b35	Lin	1	1	0.014	0.2192	0.31626	0.204059
c35	Lin	0.972	1	0.107	0.08	0.115423	
b40	Lin	0.972	1	0.107	0.2067	0.298225	0.274131
c40	Lin	0.968	1	0.114	0.1733	0.250036	
a45	Lin	0.957	1	0.132	-0.2119	-0.30573	
b45	Lin	0.905	1	0.2	-0.1638	-0.23633	-0.20945
c45	Lin	0.943	1	0.153	-0.0598	-0.08628	

31ln25C	Method	Rsq.	d.f.	Sigf	b1	Div/Day	Avg.
a25	Lin	0.501	3	0.181	-0.0727	-0.10489	-0.12574
b25	Lin	0.9	3	0.014	-0.1016	-0.14659	
a30	Lin	0.899	3	0.041	-0.0691	-0.0997	
b30	Lin	0.854	3	0.025	-0.0655	-0.0945	-0.12466
c30	Lin	0.808	3	0.038	-0.1246	-0.17977	
a35	Lin	0.863	3	0.022	-0.1089	-0.15712	
b35	Lin	0.328	3	0.313	-0.1019	-0.14702	-0.14428
c35	Lin	0.637	3	0.105	-0.0892	-0.1287	
a40	Lin	0.313	3	0.327	-0.0559	-0.08065	
b40	Lin	0.746	3	0.059	-0.1156	-0.16679	-0.1111
c40	Lin	0.279	3	0.36	-0.0595	-0.08585	
a45	Lin	0.345	3	0.297	-0.0522	-0.07531	
b45	Lin	0.089	3	0.625	-0.0249	-0.03593	-0.03708
c45	Lin	0.124	3	0.562	-0.035	-0.0505	

19ln25C	Method	Rsq.	d.f.	Sigf	b1	Div/Day	Avg.
a25	Lin	1	0		-0.265	-0.38234	
b25	Lin	1	0		-0.154	-0.22219	-0.2875
c25	Lin	1	0		-0.1788	-0.25797	
a30	Lin	0.866	2	0.069	-0.1545	-0.22291	
b30	Lin	0.894	2	0.055	-0.2458	-0.35464	-0.33112
c30	Lin	0.785	2	0.114	-0.2882	-0.41581	
a35	Lin	0.492	2	0.298	-0.1081	-0.15597	
b35	Lin	0.715	2	0.154	-0.1387	-0.20012	-0.19069
c35	Lin	0.742	2	0.138	-0.2008	-0.28971	
a40	Lin	0.043	2	0.794	-0.057	-0.08224	
b40	Lin	0.412	2	0.358	-0.1309	-0.18886	-0.12663
c40	Lin	0.5	2	0.293	-0.0754	-0.10879	
a45	Lin	0.928	2	0.037	-0.1842	-0.26576	
b45	Lin	0.558	2	0.253	-0.1035	-0.14933	-0.3165
c45	Lin	0.697	2	0.165	-0.3704	-0.53441	

Appendix H. Results of linear regression

Lin = Linear regression, d.f. = degrees of freedom, Sigf = significance, b1 = slope of line

123ln30C	Method	Rsq.	d.f.	Sigf	b1	Div/Day	Avg.
a25	Lin	0.501	1	0.499	0.0482	0.069543	0.040759
b25	Lin	0.006	1	0.952	0.0083	0.011975	
a30	Lin	0.201	1	0.704	0.0533	0.076901	
b30	Lin	0.457	1	0.527	0.1073	0.154812	0.152599
c30	Lin	0.777	1	0.313	0.1567	0.226086	
a35	Lin	0.987	1	0.072	0.2549	0.367768	
b35	Lin	0.995	1	0.045	0.2225	0.321021	0.331458
c35	Lin	1	1	0.005	0.2118	0.305584	
a40	Lin	0.95	1	0.143	0.2334	0.336748	0.363223
c40	Lin	0.939	1	0.159	0.2701	0.389698	
b45	Lin	1	0		-0.1865	-0.26908	
c45	Lin	1	0		-0.231	-0.33329	-0.30118

67ln30C	Method	Rsq.	d.f.	Sigf	b1	Div/Day	Avg.
a25	Lin	0.432	1	0.543	0.1361	0.196364	
b25	Lin	0.114	1	0.781	0.0566	0.081662	0.18891
c25	Lin	0.711	1	0.361	0.2001	0.288703	
b30	Lin	0.973	1	0.104	0.2214	0.319434	0.321959
c30	Lin	0.949	1	0.145	0.2249	0.324484	
a35	Lin	0.914	1	0.19	0.2149	0.310056	0.307315
b35	Lin	0.988	1	0.07	0.2111	0.304574	
a40	Lin	0.998	1	0.026	0.2704	0.390131	
b40	Lin	0.98	1	0.09	0.2089	0.3014	0.344828
c40	Lin	0.999	1	0.025	0.2377	0.342952	
a45	Lin	1	0		-0.4621	-0.66671	-0.5
c45	Lin	1	0		-0.231	-0.33329	

52ln30C	Method	Rsq.	d.f.	Sigf	b1	Div/Day	Avg.
a25	Lin	0.874	1	0.231	0.1323	0.190882	
b25	Lin	0.868	1	0.237	0.1815	0.261867	0.222815
c25	Lin	0.896	1	0.209	0.1495	0.215698	
a30	Lin	0.999	1	0.022	0.1748	0.2522	
b30	Lin	0.986	1	0.077	0.2008	0.289713	0.276054
c30	Lin	0.924	1	0.177	0.1984	0.28625	
a35	Lin	0.918	1	0.185	0.1302	0.187852	
b35	Lin	0.999	1	0.018	0.1722	0.248449	0.267205
c35	Lin	1	1	0.012	0.2532	0.365315	
a40	Lin	0.872	1	0.233	0.1184	0.170827	0.263959
b40	Lin	0.922	1	0.18	0.2475	0.357091	
a45	Lin	0.75	1	0.333	-0.5759	-0.8309	-0.72075
b45	Lin	0.75	1	0.333	-0.4232	-0.61059	

31ln30C	Method	Rsq.	d.f.	Sigf	b1	Div/Day	Avg.
b25	Lin	0.207	2	0.545	0.03	0.043284	-0.02575
c25	Lin	0.649	2	0.194	-0.0657	-0.09479	
b30	Lin	0.214	2	0.538	-0.0439	-0.06334	-0.08635
c30	Lin	0.268	2	0.482	-0.0758	-0.10936	
a35	Lin	0.655	2	0.191	0.1267	0.182802	0.131799
b35	Lin	0.796	2	0.108	0.056	0.080796	
a40	Lin	0.933	2	0.034	0.0548	0.079065	0.056846
b40	Lin	0.854	2	0.076	0.0634	0.091473	
a45	Lin	0.924	1	0.177	-0.3064	-0.44207	-0.27017
b45	Lin	0.873	1	0.232	-0.06811	-0.09827	

19ln30C	Method	Rsq.	d.f.	Sigf	b1	Div/Day	Avg.
a25	Lin	1	0		0.1352	0.195066	-0.06911
b25	Lin	1	0		-0.231	-0.33329	
b30	Lin	1	0		-0.3996	-0.57654	-0.40622
c30	Lin	1	0		-0.1635	-0.2359	
a35	Lin	1	0		-0.2594	-0.37426	
b35	Lin	1	0		-0.2303	-0.33228	-0.35327
a40	Lin	1	0		-0.1352	-0.19507	
b40	Lin	1	0		-0.1025	-0.14789	-0.16732
c40	Lin	1	0		-0.1102	-0.159	
a45	Lin	1	0		-0.4142	-0.5976	
b45	Lin	1	0		-0.555	-0.80075	-0.58827
c45	Lin	1	0		-0.254	-0.36647	