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

Hugo A. Magaña *, Tracy A. Villareal [1]

The University of Texas at Austin, Marine Science Institute, 750 Channel View Drive, Port Aransas TX 78373, USA

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Abstract

The red tide dinoflagellate *Karenia brevis* (Davis) G. Hansen and Moestrup is noted for causing mass mortalities of marine organisms in the Gulf of Mexico. Most research has focused on culture isolates from the eastern Gulf of Mexico. In this investigation, we examine the effects of light, temperature and salinity on the growth rate of *K. brevis* from the western Gulf of Mexico. Growth rates of *K. brevis* were determined under various combinations of irradiance (19, 31, 52, 67, and 123 μmol m^{-2} s^{-1}), salinity (25, 30, 35, 40 and 45), and temperature (15, 20, 25, and 30 °C). Maximum growth rates varied from 0.17 to 0.36 div day^{-1} with exponential growth rates increasing with increasing irradiance. Little or no growth was supported at 19 μmol photons m^{-2} s^{-1} for any experiment. Maximum growth rates at 15 °C were much lower than at other temperatures. 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} versus 0.2–1.0 div day^{-1}). The Texas clone SP3 had a very similar light saturation point compared to that of a Florida isolate (Wilson's clone) (67 μmol m^{-2} s^{-1} versus 65 μmol m^{-2} s^{-1}), and light compensation (20–30 μmol m^{-2} s^{-1}1). The upper and lower salinity tolerance of the Texas clone was similar than that of some Florida clones (45 versus 46 and 25 versus 22.5, respectively). In our study, the Texas clone had the same temperature tolerance reported for Florida clones (15–30 °C). While individual clones can vary considerably in maximum growth rates, our results indicate only minor differences exist between the Texas and Florida strains of *K. brevis* in their temperature and salinity tolerance for growth. While the literature notes lower salinity occurrences of *K. brevis* in nearby Louisiana, our isolate from the southern Texas coast has the higher salinity requirements typical of *K. brevis* in the eastern Gulf of Mexico.
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Keywords: *Karenia brevis*; Light intensity; Red tide; Salinity; Temperature

1. Introduction

Mass mortalities of marine organisms in the Gulf of Mexico (GOM) are often associated with blooms of red tide organisms. A dominant toxic dinoflagellate in the GOM is *Karenia brevis* (Davis) G. Hansen and

* Corresponding author at: U.S. Forest Service, Rocky Mountain Research Station, 333 Broadway St SE #115, Albuquerque NM 87102, USA. Tel.: +1 505 724 3682; fax: +1 505 724 3688.
E-mail addresses: hmagana@fs.fed.us (H.A. Magaña), tracy@utmsi.utexas.edu (T.A. Villareal).
[1] Tel.: +1 361 749 6736.

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Moestrup (*K. brevis*). This organism produces potent neurotoxins (brevetoxins) that are harmful to both fish and mammals, and can have significant human health impacts (Kusek et al., 1999; Kirkpatrick et al., 2004). *K. brevis* is found throughout the GOM, and at times off the southeast Atlantic coast of the U.S. (Steidinger et al., 1998) with a historical record of icthyotoxic red tides dates back to the earliest European explorers in Mexico (Magaña et al., 2003). Florida experienced *K. brevis* blooms almost yearly over the last quarter century (Kusek et al., 1999). In Texas, *K. brevis* blooms are relatively rare, but their frequency has increased over the past decade (Villareal et al., 2000). The mechanisms leading to bloom inception are unclear; recent work has focused on complex interactions of dust transport from Saharan Africa, nitrogen fixation and remineralization links to *K. brevis* blooms (Walsh and Steidinger, 2001).

Early work on salinity tolerance in *K. brevis* led Ray and Aldrich (1967) to note that there may be a salinity barrier that prevents blooms from contaminating shellfish below a salinity of 24. However, Dortch et al. (1998) noted *K. brevis* in low salinity waters near the Mississippi River. These results raise concerns that *K. brevis* in the western GOM may have different temperature-salinity requirements than isolates from the eastern GOM. The objective of this study was to define the range of environmental factors that provide optimal growth for a Texas clone of *K. brevis* under various environmental conditions (light, salinity, and temperature). With this information, we can compare the results to published data from other areas to determine if the western Gulf of Mexico populations have similar characteristics.

2. Methods and materials

K. brevis clone SP3 was obtained from the University of Texas at Austin Marine Science Institute (UTMSI), Port Aransas, Texas, as xenic, unialgal cultures. They were cultured in L1-Si media (Guillard and Hargraves, 1993) modified by replacing $NaH_2PO_4 \cdot H_2O$ with equimolar K_2HPO_4. This clone was isolated by Suzanne Pargee at UTMSI from in October, 1999 from a red tide bloom off South Padre Island, Texas. Seawater (salinity 36) used for media preparation was collected 200 miles offshore and aged in the dark for several months prior to use. This seawater was diluted with Milli-Q reagent water 18 MΩ (Millipore Corp. system MQ) to achieve treatment salinities of 25, 30, and 35. Other salinity treatment levels (40 and 45) were obtained by evaporating seawater via aeration and an aquarium heater. Salinity was measured with a Fisher Scientific refractometer (Cat. # 13-946-27) calibrated with a seawater salinity standard of 35. The L1-Si medium was prepared using sterile filtered seawater through a 0.2 μm surfactant free cellulose acetate filter (Nalgene® Co.). All glassware, culture vessels, and utensils were washed in hot water and detergent (Liquinox™) and then rinsed repeatedly with deionized water. All items were placed in a 5% HCl water bath and allowed to soak overnight to remove trace metals. Items were rinsed repeatedly with deionized water and then repeatedly rinsed with 18 MΩ deionized water and left to air dry inverted in a dish rack to reduce contamination. Borosilicate culture tubes (25 mm × 150 mm Kimble Co.) were filled with 18 MΩ deionized water and autoclaved at 121 °C at 15 psi for 30 min. Stock cultures were maintained in 1 L borosilicate bottles (Kimax Corp.) at 25 °C in a separate incubator (Hotpack Corp. model 352622) under 20 W ''daylight'' fluorescent lamps on a 12/12 light, dark photoperiod. Aliquots (150 mL) from stock cultures were transferred into 250 mL polycarbonate square bottles in a laminar flow hood. These parent cultures were acclimated in a walk-in incubator to the designated light intensity, salinity, and temperature for 2 weeks prior to inoculation.

Triplicate culture tubes (30 mL medium) were each aseptically inoculated in a laminar flow hood with 3 mL of inoculum from parent cultures. Culture tubes racks were transferred to a walk-in incubator. Inoculated tubes were then placed along the perimeter of 4 × 10 test tube rack, returned to the walk-in incubator and maintained at the appropriate temperature for each experiment (15, 20, 25, 30, or 35 °C).

Illumination was provided by overhead fluorescent ''warm white'' 40 W lights for all experiments. The height 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 over the culture tube racks. Irradiance was measured at the top, middle, and bottom level of culture tubes with a calibrated 4π

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}. Infrequently, a small (<5 μm) colorless flagellate was observed co-existing in *K. brevis* cultures (salinities of 25 and 30) during 15 and 30 °C experiments. Because this flagellate never achieved substantial abundance, it was not considered to have a negative impact on growth rate of *K. brevis*.

Culture tubes were sampled at 3-day intervals. Each culture tube was homogenized for five seconds on a vortex and a 1 mL sample extracted. Each sample was placed in a screw-top glass vial and preserved with one drop of Lugol's solution (Throndsen, 1978). Culture tubes were subsampled for a period of 14 days. Cell counts were enumerated using a stereomicroscope (Olympus Corp. model SZH-ILLD) at 64× on a grided Sedgewick-Rafter counting chamber (Guillard, 1973). Growth rates (div day^{-1}) were calculated using the slope of the line relating time and cell counts (Guillard, 1973). Growth rates-irradiance plots were curve-fit to the Jassby and Platt (1976) hyperbolic tangent function model. Evaluation of fits was determined using nonlinear least-squares regression. Light compensation (I_c) for all experiments was calculated from a Jassby and Platt (1976) hyperbolic tangent function model. Light compensation values were verified against inspection of graphs. There was little or no growth at the lowest light intensities (19 and 31 μmol m^{-2} s^{-1}) creating difficulties curve fitting the I_c. In these cases, the I_c value was determined by visual inspection.

3. Results

Growth rate curves in general followed the Jassby-Platt (J-P) formulation (Fig. 1A–D). Goodness of fit became increasingly poor at physical criteria extremes, especially at higher salinities and lower temperatures (Table 1). Exponential growth rates of *K. brevis* increased with irradiance at greater than I_c, however, the exceptions to this pattern were cultures at salinity of 45 maintained at 15, and 30 °C, which had no growth. Negative growth rates are due to the inability of *K. brevis* to grow at high salinity levels except at the highest light regime.

Maximum growth rate for all temperature/salinity combinations was 0.36 div day^{-1} at a salinity of 30 at

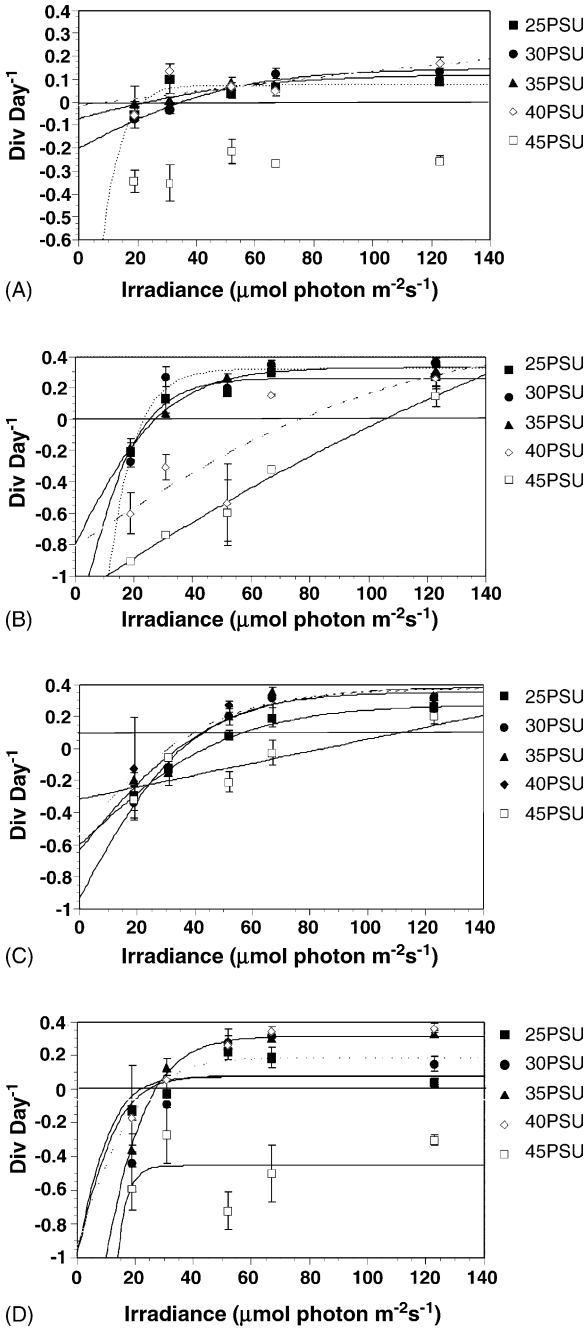

Fig. 1. (A–D) Growth rate of *Karenia brevis* Texas clone SP3 at different temperatures and irradiance levels. (A) Irradiance-growth curves 15 °C. (B) Irradiance-growth curves 20 °C. (C) Irradiance-growth curves 25 °C. (D) Irradiance-growth curves 30 °C.

Table 1
Results of μ_{max}, I_c, and R^2 for all experiments

	19 μmol m^{-2} s^{-1}	31 μmol m^{-2} s^{-1}	52 μmol m^{-2} s^{-1}	67 μmol m^{-2} s^{-1}	123 μmol m^{-2} s^{-1}
15 °C	Salinity of 25, μ_{max} = 0.09, I_c = 21.0, R^2 = 0.80	Salinity of 30, μ_{max} = 0.13, I_c = 35.0, R^2 = 0.96	Salinity of 35, μ_{max} = 0.12, I_c = 21.0, R^2 = 0.92	Salinity of 40, μ_{max} = 0.17, I_c = 7.9, R^2 = 0.45	Salinity of 45, μ_{max} = N/A, I_c = N/A, R^2 = 0.22
20 °C	Salinity of 25, μ_{max} = 0.30, I_c = 25.0, R^2 = 0.95	Salinity of 30, μ_{max} = 0.36, I_c = 23.0, R^2 = 0.92	Salinity of 35, μ_{max} = 0.32, I_c = 28.0, R^2 = 0.99	Salinity of 40, μ_{max} = 0.26, I_c = 77.0, R^2 = 0.72	Salinity of 45, μ_{max} = 0.15, I_c = 105.0, R^2 = 0.98
25 °C	Salinity of 25, μ_{max} = 0.26, I_c = 42.0, R^2 = 0.99	Salinity of 30, μ_{max} = 0.32, I_c = 35.0, R^2 = 0.98	Salinity of 35, μ_{max} = 0.36, I_c = 32.5, R^2 = 0.92	Salinity of 40, μ_{max} = 0.33, I_c = 30.0, R^2 = 0.90	Salinity of 45, μ_{max} = 0.20, I_c = 85.0, R^2 = 0.86
30 °C	Salinity of 25, μ_{max} = 0.20, I_c = 22.0, R^2 = 0.86	Salinity of 30, μ_{max} = 0.32, I_c = 25.0, R^2 = 0.88	Salinity of 35, μ_{max} = 0.33, I_c = 27.0, R^2 = 0.99	Salinity of 40, μ_{max} = 0.34, I_c = 25.0 R^2 = 0.83	Salinity of 45, μ_{max} = N/A, I_c = N/A, R^2 = 0.86

μ_{max} = div day^{-1}, I_c = μmol m^{-2} s^{-1}.

20 °C (Fig. 1B) and at a salinity of 35 at 25 °C (Fig. 1C). Maximum growth rates occurred between light intensities of 31 and 67 μmol m^{-2} s^{-1} for all experiments where there was growth. In the laboratory setting there seems to be an inability for *K. brevis* to tolerate high light at temperature/salinity extreme, and photoinhibition at the mid temperatures and highest salinity. It is a consistent pattern that *K. brevis* does not prefer high salinities, but can tolerate better in the 20–25 °C ranges. This may explain why there have been no blooms in the hypersaline Laguna Madre in the summer when the temperature exceeds 30 °C. Little or no growth was supported at a light intensity of 19 μmol m^{-2} s^{-1} at any temperature/salinity combinations.

K. brevis (SP3) could not be acclimated to salinity levels >45 or <25. Salinities that yielded the greatest growth were 30 and 35 at 20 and 25 °C, respectively (0.36 div day^{-1}). In general, the pattern for maximum growth rate was a gradual increase as salinity increased then a decrease in growth rate at the higher salinities. The upper salinity limit was abrupt at both 15 and 30 °C with near maximum growth at a salinity of 40 and no growth at 45. No growth was observed at a salinity of 45 at temperature extremes or below a light intensity of 123 μmol m^{-2} s^{-1}.

K. brevis (SP3) could not be acclimated to temperatures >30 °C. All attempts to increase the temperature >30 °C 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 maximum growth rates were 50% lower than those at higher temperatures. The maximum growth rates at temperature and salinity are as follows; 15 °C at salinity of 40 was 0.17 div day^{-1} (Fig. 1A) 20 °C at salinity of 30 was 0.36 div day^{-1} (Fig. 1B) 25 °C at salinity of 35 was 0.36 div day^{-1} (Fig. 1C) 30 °C at salinity of 40 was 0.34 div day^{-1} (Fig. 1D).

There was little variability in growth rate within the normal range of environmental conditions. The general pattern for I_c for experiments at 15–25 °C (Fig. 2A–D) was an inverse relationship between I_c and μ_{max}. At higher salinities, more light was required for growth with a maximum value of 105 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. 2A). Light compensation could not be calculated for cultures at salinity of 45 at the temperature extremes of 15 and 30 °C. A tabular summary of all growth rate and compensation light intensity data is present in Table 1.

4. Discussion

Maximum growth rates (0.17–0.36 div day^{-1}) displayed by *K. brevis* in this study fell within the range (0.2–0.5 div day^{-1}) reported by Wilson (1966) and Steidinger (1983), but were lower (0.2–1.0 div day^{-1}) than those reported by Shanley and Vargo, (1993), and higher (0.16–0.19 div day^{-1}) than those reported by Doig (1973). In the present study, growth rates increased with increasing irradiance from 31 to 67 μmol m^{-2} s^{-1}. Suboptimal temperature and light conditions resulted in an inability to tolerate the

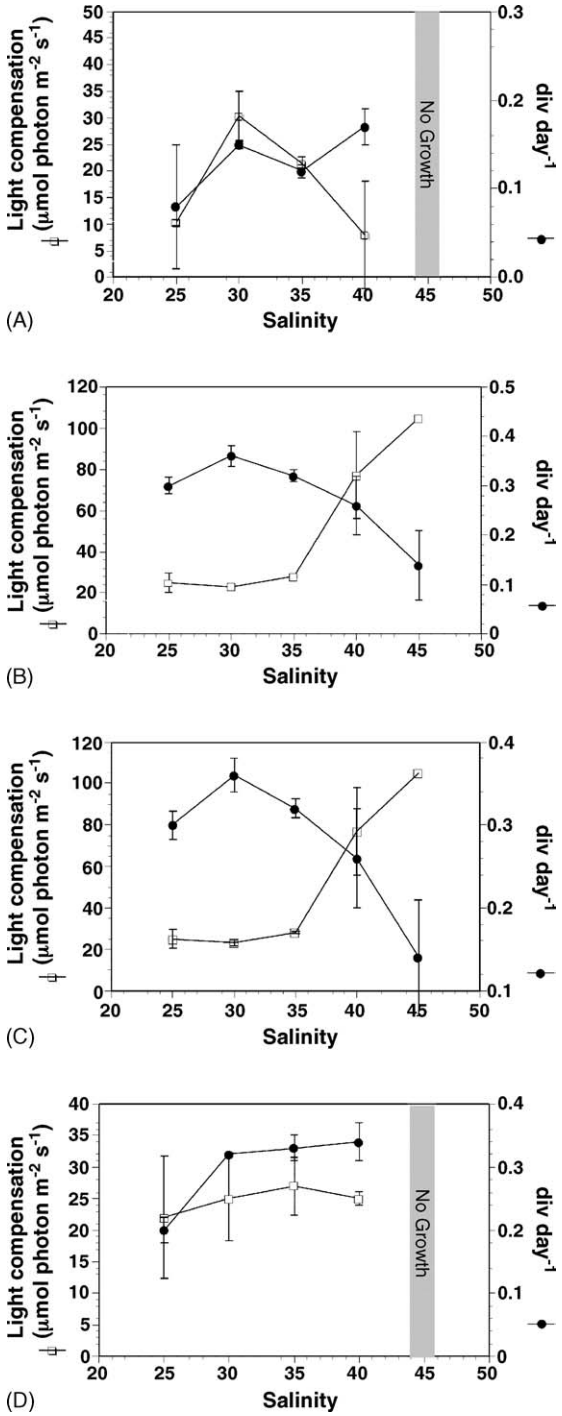

Fig. 2. (A–D) Light compensation-growth rates. (A) Light compensation-growth rates 15 °C. (B) Light compensation-growth rates 20 °C. (C) Light compensation-growth rates 25 °C. (D) Light compensation-growth rates 30 °C.

highest salinity. Results indicate that, under normal environmental conditions (20–25 °C), a light intensity of approximately 67 μmol m^{-2} s^{-1} could be the saturation point for the Texas clone. Light saturation for the Texas clone closely approximated that of a Florida clone (67 μmol m^{-2} s^{-1} versus 65 μmol m^{-2} s^{-1}, respectively) reported by Shanley and Vargo (1993).

While the results in this study showed substantial variability in growth rates of *K. brevis*, they also indicated that variation in I_c was largely a function of salinity and temperature, and that I_c was sensitive to environmental extremes. *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 (26.25–30 μmol m^{-2} s^{-1}; Wilson and Collier, 1955, 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}. However, this value was extrapolated from only two values in the light-limited section of the curve, both of which had large error bars. With the exception of this one result, our data are reasonably consistent with the results from previous studies; however, further investigations of other clones and different temperature/salinity combinations are warranted.

Results from this study indicate only a slightly narrower range of salinity tolerance (25–40) than those reported from previous studies. *K. brevis* clone SP3 could not be acclimated to salinity levels above 45 or below 25. This is a slightly narrower range than Aldrich and Wilson (1960) who reported a salinity minimum of 22.5 and a maximum of 46. Interestingly, Dortch et al. (1998) reported *K. brevis* found in the northern Gulf of Mexico at salinity as low as 5. Rounsefell and Nelson (1966) reported that the upper salinity limit of 37 for *K. brevis* appeared to vary with temperature. Growth was poor above salinity of 36, in combination with temperature in excess of 23 °C. Clone SP3 exhibited a greater tolerance for high salinity (>35) at 25 and 30 °C, although salinity of 40 was lethal at 30 °C. The data from the present study indicated an upper limit of salinity is approximately 40–45. In the present study, adequate growth of *K. brevis* was supported between salinity of 25–40, however, it did not grow at salinity of 45 except at the

highest light intensity of 123 μmol m^{-2} s^{-1} and at temperatures of 20 and 25 °C. Repeated attempts to culture SP3 at salinities >45 failed.

A temperature tolerance for Florida *K. brevis* was reported 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. Growth rates in this study were significantly lower at 15 °C than other temperatures indicating that low temperatures probably restrict its growth in Texas coastal waters in late fall and winter. The upper temperature for growth (33 °C) reported by Hitchcock (1976) is somewhat higher and does not agree with results from the present study since all attempts to culture *K. brevis* (SP3) >30 °C failed. Results compare favorably with those of Eng-Wilmot et al. (1977) who reported rapid decline in viability of cells above 31 °C. Minor variations in water quality may also contribute to variation in growth rates.

In summary, Texas *K. brevis* clone SP3 varied in growth rate when cultured under different light, salinity, and temperature criteria. The Texas clone (SP3) had a similar light saturation point compared to that of the Florida isolate (67 μmol m^{-2} s^{-1} versus 65 μmol m^{-2} s^{-1}), and similar light compensation point (20–30 μmol m^{-2} s^{-1}1) to most previous studies. The upper salinity tolerance of the Texas clone was slightly lower (45 versus 46) than that of some Florida clones; however, lower salinity tolerance was similar. The Texas clone had the same temperature tolerance as that of Florida clones (15–30 °C). Based on these laboratory studies, there appear to be no differences in the environmental range of this one Texas isolate to the data collected from Florida isolates.

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